STUDIES ON THE EFFECT OF CORIANDER AND CUMIN ON PLATELETS

A thesis submitted to the

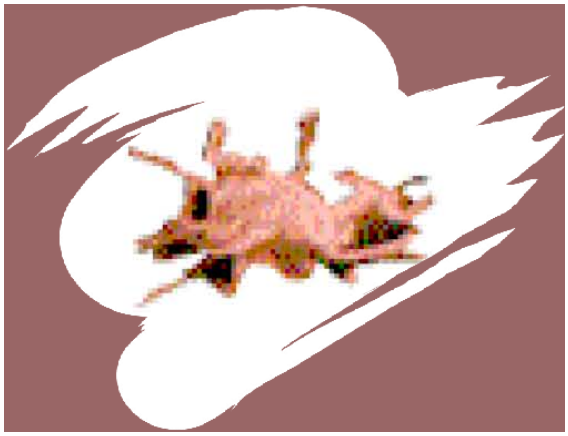
University of Mysore

for the award of the degree of

DOCTOR OF PHILOSOPHY

in

FOOD SCIENCE



By

WESLEY JESSIE SUNEETHA

Department of Biochemistry and Nutrition

Central Food Technological Research Institute

Mysore 570 020 India

April 2005

To you.....

Dear Mummy

Ms. Wesley Jessie Suneetha
Research Fellow
Dept of Biochemistry and Nutrition
Central Food Technological Research Institute
Mysore 570 020

DECLARATION

I hereby declare that the thesis entitled "**Studies on the effect of coriander and cumin on platelets**" submitted to the University of Mysore, for the award of the degree of **Doctor of Philosophy** in the Faculty of **Food Science** is the result of work carried out by me under the guidance of **Dr T.P. Krishnakantha**, Scientist, Dept of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore, during the period March 2000 to April 2005.

I further declare that the results have not been previously submitted for any other degree or fellowship.

(**Jessie Suneetha. W**)

Dr. T. P. Krishnakantha
Scientist
Dept of Biochemistry and Nutrition

CERTIFICATE

This is to certify that the thesis entitled "**Studies on the effect of coriander and cumin on platelets**" submitted to the University of Mysore, for the award of the Degree of **Doctor of Philosophy** in the Faculty of **Food Science** by Ms. Wesley Jessie Suneetha is the result of work carried out by her in the Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore under my guidance during the period March 2000 to April 2005.

(T.P. Krishnakantha)
Research Supervisor

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First and foremost I would like to thank **God Almighty** for His abundant Grace and Blessings through out the course of this work.

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At this juncture, **Dear Mummy,**

All I can say is **"Thank you"**

Jessie Suneetha. W

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List of Abbreviations

°C	: degrees Celsius
μg	: microgram
μl	: microlitre
μm	: micromole
12-HETE	: 12 L-hydroxy-5, 8,10,14-eicosatetraenoic acid
12-HHT	: 12 L-hydroxy 5,8,10-hepta decatrienoic acid
12-HPETE	: 12 L-hydroperoxyl-5, 8,10,14-eicosatetraenoic acid
$Ca^{+2}Mg^{+2}$ATPase	: Calcium Magnesium activated Adenosine triphosphatase
g	: gram
hr	: hour
M	: Molar
MDA	: Malondialdehyde
mg	: milligram
min	: minute
N	: Normal
$Na^{+}K^{+}$ATPase	: Sodium Potassium activated Adenosine triphosphatase
nm	: nanomole
OD	: Optical Density
PG	: Prostaglandin
Pi	: Inorganic Phosphate
TXA_2	: Thromboxane A_2
TXB_2	: Thromboxane B_2
v/v	: volume by volume

SYNOPSIS

Platelets are the tiny corpuscular cells found in the blood along with erythrocytes and leukocytes. They are continuously surveying the inner layers of intact blood vessels and play an important role in haemostasis. Whenever there is any break / damage in the blood vessels, platelets are exposed to the damaged tissue resulting in a cascade of events like shape change, secretion and aggregation leading to the formation of a precise haemostatic plug. But if these synergistic interactions take place *in vivo*, it results in the blockage of blood supply to essential organs causing cardio-vascular or cerebro-vascular complications. It is reported that patients with atherosclerosis possess increased platelet activity.

Platelets are activated by a variety of agonists like ADP, epinephrine, collagen, thrombin, arachidonic acid, calcium ionophore A 23187 and ristocetin resulting in the shape change from discoidal to spherical. Also platelets possess a unique property of 'stickiness', which is seen once they are activated resulting in the adhesion of platelets to one another.

Platelet aggregation is a membrane-associated event and the involvement of platelet membranes is crucial due to their interaction with the various agonists. A variety of dietary components influence the membrane characteristics like fluidity, stability and susceptibility to oxidative damage. Membrane lipids are highly susceptible to peroxidation resulting in the damage to the membrane structure and function. Spices the known food adjuncts possess anti-diabetic, anti-inflammatory, hypolipidemic and anti - lithogenic effect as per Ayurveda, the indigenous system of Indian medicine.

Reports indicate that aqueous extracts of onion and garlic inhibited platelet aggregation by acting on the arachidonic acid metabolism. Similarly,

aqueous extract of ginger inhibited ADP, epinephrine, collagen and arachidonic acid induced platelet aggregation by reducing the formation of thromboxanes.

A dietary survey indicated that red chillies, turmeric, garlic, coriander and cumin are the most commonly used spices in Indian households. Hence two of these – **coriander** and **cumin** were selected for the present investigation, as no information is available on the effect of these spices at present. In principle the effect of these spices on human platelet aggregation, its membrane structure and function were investigated *in vitro*. Experiments were carried out *in vitro* using platelets isolated from human blood.

The results of this investigation are presented in three chapters.

Chapter 1:

This chapter presents a brief review of existing literature on platelets, its formation, role in haemostasis and blood coagulation, platelet aggregation, lipid peroxidation, membrane bound enzymes like $Na^{+}K^{+}$ATPase and $Ca^{+2}Mg^{+2}$ATPase, membrane fluidity, fluorescence anisotropy, probes of membrane structure, anti platelet aggregating agents, abnormalities of platelet functions.

A brief history of role of spices and their pharmacological role along with that of coriander and cumin is also presented.

Chapter 2:

In this chapter the experimental methods employed in the present investigation is detailed.

Chapter 3:

The results of the present investigation haven been discussed in this chapter under five sub headings, which are as follows:

1. Effect of coriander and cumin extracts on human platelets.
2. Effect of coriander and cumin extracts on human platelet membrane lipid peroxidation and membrane fluidity.
3. Effect of the coriander and cumin extracts on the kinetics of human platelet membrane bound enzymes.
4. Sephadex fractionation of cumin and coriander extracts.
5. Effect of other spices on human platelets.

1. Effect of coriander and cumin extracts on human platelets:

(a) Aqueous extracts of coriander and cumin were tested on human platelets for their inhibitory effect at different concentrations with a variety of agonists like ADP, epinephrine, collagen, calcium ionophore A 23187 and ristocetin to obtain the IC_{50} at one-minute incubation.

(b) The effect of increase in the duration of incubation from one minute to 2, 4 and 8 min were determined at the IC_{50}.

(c) After aggregation the products like malondialdehyde (MDA) and serotonin released were assayed for all the spice extracts.

(i) Raw extracts of coriander and cumin were tested on human platelets for their inhibitory effect and steps 1a – 1c were carried out.

(ii) Heat treatment of coriander and cumin.

The stability of the inhibitory activity of coriander and cumin were tested by subjecting them to heat treatment (boiling and roasting) as they may

be susceptible to changes due to their chemical nature. These extracts were used on human platelets and steps 1a – 1c were carried out.

(iii) Combination of coriander and cumin.

Spices are generally used in combinations. In order to determine their synergistic effect, these two spices were taken in the ratio of 1:1. Raw and heat treated extracts were used on human platelets and steps 1a – 1c were carried out.

(iv) Processed coriander dhal extract was tested on human platelets and steps 1a – 1c were carried out.

(v) The pure isolated and selected components of coriander and cumin available commercially were tested on human platelets to assess their inhibitory effect and steps 1a – 1c were carried out.

(vi) Effect of extracts of coriander and cumin at IC_{50} were used to study their effect on washed platelets with agonists like ADP, collagen and A 23187.

2. Effect of coriander and cumin extracts on human platelet membrane lipid peroxidation and membrane fluidity:

Since lipid peroxidation adversely affects the biosystems, human platelet membrane lipid peroxidation was carried *in vitro* using iron-ascorbic acid system to assess the protective effect of extracts of coriander and cumin on lipid peroxidation.

Membrane fluidity using fluorescent probes was studied to understand the interaction of the extracts of coriander and cumin with human platelet membranes and its relationship to their inhibitory effect.

3. **Effect of the coriander and cumin extracts on the kinetics of human platelet membrane bound enzymes:**

As platelet aggregation is a membrane-associated phenomenon, studies were carried out to determine the effect of the raw and heat-treated coriander and cumin extracts on membrane bound enzymes $Na^{+}K^{+}$ATPase and $Ca^{+2}Mg^{+2}$ATPase. K_m and K_I were determined for each of the extracts of coriander, cumin and their combination.

4. **Sephadex fractionation of cumin and coriander extracts:**

The extracts of cumin and coriander were fractionated on sephadex G-50 and G-25, which yielded fractions with poor inhibitory activity.

5. **Effect of other spices on human platelets:**

Initially other spices like cardamom, saffron, swallowroot, coriander leaf and curry leaf were screened for their inhibitory effect on human platelets. Apart from that other reported spices like turmeric, ginger, garlic, cloves and cinnamon were also tested with agonists like ADP, epinephrine, collagen, A 23187 or ristocetin for comparison.

Summary and Conclusion:

This section summarises the findings of this investigation and possible mode of inhibitory action of coriander and cumin on human platelets.

A collective **Bibliography** is given at the end of the thesis followed by the **Appendix**.

Materials and Methods

Materials:

All the spices, including coriander and cumin seeds used for preparing the extracts, were purchased from local market. Processed coriander dhal was obtained from Grain Science and Technology department, CFTRI, Mysore.

Chemicals:

Adenosine diphosphate (ADP), adenosine triphosphate (ATP), Bovine Serum Albumin (BSA), epinephrine, collagen, calcium ionophore A 23187, ristocetin, 1,6-Diphenyl- 1, 3, 5-Hexatriene (DPH), Ouabain (Strophanthin-G), Ethylene glycol (b-aminoethyl ether)- N, N, NO, NO- tetra acetic acid (EGTA), Thiobarbituric acid (TBA) along with α-Linalool (coriander) and α- Terpinene (cumin) were purchased from Sigma Chemical Co., St. Louis, UO, USA. Trichloroacetic acid (TCA), ascorbic acid, O-phthaldialdehyde and tetrahydrofuran were purchased from SISCO Research Laboratories Pvt. Ltd, Mumbai, India.

All other chemicals, including solvents were of extra pure analytical grade, available either from Glaxo Laboratories (India) Ltd or Sarabhai M. Chemicals, Baroda, India or Rankhem Fine Chemicals Limited, SAS Nagar, Punjab, India or Merck Limited, Mumbai, India.

METHODS:

Isolation of Platelets:

Venous blood was collected from healthy human volunteers with oral consent (who had not taken any drugs like aspirin that may affect platelet aggregation at least for the past 10 days) in 3.8% tri-sodium citrate (9:1 v/v) and was used within 3 hr of collection. This citrated blood was centrifuged using a Remi R8C clinical centrifuge at 1100 rpm for 20 min to obtain Platelet Rich Plasma (PRP), which was aspirated carefully into a polypropylene tube kept at 37 °C till use. The isolation of platelets was done using polypropylene tubes or siliconised glass tubes. The residual blood was again centrifuged at 2500 rpm for 20 min to obtain the homologous Platelet Poor Plasma (PPP). Platelet count was adjusted to 1.6 X 10^8 platelets per ml of PRP using a haemocytometer (Gerrad, 1982).

Preparation of washed platelets:

Washed platelets were prepared by applying citrated PRP to a Sepharose 2B column. First Sepharose 2B column was thoroughly washed with acetone and 0.9% saline after the gel was deaerated. The column was equilibrated with buffer and then platelets were applied. The size of column depends on the amount of PRP to be applied and degree of separation from plasma proteins. It was usually seen that 5-10 times the plasma volume for good separation. The platelets, which were eluted at the void volume, were collected by visual observation of the change in opacity of the column effluent.

The Ca^{+2} free Tyrodes buffer consists of NaCl 137 mM, KCl 2.7 mM, $NaHCO_3$ 12 mM, EDTA 1 mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 1 mM and glucose 5.6 mM along with 2 g of bovine serum albumin, pH 7.3 which was used as the elutant (Gerrad, 1982).

Preparation of platelet membranes:

PRP was mixed with double the volume of saline and centrifuged at 2500 rpm for 10 min. To the platelet pellet, 5 ml volume of Tyrodes buffer (NaCl 137 mM, KCl 2.7 mM, $NaHCO_3$ 12 mM, EDTA 1 mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 1 mM and glucose 5.6 mM, pH 7.3) was added; washed twice and final wash was done with 5 ml of 0.1 M Tris-HCl pH 7.4. The platelet pellet formed was homogenized finally in 0.1 M Tris-HCl and kept frozen till use (Brunauer and Huestis, 1993).

Preparation of platelet membranes from agonist challenged platelets:

PRP was subjected to aggregation with ADP, epinephrine, collagen, A 23187 or ristocetin in the presence and absence of raw coriander and cumin as well as their processed extracts, α-Linalool and α-Terpinene at their respective IC_{50}. The platelets after aggregation were used to prepare platelet membranes as per the modified procedure of Brunauer and Huestis, 1993.

The plasma containing platelet aggregates was mixed with double the volume of saline and centrifuged at 2500 rpm for 10 min. To the platelet pellet, 2 ml of Tyrodes buffer (NaCl 137 mM, KCl 2.7 mM, $NaHCO_3$ 12 mM, EDTA 1 mM, NaH_2PO_4 0.4 mM, $MgCl_2$ 1 mM and glucose 5.6 mM,

pH 7.3) was added, washed twice and final wash was done with 1.5 ml 0.1 M Tris-HCl pH 7.4. The platelet pellet formed was homogenized in 0.1 M Tris-HCl and kept frozen till use. These platelet membranes were used to assay $Na^{+}K^{+}$ATPase, $Ca^{2+}Mg^{2+}$ATPase activities and for lipid peroxidation inhibition studies.

Platelet Aggregation:

The aggregation experiments were carried out turbidimetrically in a Dual Path Aggro-meter (Chrono-Log Corporation, Havertown, PA, USA). PRP of 0.45 ml was kept stirred at 1200 rpm and temperature was maintained at 37 ^{o}C. Aggregation was induced by adding agonists like ADP (61 μM), epinephrine (76 μM), collagen (11 μg / ml), calcium ionophore A 23187 (6 μM) or ristocetin (1.25 μg / ml) to record the progression of aggregation on a chart paper for atleast 4 to 5 min and this was used as the control. The slope of the line and percent aggregation were calculated.

Inhibition of platelet aggregation:

PRP (0.45 ml) was pre-incubated with a known amount of the various extracts for 1 min 37 ^{o}C with constant stirring to expose the platelets to the extracts. After 1 min platelet aggregation was induced by the addition of the agonists like ADP, epinephrine, collagen, A 23187 or ristocetin. The reaction was followed for atleast 4 to 5 min. The slope of the line and percent inhibition were calculated.

The concentration dependent inhibition of platelet aggregation was used to determine the IC_{50} for each of the extracts. Time course of incubation was studied by pre-incubating the PRP with IC_{50} of each of the

extracts by increasing the duration of incubation to 2, 4 and 8 min followed by induction of platelet aggregation by the respective agonists. This was also followed for 4 to 5 min.

Estimation of protein in platelet homogenate:

Principle:

The reaction between the –CONH group of protein molecule with the copper ion in alkali and reduction of phosphomolybdic-phosphotungstic acid reagent by tyrosine and tryptophan present in the treated proteins results in a blue colour in solution proportional to the amount of protein present in the assay at 650 nm in a Spectrophotometer.

Procedure:

Protein concentration was determined in platelet membranes and agonist challenged platelet membranes by modified Folin-phenol method. To the protein sample, 1 ml of solution A (2 g of sodium potassium tartarate and 100 g of sodium carbonate dissolved in 500 ml of 1 N sodium hydroxide and diluted to 1 L) was added and left at 50 ^{o}C for 10 min. This was cooled to room temperature and 0.1 ml of solution B (2 g of sodium tartarate and 1 g of copper sulphate dissolved in 90 ml water and 10 ml of 1 N sodium hydroxide) was added and left at room temperature for 10 min followed by the addition of 3 ml of solution C (1 volume of Folin-Coicaltaeu reagent diluted to 15 volumes with water) forcibly.

The tubes were then left again at 50 ^{o}C for 10 min, cooled to room temperature and the absorbance was read at 650 nm. Bovine serum albumin was used as the standard (Hartree, 1972).

Estimation of malondialdehyde (*MDA*) in platelets after aggregation:

Platelets after aggregation (0.45ml) were transferred to an Eppendorf tube and 0.02 ml of 1% BHT in ethanol plus 0.1 ml of 100% TCA in 3 N HCl were added. The precipitate was centrifuged at 10000 rpm for 10 min. From this 0.5 ml of supernatant was taken and mixed with 0.1 ml of TBA reagent (0.12 M TBA in 0.26 M Tris-HCl). The contents were left in boiling water bath for 30 min and the chromophore generated was measured at 532 nm. The malondialdehyde (*MDA*) was calculated by using the following equation –

Amount of MDA formed = (Absorbance/156) X

(Total volume/mg of protein/ml)

MDA was expressed in nmoles of MDA formed/mg protein/hr (Maguire and Csona-Khalifah, 1987).

Estimation of serotonin in platelets after aggregation:

Platelets after aggregation (0.45 ml) was mixed with 0.1 ml of 6 M Trichloroacetic acid to precipitate the protein and centrifuged at 10000 rpm for 10 min. After centrifugation 0.5 ml of supernatant was mixed with 2 ml of O-phthaldialdehyde reagent (0.5% O-phthaldialdehyde in ethanol and 8 N HCl). These mixtures were placed in boiling water bath for 10 min, cooled on ice and washed twice with chloroform. It was read in spectrofluorimeter with excitation 360 nm and emission 475 nm. The values were expressed as nmoles of serotonin released/mg protein/hr (Holsmen and Dangelmaier, 1989).

Estimation of inorganic phosphate:

Principle:

The inorganic phosphate reacts with the acidic molybdic acid to form phosphomolybdate complex, which is reduced by ascorbic acid. This method is about seven times more sensitive than Fiske-Subbarow procedure and also involves less pipetting. One can easily determine 0.01 μmole of phosphate liberated in solution.

Procedure:

To 0.3 ml of reaction mixture containing liberated inorganic phosphate, 0.7 ml of solution A (1 part of 10% ascorbic acid and 6 parts of 0.42% ammonium molybdate in 1 N H_2SO_4) was added and left at 37 ^oC for 1 hr. It was cooled to room temperature and absorbency was read at 820 nm (Ames, 1966).

Assay of Na^+K^+ATPase:

Principle:

Na^+K^+ATPase transports Na^+ and K^+ ions against the gradient at the cost of ATP molecules liberating inorganic phosphate, which is estimated using Ames method.

Method:

The activity of Na^+K^+ATPase (E.C. No.3.6.1.3) in platelet membranes was assayed in which the reaction mixture contained 140 mM NaCl, 14 mM KCl, 3 mM $MgCl_2$, 0.2 mM EDTA, 3 mM ATP, 20 mM Tris (pH 7.0) and 100 μg of platelet membrane protein in a final reaction volume of 0.5 ml. The reaction was initiated by adding 3 mM ATP to the reaction

mixture and incubated at 37 °C for 1 hour in the presence and absence of 2 mM Ouabain, an inhibitor of this enzyme. The reaction was terminated by the addition of 0.5 ml of 10 % TCA followed by cooling on ice. The amount of inorganic phosphate liberated was estimated by the method of Ames (1966).

The enzyme activity is expressed as μmoles of phosphate liberated/hr/ mg protein (Kaplay, 1978).

Assay of $Ca^{+2}Mg^{+2}$ATPase:

$Ca^{+2}Mg^{+2}$ stimulated ATPase was estimated by modified method of Kaser-Glanzmann *et al.*, (1978). The reaction mixture contained 100 mM KCl, 5 mM $MgCl_2$, 0.1mM $CaCl_2$ and 20 mM Tris-HCl (pH 7.0) and 0.2 mg of platelet membranes in final volume of 0.5 ml. The reaction was initiated by adding 2 mM ATP to the reaction mixture and incubated at 37 °C for 1 hour in the presence and absence of 2 mM EGTA an inhibitor of this enzyme. The reaction was terminated by the addition of 0.5 ml of 10 % TCA followed by cooling in ice. The amount of inorganic phosphate liberated was estimated by method of Ames (1966).

The enzyme activity is expressed as μmoles of phosphate liberated/hr/ mg protein.

Enzyme kinetics:

Kinetic parameter K_m was determined in platelet membranes in the presence of the various spice extracts as above by assaying the activities at various concentrations of ATP ranging from 0.45 mM to 1.5 mM for

$Na^{+}K^{+}$ATPase and 0.8 to 2.4 mM for $Ca^{+2}Mg^{+2}$ ATPase. The rate of the reaction was computed by determining the liberated inorganic phosphate.

Reciprocal plots (Lineweaver-Burk plots) of the rate of reaction (1/V) versus substrate concentration (1/S) were constructed from which the above parameter was derived. K_I values were also determined for each of the spice extracts.

Platelet membrane lipid peroxidation:

The extent of lipid peroxidation can be estimated using malondialdehyde (MDA) and conjugated dienes formed. The most common method is the estimation of MDA formed.

Estimation of malondialdehyde (MDA) formed:

Platelet membranes (1.5 mg/ml of 0.1 M Tris-HCl buffer pH 7.4) were dispersed in a medium containing 0.15 M KCl, 0.025 M Tris-HCl, 2 mM ADP and 10 μm $FeSO_4$. The contents were incubated for 5 min at 37 °C. Lipid peroxidation was initiated by adding 0.1 mM ascorbic acid. The final reaction volume was 1 ml. Appropriate blanks without platelet membranes were also incubated along with samples for 30 min at 37 °C. The reaction was terminated after 30 min by adding 2 ml of TBA reagent (0.375% TBA in 15% TCA and 0.2 N HCl) and again left for 30 min in boiling water bath. The MDA formed was measured at 535 nm.

Lipid peroxidation is expressed as μmole of MDA formed/mg protein/hr. MDA formed was calculated using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$ (Miller & Aust, 1989).

Estimation of conjugated dienes formed:

Platelet membranes (1.5 mg/ml of 0.1 M Tris-HCl Buffer pH 7.4) were dispersed in a medium containing 0.15 M KCl, 0.025 M Tris-HCl, 2 mM ADP and 10 μm $FeSO_4$. The contents were incubated for 5 min at 37 °C. Lipid peroxidation was initiated by adding 0.1 mM ascorbic acid. The final reaction volume was 1 ml. Appropriate blanks without platelet membranes were also incubated along with samples for 30 min at 37 °C. The reaction was terminated after 30 min by adding 2 ml of TBA reagent (0.375% TBA in 15% TCA and 0.2 N HCl) and left for 30 min in boiling water bath (Miller & Aust, 1989). These samples were allowed to cool, and 2 ml of chloroform: methanol (2:1) was added, vortexed, the upper layer extracted and dried under nitrogen. To this dried sample 2 ml of hexane was added and absorbancy read at 234 nm for 5-HETE formed.

Molar absorption co-efficient ä for conjugated dienes at 234 nm is 2.5 X 10^4 / M / cm^{-1}. This is a modified method of Stoffel and Ahrens, 1958 (Salimath, *et al.*, 1986).

Fluorescence polarisation measurements:

This was determined by steady state fluorescence polarisation with the fluorescent probe 1,6-Diphenyl-1,3,5-Hexatriene (DPH) using a spectrofluorimeter (Shimadzu RF-5000) coupled with a polarization unit. A stock solution of 2 mM DPH in tetrahydrofuran was prepared, stored under refrigeration, well protected from light. Equal volumes of platelet membrane preparation (100 μg protein/ml) and DPH dispersion in tetrahydrofuran diluted 1000 fold to 2 μM were mixed and incubated at 37 °C for 30 min.

Fluorescence was measured at a temperature of 37 °C with constant stirring. Excitation and emission wavelengths were 355 and 432 nm respectively. The results were corrected for light scattering. The equation used to determine the anisotropy (r) was:

$$r = Ivv-G.Ivh / Ivv+Ivh$$

where G = Ihv/Ihh, the instrument correction factor, Ivv and Ivh are the emitted light components polarised vertically (v) and horizontally (h) with reference to the polarisation orientation of the excitation light. The anisotropy parameter $[(r_o/r)-1]^{-1}$ was calculated using the limiting anisotropy of DPH (r_o = 0.362). Membrane lipid fluidity is inversely proportional to fluorescence anisotropy (Hayam, *et al.*, 1993).

Preparation of coriander extracts:

(a) Preparation of raw coriander extract:

Coriander seeds (10 g) were taken, ground and sieved to obtain a fine powder of particle size 200 μ from which 4 g was taken and made into slurry with 10 ml water and left over night at 4 °C. Next day the raw extract was obtained by centrifuging at 10000 rpm for 15 min. This clear extract was lyophilised completely and 12 mg of the lyophilised sample was dissolved in 1 ml water to be used as the inhibitor. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

(b) Preparation of boiled coriander extract:

Coriander seeds (10 g) were taken, ground and sieved to obtain a fine powder of particle size 200 μ from which 4 g was taken and made into

slurry with 20 ml water. This slurry was boiled at 98 °C for 15 min and 30 min respectively, cooled and left over night at 4 °C. Next day the boiled extracts were obtained by centrifuging at 10000 rpm for 15 min. These clear extracts were lyophilised completely and 12 mg of each was dissolved in 1 ml water to be used as inhibitors. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

(c) Preparation of roasted coriander extract:

Coriander seeds (10 g) were taken, roasted at 120.5 °C for 4 min and 8 min respectively, cooled, ground and sieved to obtain a fine powder of particle size 200 μ from which 4 g each were taken and made into slurry with 10 ml water. These slurries were left over night at 4 °C. Next day the roasted extracts were obtained by centrifuging the slurries at 10000 rpm for 15 min. The clear extracts were lyophilised completely and 12 mg of each was dissolved in 1 ml water to be used as inhibitors. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

Preparation of cumin extracts:

(a) Preparation of raw cumin extract:

Cumin seeds (10 g) were taken, ground and sieved to obtain a fine powder of particle size 200 μ from which 4 g was taken and made into slurry with 10 ml water and left over night at 4 °C. Next day the raw extract was obtained by centrifuging at 10000 rpm for 15 min. This clear extract was lyophilised completely and 16 mg of the lyophilised sample was

dissolved in 1 ml water to be used as the inhibitor. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

(b) Preparation of boiled cumin extract:

Cumin seeds (10 g) were taken, ground and sieved to obtain a fine powder of particle size 200 μ from which 4 g was taken and made into slurry with 20 ml water. This slurry was boiled at 98 °C for 15 min and 30 min respectively, cooled and left over night at 4 °C. Next day the boiled extracts were obtained by centrifuging at 10000 rpm for 15 min. The clear extracts were lyophilised completely and 16 mg of each was dissolved in 1 ml water to be used as inhibitors. The amount of extract used varied between 5 to 50 μl. pH of the extracts ranged between 7.0 to 7.4.

(c) Preparation of roasted cumin extract:

Cumin seeds (10 g) were taken, roasted at 125.5 °C for 4 min and 8 min respectively, cooled, ground and sieved to obtain fine powders of particle size 200 μ from which 4 g was taken and made into slurry with 10 ml water. These slurries were left over night at 4 °C. Next day the roasted extracts were obtained by centrifuging at 10000 rpm for 15 min. The clear extracts were lyophilised completely and 16 mg of each was dissolved in 1 ml water to be used as inhibitors. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

Preparation of coriander and cumin mix extracts:

It is obtained by taking coriander and cumin seeds in the ratio of 1:1.

(a) Preparation of raw coriander and cumin mix extract:

Both these seeds (10 g each) were ground together and sieved to obtain a fine powder of particle size 200 μ from which 4 g was taken and made into slurry with 10 ml water and left over night at 4 °C. Next day the raw extract of the mix was obtained by centrifuging at 10000 rpm for 15 min. This clear extract was lyophilised completely and 14 mg of the lyophilised sample was dissolved in 1 ml water to be used as the inhibitor. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

(b) Preparation of boiled coriander and cumin mix extract:

Both these seeds (10 g each) were ground together and sieved to obtain a fine powder of particle size 200 μ from which 4 g each were taken and made into slurries with 20 ml water. The slurries were boiled at 98 °C for 15 min and 30 min respectively, cooled and left over night at 4 °C. Next day the boiled extracts were obtained by centrifuging at 10000 rpm for 15 min. The clear extracts were lyophilised completely and 14 mg of each was dissolved in 1 ml water to be used as inhibitors. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

(c) Preparation of roasted coriander and cumin mix extract:

Both coriander and cumin seeds (10 g each) were roasted separately at 120.5 and 125.5 °C for 4 min and 8 min respectively, cooled, then ground together and sieved to obtain a fine powder of particle size 200 μ. From this 4 g each was taken and made into slurries with 10 ml water.

These slurries were left over night at 4 °C. Next day the roasted extracts were obtained by centrifuging them at 10000 rpm for 15 min. The clear extracts were lyophilised completely and 14 mg of each was dissolved in 1 ml water to be used as inhibitors. The amount of extract used varied between 5 to 50 μl. pH of the extracts ranged between 7.0 to 7.4.

Preparation of Coriander Dhal:

Coriander seeds were taken, cleaned, dehusked and dry polished with paddy husk. Then to this water at 2½ to 3% was added and polished again to get the processed dhal, which is free of fiber content. This dhal is commercially used as a mouth freshener. Here it was used to make the extract.

Preparation of Coriander Dhal extract:

About 10 g of this processed dhal was taken, ground and sieved to obtain a fine powder of particle size 150 μ from which 4 g was taken and made into slurry with 10 ml water and left over night at 4 °C. Next day the extract was obtained by centrifuging at 10000 rpm for 15 min. This clear extract was lyophilised completely and 8 mg of the lyophilised sample was dissolved in 1 ml water to be used as the inhibitor. The amount of extract used varied between 5 to 50 μl. pH of the extract ranged between 7.0 to 7.4.

Partial purification of coriander and cumin extracts by gel filtration:

Aqueous extracts of raw coriander and cumin (100 mg in 1 ml) was partially purified to isolate the fraction, which may contain the inhibitory

activity. Coriander extract was loaded on sephadex G-50 column and not fractions were obtained. Similarly cumin extract was loaded on sephadex G-25 and G-50 column to obtain three (a, b and c) and two fractions (1 and 2) respectively. These fractions did not show any significant inhibition of human platelet aggregation.

Results and Discussion

Results:

Spices are a group of esoteric food adjuncts, which have been used in foods for thousands of years. As per Ayurveda, the Indian system of medicine, these spices are known to possess anti-diabetic, anti-inflammatory, hypolipidemic, anti-arthritic, anti-carcinogenic and anti-lithogenic properties to name a few (Nadkarni, 1978). A few of the spices are used for the following reasons also –

- ☞ Cardamom is used as a carminative, diuretic, stomachic and cardiac stimulant.
- ☞ Saffron is used as an anti-spasmodic, aphrodisiac, carminative and stimulative agent.
- ☞ Swallowroot is used as an appetizer and blood purifier.
- ☞ Coriander and curry leaves are used to enhance the palatability of foods.
- ☞ Coriander is used as an anti-bilious, aphrodisiac, carminative, diaphoretic and diuretic agent.
- ☞ Cumin is used as a stimulant, carminative, loctogogue, diuretic stomachic and an astringent useful in dyspepsia and diarrhea.

Though a lot has been said about the beneficial effects of spices from times immemorial, experimental evidences are being provided to this ancient knowledge in recent times through various investigations.The principle objective of this investigation is to envisage the inhibitory effect of the two of the extensively used spices in a variety of cuisine through out the

world – **CORIANDER** and **CUMIN** on human platelets by *in vitro* studies. This study covers –

1. Effect of coriander and cumin extracts on human platelets.
2. Effect of coriander and cumin extracts on human platelet membrane lipid peroxidation and membrane fluidity.
3. Effect of the coriander and cumin extracts on the kinetics of human platelet membrane bound enzymes.
4. Sephadex fractionation of cumin and coriander extracts.
5. Effect of other spices on human platelets.

1. EFFECT OF CORIANDER AND CUMIN EXTRACTS ON HUMAN PLATELETS:

Normally platelets when exposed to external stimuli undergo the phenomenon of aggregation or clumping together as a sequel to bleeding injuries. A deep cut in the skin exposes the skin collagen to flowing platelets causing aggregation to stop further bleeding. If the same events occur inside the blood vessels for reasons other than and in addition to injury, platelets do aggregate at a localized site inside the blood vessel forming a thrombus. This can further develop with time to a bigger one and eventually clog the blood vessel causing cessation of blood flow.

The consequences are fatal if it happens in the coronary arteries and cause physiological disturbances if it occurs in the brain or heart or somewhere else. In persons who are physiologically prone to thrombus formation, drugs are given to prevent platelet aggregation. These medications inhibit platelet aggregation internally. Several inhibitors are

known today. Mention may be made of aspirin, indomethacin, clopidogrel, tirofiban, phenylbutazone, oxyphenbutazone, ibuprofen, fenoprofen, tolmetin, sulfinpyrazone and sulindac. The pharmacogical effect of these platelet aggregation inhibitors has been studied *in vitro* using human or animal blood.

The beneficial health effect of several food components has been well documented. Among these, platelet aggregation inhibitors of food origin are also available. Examples are active components like curcumin from turmeric, capsaicin from red pepper and eugenol and acetyl eugenol from cloves to name a few. The present study deals with the inhibitory activity of coriander and cumin extracts on human platelet aggregation *in vitro*.

These spice extracts are used at concentrations varying between 5 to 50 μl at one-minute incubation to obtain IC_{50} with a variety of agonists like ADP, epinephrine, collagen, A 23187 and ristocetin. Each of these agonists has their own mechanism of stimulating the platelet aggregation apart from the synergistic effect of each other. The extracts of coriander and cumin have more or less inhibited the platelet aggregation.

Table 1 - Inhibition of human platelet aggregation by Coriander (raw) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.12	3.90 ± 0.12	20.0 ± 3.1
″	0.24	3.31 ± 0.11	32.2 ± 3.3
″	0.36	2.44 ± 0.08	50.0 ± 3.2
″	0.48	1.74 ± 0.05	64.3 ± 2.9
″	0.60	1.51 ± 0.04	69.1 ± 2.6
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.06	1.22 ± 0.07	12.6 ± 5.4
″	0.12	1.0 ± 0.06	28.2 ± 6.0
″	0.18	0.78 ± 0.05	44.1 ± 5.8
″	0.24	0.55 ± 0.03	60.7 ± 5.8
″	0.30	0.46 ± 0.02	67.1 ± 4.4
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.06	5.02 ± 0.17	10.4 ± 3.4
″	0.12	4.11 ± 0.14	26.6 ± 3.4
″	0.18	3.36 ± 0.13	40.0 ± 3.9
″	0.24	2.90 ± 0.10	48.2 ± 3.5
″	0.30	2.44 ± 0.06	56.5 ± 2.5
	0.36	2.30 ± 0.03	59.1 ± 1.3
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.12	4.63 ± 0.18	20.8 ± 3.9
″	0.24	3.50 ± 0.16	40.0 ± 4.6
″	0.36	2.58 ± 0.10	55.8 ± 3.8
″	0.48	2.04 ± 0.07	65.0 ± 3.4
″	0.60	1.98 ± 0.05	66.1 ± 2.5
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.12	5.76 ± 0.23	18.2 ± 4.0
″	0.24	4.40 ± 0.20	37.5 ± 4.6
″	0.36	3.60 ± 0.16	48.9 ± 4.4
″	0.48	2.80 ± 0.11	60.0 ± 3.9
″	0.60	2.0 ± 0.06	71.6 ± 3.0

Values are Mean ± S.D of triplicates.

Raw extract of coriander inhibited platelet aggregation with ADP (20 to 69%), epinephrine (12 to 67%), collagen (10 to 59%), A 23187 (20 to

66%) and ristocetin (18 to 72%) as shown in table 1 to obtain IC_{50}, which was shown in table 2 for the respective agonists.

Table 2 - IC_{50} for inhibition of human platelet aggregation by coriander (raw) extract

Agonist	IC_{50}
ADP	0.360
Epinephrine	0.198
Collagen	0.306
A 23187	0.324
Ristocetin	0.372

Values are expressed in mg.
Values are mean of triplicates.

These IC_{50} were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation to determine whether longer exposure of the platelets to the inhibitors would reveal better inhibition. A maximum inhibition of 64.7, 77.4,64.2, 63.0 and 96.2 % was seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 1.

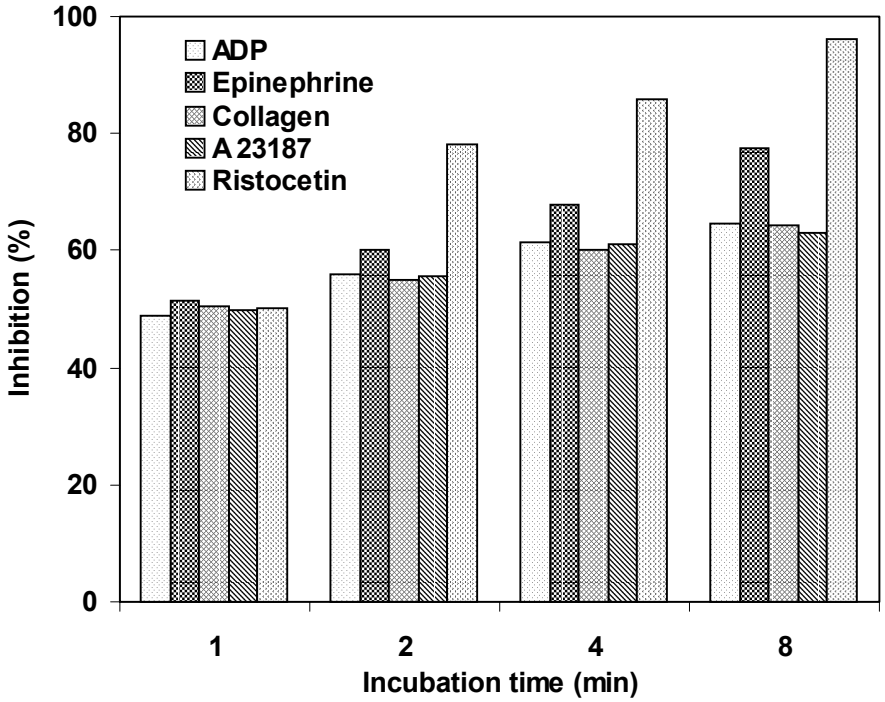


Figure 1 – Effect of coriander (raw) extract on human platelet aggregation at IC_{50}

Similarly raw extract of cumin inhibited platelet aggregation with ADP (16 to 74%), epinephrine (17 to 79%), collagen (21 to 95%), A 23187 (15 to 53%) and ristocetin (16 to 69%) as shown in table 3 to obtain IC_{50}.

Table 3 - Inhibition of human platelet aggregation by Cumin (raw) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.16	4.12 ± 0.16	15.7 ± 3.9
″	0.32	3.23 ± 0.14	33.9 ± 4.3
″	0.48	2.52 ± 0.09	48.4 ± 3.6
″	0.64	1.89 ± 0.06	61.2 ± 3.1
″	0.80	1.26 ± 0.05	74.2 ± 4.0
+ Epinephrine	0.0	1.40 ± 1.17	0.0
″	0.16	1.17 ± 0.08	16.7 ± 6.9
″	0.32	0.76 ± 0.04	45.8 ± 4.8
″	0.48	0.64 ± 0.02	54.2 ± 3.1
″	0.64	0.35 ± 0.01	75.0 ± 2.1
″	0.80	0.30 ± 0.01	78.6 ± 2.0
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.16	4.42 ± 0.17	21.1 ± 3.9
″	0.32	3.42 ± 0.17	38.9 ± 5.0
″	0.48	2.69 ± 0.10	52.0 ± 3.7
″	0.64	1.25 ± 0.04	77.6 ± 4.0
″	0.80	0.30 ± 0.01	94.7 ± 3.3
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.16	4.96 ± 0.17	15.0 ± 3.4
″	0.32	4.08 ± 0.16	30.2 ± 3.9
″	0.48	3.26 ± 0.10	44.2 ± 3.1
″	0.64	2.88 ± 0.04	50.8 ± 1.4
″	0.80	2.77 ± 0.03	52.5 ± 1.1
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.16	5.92 ± 0.20	15.9 ± 3.4
″	0.32	4.64 ± 0.19	34.4 ± 4.1
″	0.48	3.68 ± 0.12	47.7 ± 3.3
″	0.64	3.0 ± 0.07	57.4 ± 2.3
″	0.80	2.16 ± 0.04	69.3 ± 1.9

Values are Mean ± S.D of triplicates.

The IC_{50} of cumin (raw) extract is given in table 4 with the respective agonists.

Table 4 - IC_{50} for inhibition of human platelet aggregation by cumin (raw) extract

Agonist	IC_{50}
ADP	0.360
Epinephrine	0.198
Collagen	0.306
A 23187	0.324
Ristocetin	0.372

Values are expressed in mg.
Values are mean of triplicates.

These IC_{50} were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 70.0, 74.2, 69.5, 60.2 and 70.0% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 2.

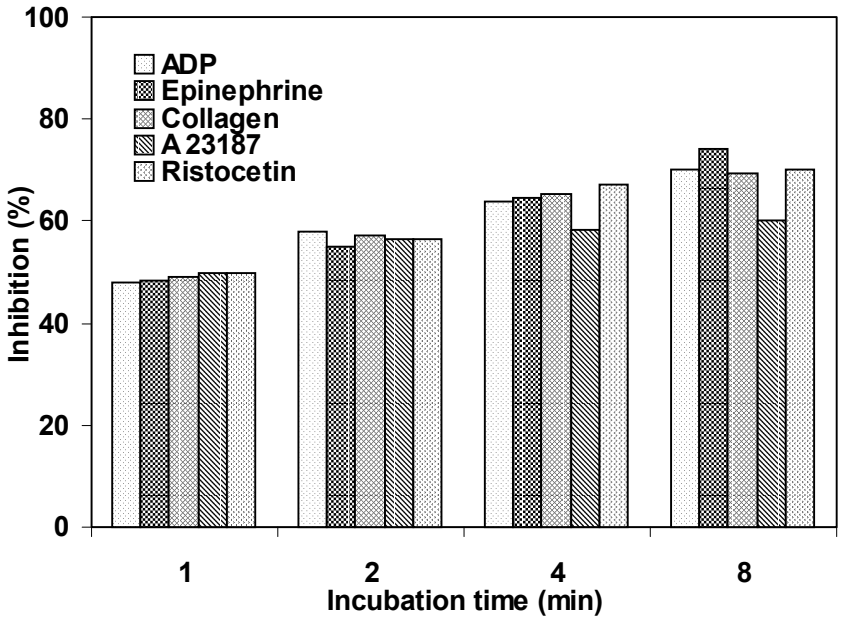


Figure 2 - Effect of cumin (raw) extract on human platelet aggregation at IC_{50}

The common practice is that spices are subjected to a variety of processing like boiling, pressure-cooking, steaming, roasting, frying, etc to name a few. Here two methods were selected namely **BOILING** and **ROASTING** to see if they in any way affect the inhibitory properties of these two spices coriander and cumin. Studies have shown that generally about 86 to 91% of active principle was lost from turmeric, 13 to 17% in black pepper powder and 4 to 19% in red pepper powder when boiled for 15 min (Srinivasan *et al.*, 1992). Similarly garlic and onion, which are used for seasoning, have shown a two-fold decrease in the consistency range when the temperature of processing was increased (Ahmed, 2000).

Coriander and cumin slurry were boiled carried for 15 min and 30 min at 98 °C. Boiled extract of coriander (15 min) inhibited human platelet aggregation with ADP (14 to 59%), epinephrine (19 to 91%), collagen (24 to 73%), A 23187 (14 to 60%) and ristocetin (13 to 59%) as shown in table 5 and boiled extract of cumin (15 min) inhibited platelet aggregation with ADP (15 to 68%), epinephrine (11 to 53%), collagen (22 to 86%), A 23187 (11 to 50%) and ristocetin (22 to 71%) as shown in table 6 to obtain IC_{50}. The boiling time was increased to 30 min, a decrease in the inhibition was observed, which ranged between 19 to 30% as shown below in Table 9 and 10 for coriander and cumin extracts respectively indicating that longer duration of boiling was significantly affecting the ability of these two extracts to inhibit human platelet aggregation.

Table 5 - Inhibition of human platelet aggregation by Coriander (boiled) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.12	4.49 ± 0.15	14.2 ± 3.6
″	0.24	3.50 ± 0.13	28.3 ± 3.7
″	0.36	3.0 ± 0.09	38.7± 3.0
″	0.48	2.44 ± 0.05	50.0 ± 2.1
″	0.60	2.02 ± 0.04	58.5 ± 2.0
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.06	1.14 ± 0.09	18.9 ± 7.8
″	0.12	0.88 ± 0.07	37.8 ± 7.4
″	0.18	0.68 ± 0.04	51.1 ± 5.0
″	0.24	0.46 ± 0.02	67.7 ± 4.6
″	0.30	0.28 ± 0.02	80.7 ± 5.7
″	0.36	0.14 ± 0.01	90.6 ± 3.6
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.12	4.24 ± 0.18	24.2 ± 4.3
″	0.24	3.56 ± 0.13	36.4 ± 3.7
″	0.36	2.86 ± 0.09	48.9 ± 3.1
″	0.48	2.12 ± 0.05	62.1 ± 2.8
″	0.60	1.53 ± 0.05	72.7 ± 3.2
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.12	5.02 ± 0.19	14.1 ± 3.8
″	0.24	4.30 ± 0.17	26.5 ± 3.9
″	0.36	3.61 ± 0.12	38.2 ± 3.3
″	0.48	2.76 ± 0.07	52.9 ± 2.5
″	0.60	2.34 ± 0.07	60.0 ± 3.0
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.12	6.12 ± 0.25	13.2 ± 4.1
″	0.24	5.16 ± 0.23	26.7 ± 4.4
″	0.36	4.22 ± 0.15	40.0 ± 3.5
″	0.48	3.32 ± 0.10	52.8 ± 3.0
″	0.60	2.86 ± 0.07	59.4 ± 2.5

Values are Mean ± S.D of triplicates.
Coriander slurry was boiled for 15 min at 98 °C.

Table 6 - Inhibition of human platelet aggregation by Cumin (boiled) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.16	4.14 ± 0.17	15.2 ± 4.1
″	0.32	3.56 ± 0.13	27.2 ± 3.7
″	0.48	2.86 ± 0.09	41.3 ± 3.2
″	0.64	2.25 ± 0.04	53.8 ± 1.8
″	0.80	1.62 ± 0.03	66.7 ± 1.9
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.03	1.29 ± 0.02	10.5 ± 1.9
″	0.06	1.18 ± 0.02	18.4 ± 1.9
″	0.10	1.07 ± 0.02	23.7 ± 1.8
″	0.12	0.88 ± 0.01	36.8 ± 1.3
″	0.16	0.78 ± 0.01	44.1 ± 1.0
″	0.19	0.66 ± 0.01	52.6 ± 0.9
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.16	4.37 ± 0.20	21.9 ± 4.6
″	0.32	3.03 ± 0.19	45.9 ± 4.3
″	0.48	2.03 ± 0.09	63.8 ± 4.4
″	0.64	0.86 ± 0.04	84.7 ± 4.7
″	0.80	0.79 ± 0.01	85.8 ± 1.3
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.16	5.21 ± 0.14	10.8 ± 2.7
″	0.32	4.47 ± 0.11	23.5 ± 2.5
″	0.48	3.98 ± 0.09	31.8 ± 2.3
″	0.64	3.43 ± 0.07	41.2 ± 2.1
″	0.80	2.92 ± 0.04	50.0 ± 1.4
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.16	5.48 ± 0.26	22.1 ± 4.8
″	0.32	4.05 ± 0.14	42.4 ± 4.1
″	0.48	2.77 ± 0.09	60.6 ± 3.3
″	0.64	2.22 ± 0.04	68.4 ± 1.8
″	0.80	2.04 ± 0.03	71.0 ± 1.5

Values are Mean ± S.D of triplicates.
Cumin slurry was boiled for 15 min at 98 °C.

The IC_{50} of coriander (boiled) extract is given in table 7 with the respective agonists.

Table 7 - IC_{50} for inhibition of human platelet aggregation by coriander (boiled) extract

Agonist	IC_{50}
ADP	0.480
Epinephrine	0.168
Collagen	0.372
A 23187	0.456
Ristocetin	0.456

Values are expressed in mg.
Values are mean of triplicates.

The IC_{50} of cumin (boiled) extract is given in table 8 with the respective agonists.

Table 8 - IC_{50} for inhibition of human platelet aggregation by cumin (boiled) extract

Agonist	IC_{50}
ADP	0.608
Epinephrine	0.182
Collagen	0.344
A 23187	0.800
Ristocetin	0.416

Values are expressed in mg.
Values are mean of triplicates.

Table 9 - Inhibition of human platelet aggregation by Coriander (boiled) extract

Extract	IC_{50} in mg	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.480	3.80 ± 0.06	22.2 ± 1.6
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.168	1.0 ± 0.02	28.9 ± 2.4
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.372	3.90 ± 0.07	30.4 ± 1.8
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.456	4.28 ± 0.07	26.7 ± 1.6
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.456	5.37 ± 0.10	23.5 ± 1.9

Values are Mean ± S.D of triplicates.
Coriander slurry was boiled for 30 min at 98 °C.

Table 10 - Inhibition of human platelet aggregation by Cumin (boiled) extract

Extract	IC_{50} in mg	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.608	3.64 ± 0.05	25.4 ± 1.4
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.182	0.96 ± 0.02	31.2 ± 2.9
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.344	3.94 ± 0.09	29.7 ± 2.3
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.80	4.64 ± 0.13	20.6 ± 2.8
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.416	5.37 ± 0.14	23.8 ± 2.6

Values are Mean ± S.D of triplicates.
Cumin slurry was boiled for 30 min at 98 °C.

The IC_{50} of boiled extract of coriander (15 min) were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 76.8, 72.7, 78.7, 63.0 and 68.6% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 3.

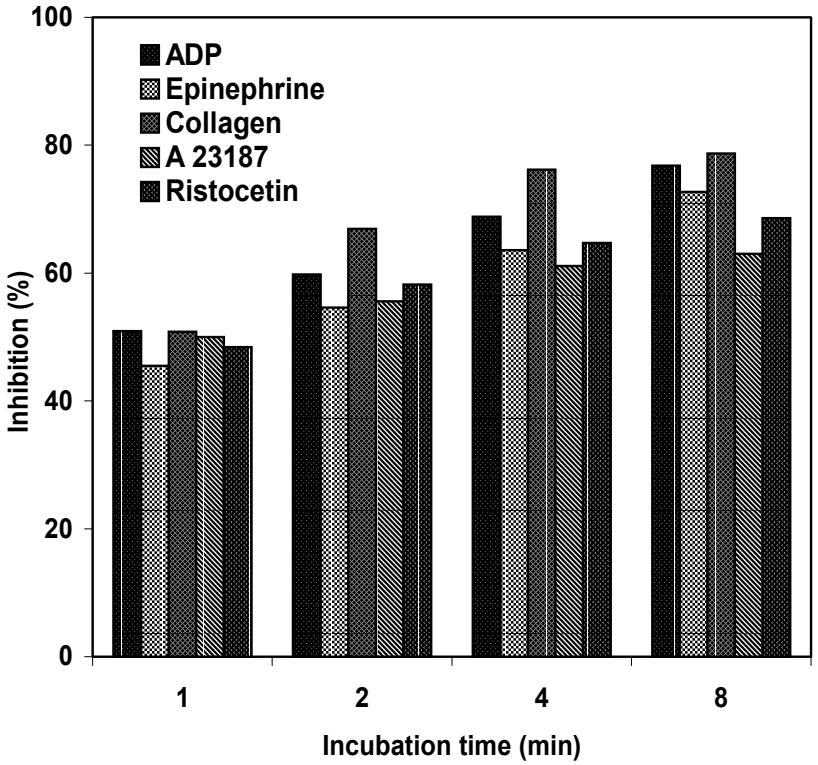


Figure 3 - Effect of coriander (boiled) extract on human platelet aggregation at IC_{50}

The IC_{50} of boiled extract of cumin (15min) were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 73.8, 77.1, 79.2, 55.8 and 72.1% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 4.

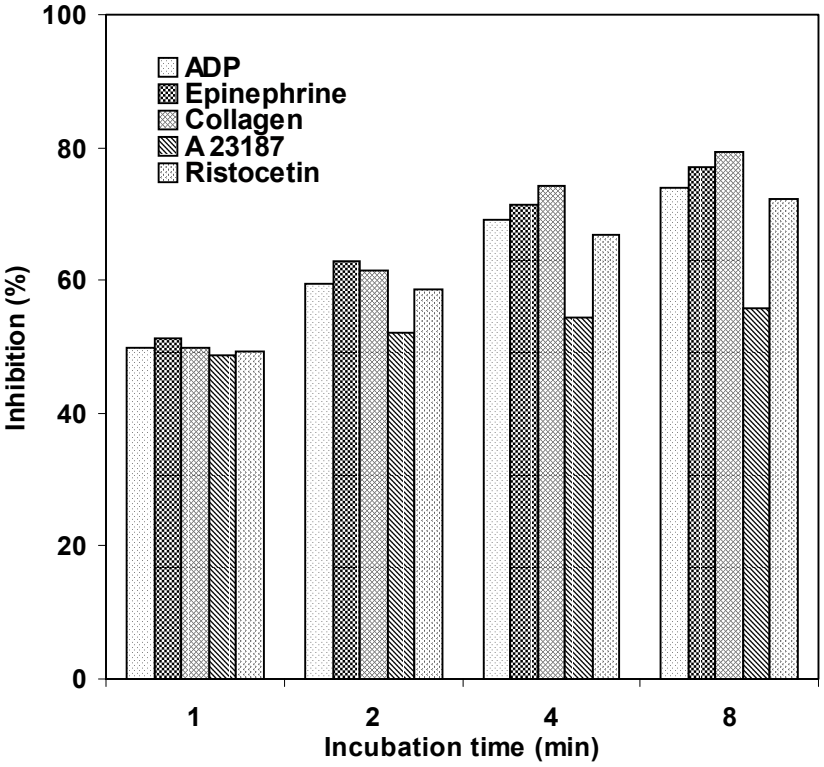


Figure 4 - Effect of cumin (boiled) extract on human platelet aggregation at IC_{50}

Coriander and cumin were subjected to roasting because the curry or sambar powders contain these spices subjected to roasting so that the characteristic aroma may be obtained to enhance the palatability of the food to which they are added.

Roasting of coriander seeds was carried for 4 min at 120.5 °C. An extract from this inhibited platelet aggregation with ADP (14 to 59%), epinephrine (19 to 91%), collagen (24 to 73%), A 23187 (14 to 60%) and ristocetin (13 to 59%) as shown in table 11 to obtain IC_{50}.

Table 11 - Inhibition of human platelet aggregation by Coriander (roasted) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.12	4.22 ± 0.15	13.6 ± 3.6
″	0.24	3.53 ± 0.12	27.6 ± 3.4
″	0.36	2.96 ± 0.08	39.3 ± 2.7
″	0.48	2.44 ± 0.06	50.0 ± 2.5
″	0.60	2.02 ± 0.04	58.6 ± 2.0
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.06	1.09 ± 0.06	22.4 ± 5.3
″	0.12	0.72 ± 0.03	48.2 ± 4.2
″	0.18	0.56 ± 0.02	60.0 ± 4.3
″	0.24	0.33 ± 0.01	76.5 ± 3.0
″	0.30	0.28 ± 0.01	80.1± 1.8
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.12	4.49 ± 0.20	19.8 ± 4.5
″	0.24	3.45 ± 0.16	38.4 ± 4.6
″	0.36	2.55 ± 0.16	54.4 ± 3.9
″	0.48	1.69 ± 0.07	69.8 ± 4.1
″	0.60	0.84 ± 0.03	85.0 ± 3.6
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.12	5.15 ± 0.20	11.8 ± 3.9
″	0.24	4.43 ± 0.17	24.1 ± 3.8
″	0.36	3.99 ± 0.13	31.7 ± 3.3
″	0.48	3.12 ± 0.09	46.5 ± 2.9
″	0.60	2.81 ± 0.04	51.8 ± 1.4
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.12	6.08 ± 0.25	13.7 ± 4.1
″	0.24	4.95 ± 0.20	29.7 ± 4.0
″	0.36	3.71 ± 0.15	47.3 ± 4.0
″	0.48	2.70 ± 0.09	61.6 ± 3.3
″	0.60	2.16 ± 0.06	69.3 ± 2.8

Values are Mean ± S.D of triplicates.
Coriander seeds were roasted for 4 min at 120.5 °C.

The IC_{50} of coriander (roasted) extract is given in table 12 with the respective agonists.

Table 12 - IC_{50} for inhibition of human platelet aggregation by coriander (roasted) extract

Agonist	IC_{50}
ADP	0.480
Epinephrine	0.156
Collagen	0.336
A 23187	0.528
Ristocetin	0.384

Values are expressed in mg.
Values are mean of triplicates.

The IC_{50} of roasted extract of coriander were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 69.1, 82.0, 81.7, 63.9 and 66.9% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 5.

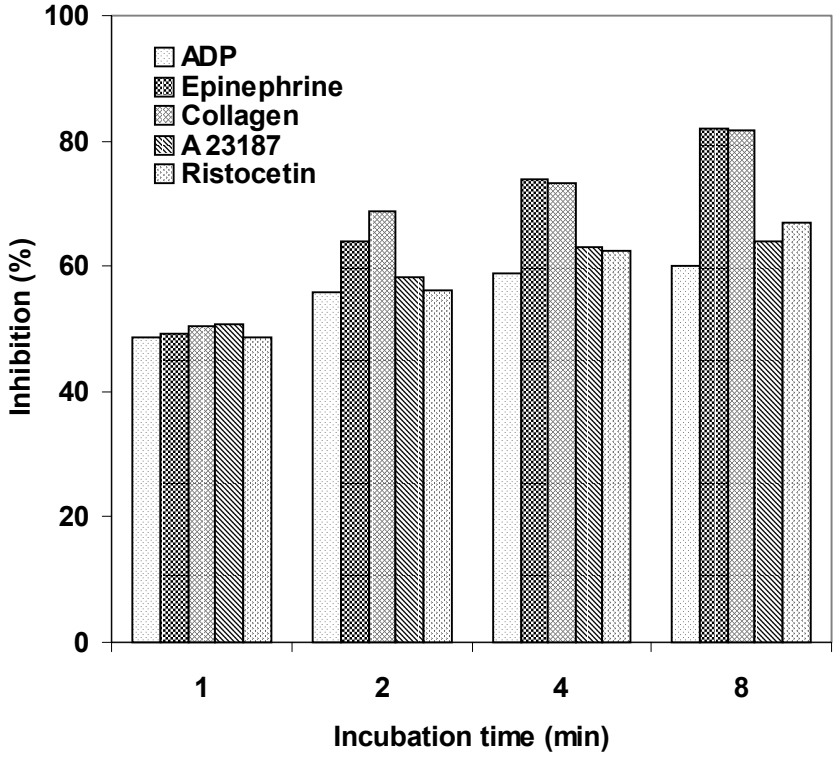


Figure 5 - Effect of coriander (roasted) extract on human platelet aggregation at IC_{50}

Roasting of cumin seeds was carried for 4 min at 125.5 °C. An extract from this inhibited platelet aggregation with ADP (12 to 60%), epinephrine (14 to 78%), collagen (20 to 95%), A 23187 (11 to 50%) and ristocetin (16 to 67%) as shown in table 13 to obtain IC_{50}.

Table 13 - Inhibition of human platelet aggregation by Cumin (roasted) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.16	4.29 ± 0.16	12.1 ± 3.7
″	0.32	3.70 ± 0.12	24.3 ± 3.2
″	0.48	3.23 ± 0.10	33.9 ± 3.1
″	0.64	2.54 ± 0.06	47.9 ± 2.4
″	0.80	1.95 ± 0.05	60.0 ± 2.6
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.02	1.21 ± 0.06	13.6 ± 4.9
″	0.03	1.02 ± 0.04	27.3 ± 4.1
″	0.05	0.88 ± 0.03	38.6 ± 3.2
″	0.06	0.63 ± 0.02	54.8 ± 3.5
″	0.07	0.31 ± 0.02	78.0 ± 6.1
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.16	4.47 ± 0.19	20.2 ± 4.3
″	0.32	3.49 ± 0.16	37.6 ± 4.6
″	0.48	2.31 ± 0.10	58.7 ± 4.3
″	0.64	1.08 ± 0.05	80.7 ± 4.6
″	0.80	0.26 ± 0.02	95.4 ± 7.7
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.16	5.21 ± 0.18	10.8 ± 3.6
″	0.32	4.47 ± 0.18	23.5 ± 4.0
″	0.48	3.98 ± 0.14	31.8 ± 3.8
″	0.64	3.43 ± 0.10	41.2 ± 2.9
″	0.80	2.90 ± 0.09	51.8 ± 3.1
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.16	5.92 ± 0.28	15.9 ± 4.7
″	0.32	5.02 ± 0.22	28.6 ± 4.4
″	0.48	4.06 ± 0.16	42.3 ± 3.9
″	0.64	3.13 ± 0.11	55.5 ± 3.5
″	0.80	2.32 ± 0.08	67.0 ± 3.5

Values are Mean ± S.D of triplicates.
Cumin seeds were roasted for 4 min at 125.5 °C.

The IC_{50} of cumin (roasted) extract is given in table 14 with the respective agonists.

Table 14 - IC_{50} for inhibition of human platelet aggregation by cumin (roasted) extract

Agonist	IC_{50}
ADP	0.656
Epinephrine	0.061
Collagen	0.40
A 23187	0.720
Ristocetin	0.576

Values are expressed in mg.
Values are mean of triplicates.

The IC_{50} of roasted extract of cumin were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 83.9, 73.3, 91.8, 61.1 and 62.7% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 6.

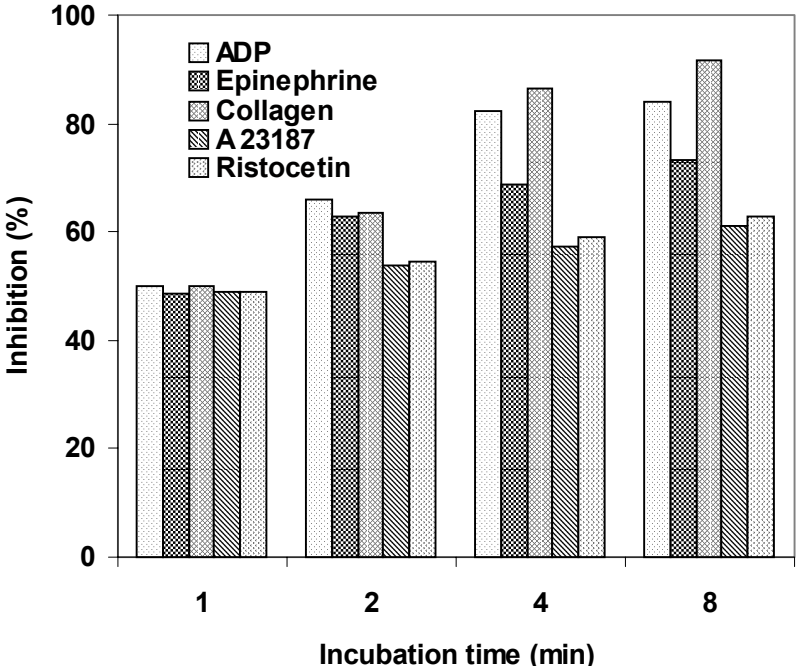


Figure 6 - Effect of cumin (roasted) extract on human platelet aggregation at IC_{50}

When the time of roasting was increased from 4 to 8 min, the decrease in inhibition ranged between 30 to 35% for coriander and cumin respectively as shown below in Table 15 and 16. But for roasted cumin extract on epinephrine induced aggregation at 8 min roasting the decrease was slightly less (25.8%) compared to all others.

Table 15 - Inhibition of human platelet aggregation by Coriander (roasted) extract

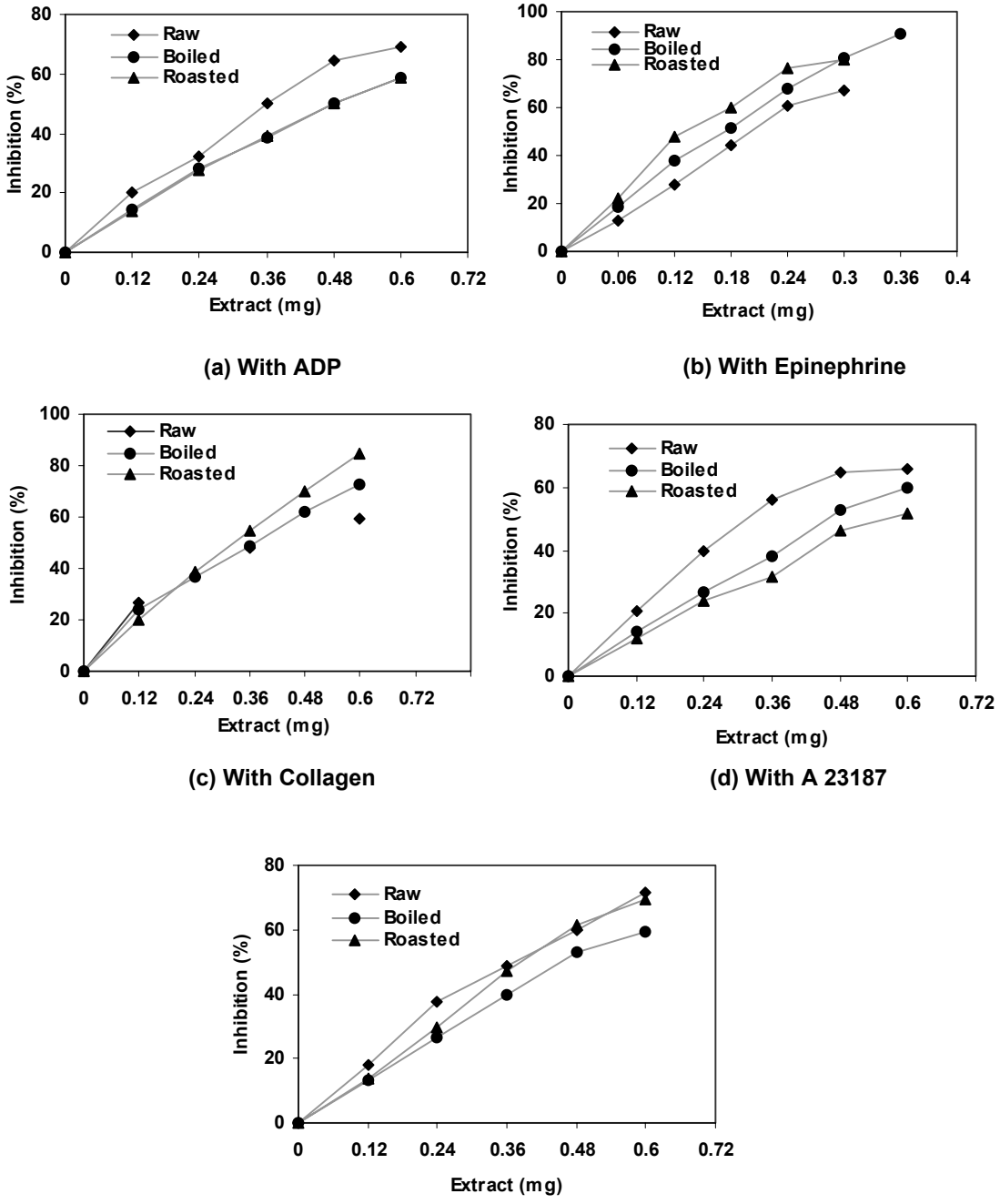
Extract	IC_{50} in mg	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.480	3.97 ± 0.09	16.8 ± 2.3
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.156	1.16 ± 0.04	17.5 ± 3.0
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.336	4.52 ± 0.11	19.3 ± 2.4
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.528	4.97 ± 0.13	14.9 ± 2.6
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.384	5.77 ± 0.12	18.0 ± 2.1

Values are Mean ± S.D of triplicates.
Coriander seeds were roasted for 8 min at 120.5 °C.

Table 16 - Inhibition of human platelet aggregation by Cumin (roasted) extract

Extract	IC_{50} in mg	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.656	3.97 ± 0.10	18.1 ± 2.5
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.061	1.06 ± 0.03	24.2 ± 2.8
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.40	4.48 ± 0.12	20.0 ± 2.7
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.720	4.88 ± 0.15	16.4 ± 3.1
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.576	5.65 ± 0.16	19.7 ± 2.8

Values are Mean ± S.D of triplicates.
Cumin seeds were roasted for 8 min at 125.5 °C.



(a) With ADP

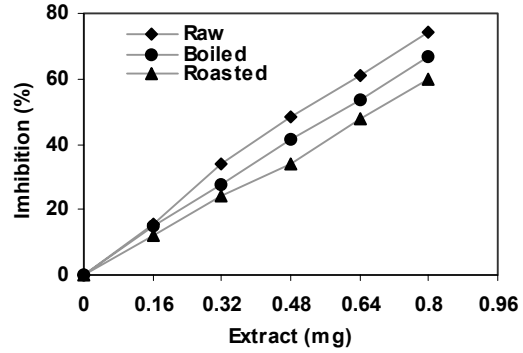
(b) With Epinephrine

(c) With Collagen

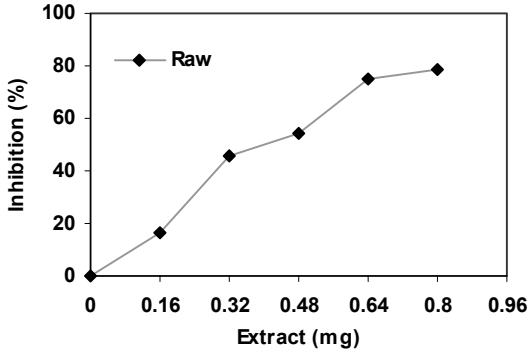
(d) With A 23187

(e) With Ristocetin

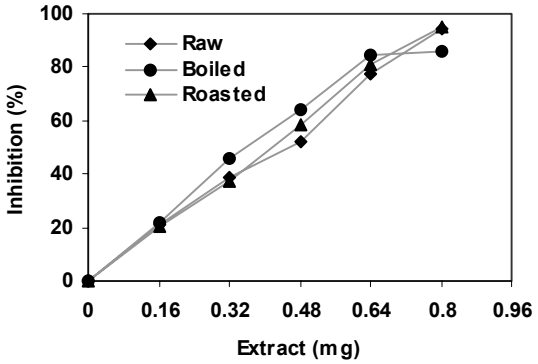
Figure 7 – Effect of raw and processed coriander extracts on human platelet aggregation with various agonists



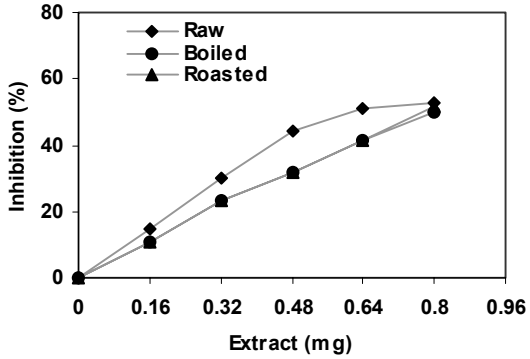
(a) With ADP



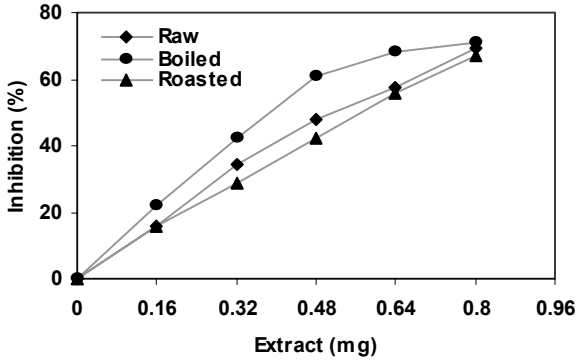
(b) With Epinephrine



(c) With Collagen



(d) With A 23187



(e) With Ristocetin

Figure 8 – Effect of raw and processed cumin extracts on human platelet aggregation with various agonists

The two spices coriander and cumin that were used in the present investigation were used as such in the raw form, followed by processing them with heat treatment like boiling and roasting with a variety of agonists like ADP, epinephrine, collagen, calcium ionophore A 23187 and ristocetin at various concentrations ranging between 5 to 50 μl (12 mg/ml of extract) at one minute incubation. The percent inhibitions were linear with concentration as shown in figures 7 and 8. Respective IC_{50} were deduced from these graphs.

It was observed that boiled and roasted extracts of coriander had more or less similar inhibitions on ADP induced aggregation. Also the raw and processed extracts of coriander were required in low concentrations ranging between 0.06 to 0.40 mg. At 0.40 mg boiled coriander extract inhibited epinephrine induced aggregation upto 90% as shown in figure 7 (b). Similarly very low concentration of 0.06 to 0.36 mg of raw extract of coriander was required to inhibit collagen induced platelet aggregation as shown in figure 7 (c). Raw extract showed better inhibition on A 23187 induced aggregation as shown in figure 7 (d).

With boiled and roasted cumin extracts, it may be seen that very low concentrations of the extract were required to inhibit epinephrine induced platelet aggregation. Raw and roasted extract of cumin had similar inhibitions with collagen induced aggregation as shown in figure 8 (c) where as with A 23187 boiled and roasted cumin extracts more or less similar inhibitions as shown in figure 8 (d). Ristocetin induced aggregation was better for boiled extract of cumin as shown in figure 8 (e).

Apart from roasting, the spices are usually used in combination like in rasam powder, curry powder, sambar powder and chutney powder where in a variety of spices like turmeric, black pepper, red pepper, fenugreek, cloves, cinnamon, cumin and coriander along with red gram dhal, roasted bengal gram dhal and black gram dhal are used. Further there are several food preparations contain these two spices in the raw as well as in processed forms separately. Hence it was of interest to investigate coriander and cumin taken in the ratio of 1:1 to see their synergistic effect, if any, on platelet aggregation. It was observed that a mixture of coriander and cumin (raw) extract inhibited the human platelet aggregation with ADP (12 to 54%), epinephrine (19 to 93%), collagen (18 to 88%), A 23187 (8 to 51%) and ristocetin (20 to 78%) as shown in table 17. The percent inhibitions were used to determine the IC_{50}.

Table 17 - Inhibition of human platelet aggregation by Coriander + Cumin mix (raw) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.14	4.30 ± 0.12	12.0 ± 2.8
″	0.28	3.69 ± 0.10	24.3 ± 2.7
″	0.42	2.93 ± 0.07	40.0 ± 2.4
″	0.56	2.51 ± 0.03	48.6 ± 1.2
″	0.70	2.23 ± 0.03	54.3 ± 1.3
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.14	1.14 ± 0.07	18.5 ± 6.3
″	0.28	0.93 ± 0.07	33.3 ± 5.2
″	0.42	0.62 ±0.02	55.6 ± 3.9
″	0.56	0.31 ± 0.02	77.8 ± 5.2
″	0.70	0.10 ± 0.01	92.6 ± 6.0
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.14	4.59 ± 0.19	18.0 ± 4.1
″	0.28	3.51 ± 0.18	37.4 ± 5.1
″	0.42	2.36 ± 0.10	57.8 ± 4.2
″	0.56	1.21 ± 0.05	78.4 ± 4.1
″	0.70	0.67 ± 0.03	88.0 ± 4.5
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.14	5.36 ± 0.25	8.2 ± 4.7
″	0.28	4.55 ± 0.19	22.1 ± 4.2
″	0.42	3.71 ± 0.15	36.4 ± 4.0
″	0.56	3.12 ± 0.11	46.6 ± 3.5
″	0.70	2.86 ± 0.09	51.0 ± 3.2
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.14	5.65 ± 0.27	19.8 ± 4.8
″	0.28	4.40 ± 0.22	37.6 ± 5.0
″	0.42	3.07 ± 0.16	56.4 ± 5.2
″	0.56	1.98 ±0.09	71.9 ± 4.6
″	0.70	1.53 ± 0.05	78.2 ± 3.3

Values are Mean ± S.D of triplicates.
Extract from 1:1 coriander and cumin mix.

The IC_{50} of coriander and cumin (raw) mix extract is given in table 18 with the respective agonists.

Table 18 - IC_{50} for inhibition of human platelet aggregation by Coriander and Cumin (raw) mix extract

Agonist	IC_{50}
ADP	0.546
Epinephrine	0.364
Collagen	0.392
A 23187	0.644
Ristocetin	0.378

Values are expressed in mg.
Values are mean of triplicates.

The IC_{50} of raw extract of coriander and cumin mix were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 66.0, 71.6, 59.1, 71.1 and 73.3% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 9.

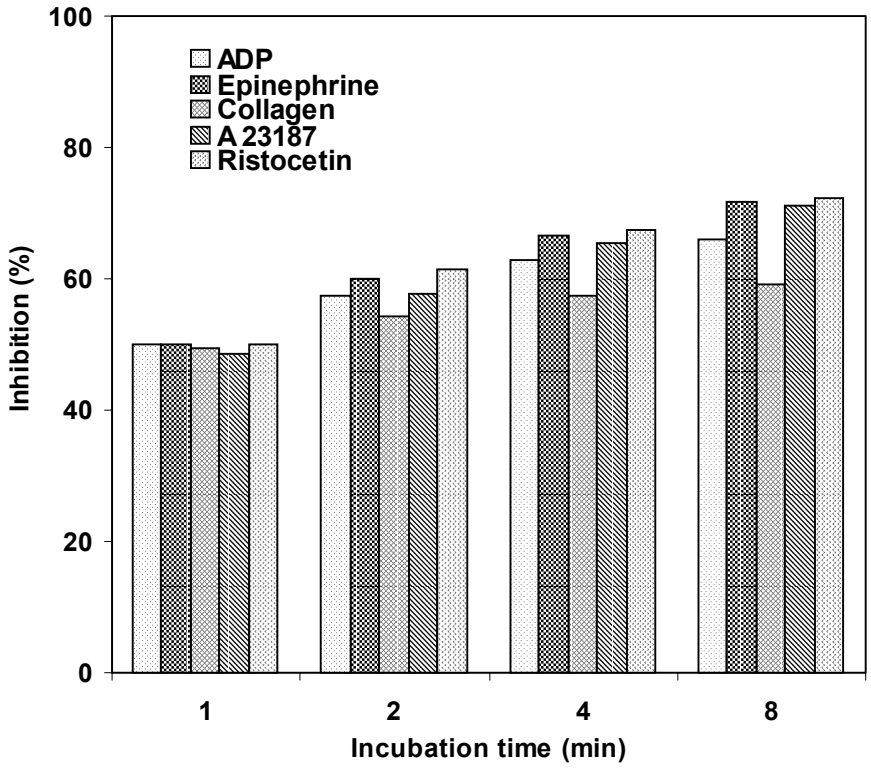


Figure 9 - Effect of coriander and cumin mix (raw) extract on human platelet aggregation at IC_{50}

Another method of processing the spice mix is that they are subjected to boiling in preparations like sambar or rasam powders. Hence the combination of coriander and cumin mix (1:1) was subjected to boiling for 15 min at 98 °C to see if heat treatment affects inhibition of platelet aggregation. The boiled extract inhibited human platelet aggregation with ADP (27 to 74%), epinephrine (20 to 69%), collagen (22 to 82%), A 23187 (20 to 54%) and ristocetin (16 to 64%) as shown in table 19.

Table 19 - Inhibition of human platelet aggregation by Coriander + Cumin mix (boiled) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.07	3.56 ± 0.16	27.0 ± 4.5
″	0.14	2.49 ± 0.11	48.9 ± 4.4
″	0.21	2.07 ± 0.08	57.6 ± 3.9
″	0.28	1.64 ± 0.04	66.3 ± 2.4
″	0.35	1.27 ± 0.03	73.9 ± 2.4
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.07	1.13 ± 0.05	19.5 ± 4.6
″	0.14	0.94 ± 0.04	32.9 ± 4.2
″	0.21	0.73 ± 0.02	48.0 ± 3.3
″	0.28	0.50 ± 0.01	64.0 ± 2.4
″	0.35	0.44 ± 0.01	68.8 ± 1.6
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.07	4.36 ± 0.21	22.1 ± 4.8
″	0.14	3.39 ± 0.17	39.4 ± 5.0
″	0.21	2.37 ± 0.10	54.9 ± 4.2
″	0.28	1.57 ± 0.06	71.8 ± 3.8
″	0.35	1.06 ± 0.04	81.7 ± 3.8
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.14	4.65 ± 0.17	20.4 ± 3.7
″	0.28	3.63 ± 0.14	37.9 ± 3.9
″	0.42	3.14 ± 0.10	46.2 ± 3.2
″	0.56	2.76 ± 0.05	52.7 ± 1.8
″	0.70	2.68 ± 0.03	54.1 ± 1.1
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.14	5.90 ± 0.22	16.2 ± 3.7
″	0.28	4.80 ± 0.18	31.8 ± 3.8
″	0.42	3.75 ± 0.13	46.7 ± 3.5
″	0.56	2.79 ± 0.06	60.3 ± 2.2
″	0.70	2.53 ± 0.04	64.0 ± 1.6

Values are Mean ± S.D of triplicates.
Coriander and cumin mix (1:1) was boiled for 15 min at 98 °C.

The IC_{50} of coriander and cumin (boiled) mix extract is given in table 20 with the respective agonists.

Table 20 - IC_{50} for inhibition of human platelet aggregation by Coriander and Cumin (boiled) mix extract

Agonist	IC_{50}
ADP	0.147
Epinephrine	0.217
Collagen	0.189
A 23187	0.392
Ristocetin	0.448

Values are expressed in mg.
Values are mean of triplicates.

The effect of increasing the duration of incubation to 2, 4 and 8 min on platelet aggregation was studied at IC_{50} for boiled extract of coriander and cumin mix. A maximum inhibition of 61.2, 70.8, 60.8, 63.5 and 65.0% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 10.

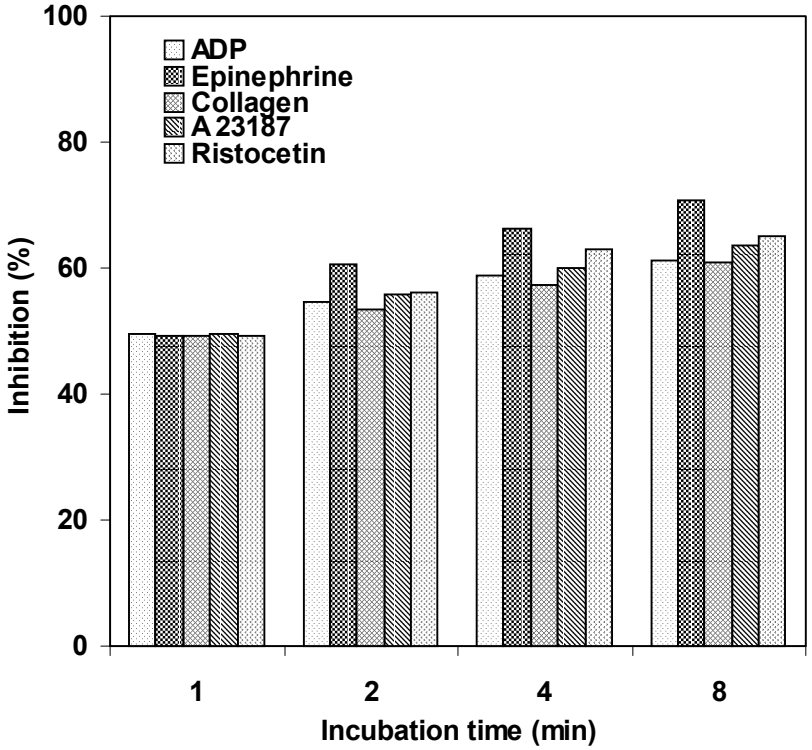


Figure 10 - Effect of coriander and cumin mix (boiled) extract on human platelet aggregation at IC_{50}

The IC_{50} at 15 min boiling was used to further study the effect of 30 min boiling on this mix at 98 °C. It may be seen that a decrease in the inhibition varied between 18 to 25%. This is shown in table 21.

Table 21 - Inhibition of human platelet aggregation by Coriander and Cumin mix (boiled) extract

Extract	IC_{50} in mg *	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.147	3.30 ± 0.08	32.3 ± 2.4
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.217	1.01 ± 0.03	28.1 ± 3.1
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.189	3.82 ± 0.09	31.8 ± 2.4
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.392	4.29 ± 0.12	26.6 ± 2.8
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.448	5.33 ± 0.17	24.3 ± 3.2

Values are Mean ± S.D of triplicates.
Coriander and cumin slurry was boiled for 30 min at 98 °C.

Apart from boiling, this mix was subjected to roasting as done for making curry and chutney powders. Roasting of the spices was done before making a fine powder so that it can be used in a variety of dishes. Coriander and cumin seeds were roasted at 120.5 and 125.5 °C respectively for 4 min before making a powder of this combination in the ratio of 1:1 followed by preparing the extract. This roasted extract inhibited human platelet aggregation with ADP (39 to 89%), epinephrine (26 to 63%), collagen (36 to 97%), A 23187 (18 to 70%) and ristocetin (20 to 74%) as shown in table 22. IC_{50} was determined for each of the above agonists.

Table 22 - Inhibition of human platelet aggregation by Coriander + Cumin mix (roasted) extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.14	3.0 ± 0.15	38.5 ± 5.0
″	0.28	1.64 ± 0.07	66.3 ± 4.3
″	0.42	1.19 ± 0.04	75.6 ± 3.4
″	0.56	0.74 ± 0.02	84.9 ± 2.7
″	0.70	0.53 ± 0.01	89.1 ± 1.9
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.03	1.04 ± 0.04	25.9 ± 4.2
″	0.06	0.87 ± 0.03	37.9 ± 3.3
″	0.08	0.72 ± 0.02	48.2 ± 2.8
″	0.11	0.62 ± 0.01	55.6 ± 1.9
″	0.14	0.52 ± 0.01	63.0 ± 1.5
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.14	3.58 ± 0.17	36.6 ± 4.8
″	0.28	1.95 ± 0.09	65.1 ± 4.6
″	0.42	0.97 ± 0.03	82.6 ± 3.1
″	0.56	0.39 ± 0.01	93.0 ± 2.6
″	0.70	0.18 ± 0.0	96.7 ± 0.0
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.14	4.78 ± 0.18	18.2 ± 3.8
″	0.28	3.74 ± 0.15	36.0 ± 4.0
″	0.42	2.54 ± 0.08	56.5 ± 3.2
″	0.56	1.94 ± 0.04	66.8 ± 2.1
″	0.70	1.73 ± 0.02	70.3 ± 1.2
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.14	5.62 ± 0.20	20.1 ± 3.6
″	0.28	4.24 ± 0.18	39.8 ± 4.2
″	0.42	2.84 ± 0.11	59.6 ± 3.9
″	0.56	2.10 ± 0.07	70.2 ± 3.3
″	0.70	1.84 ± 0.04	73.9 ± 2.2

Values are Mean ± S.D of triplicates.
Coriander and cumin seeds were roasted for 4 min at 120.5 and 125.5 °C respectively.

The IC_{50} of coriander and cumin (roasted) mix extract is given in table 23 with the respective agonists.

Table 23 - IC_{50} for inhibition of human platelet aggregation by Coriander and Cumin (roasted) mix extract

Agonist	IC_{50}
ADP	0.210
Epinephrine	0.092
Collagen	0.210
A 23187	0.364
Ristocetin	0.350

Values are expressed in mg.
Values are mean of triplicates.

The effect of increasing the duration of incubation to 2, 4 and 8 min on platelet aggregation was done at IC_{50} for roasted coriander and cumin mix extract. A maximum inhibition of 74.0, 71.2, 73.0, 72.6 and 72.2% were seen with ADP, epinephrine, collagen, A 23187 and ristocetin respectively as shown in Figure 11.

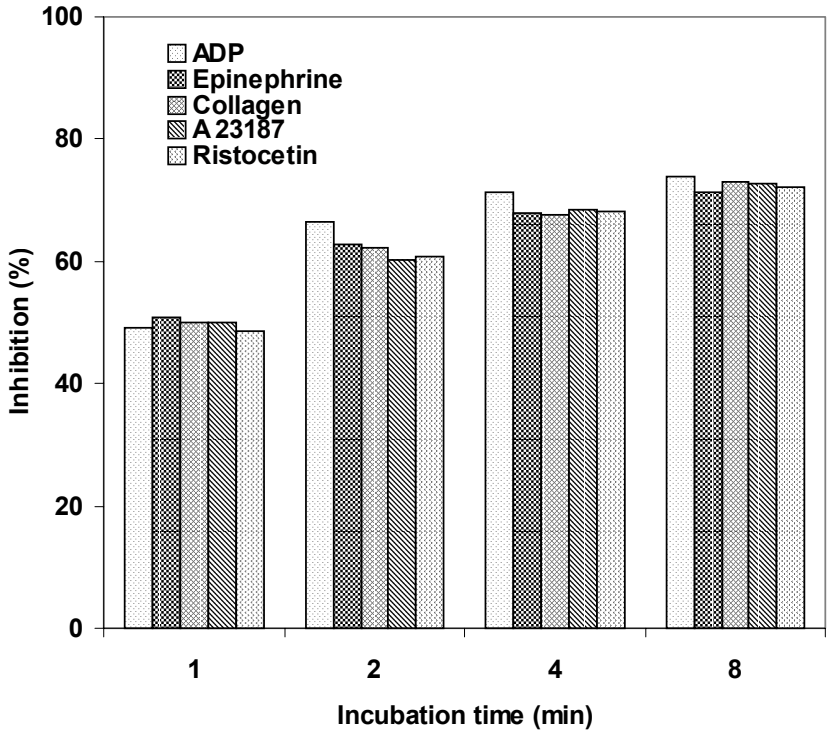


Figure 11 - Effect of coriander and cumin mix (roasted) extract on human platelet aggregation at IC_{50}

The effect of 8 min roasting of this mix at 125.5 °C was studied at IC_{50} obtained with 4 min roasting observations. It was observed that a decrease in the inhibition varied between 26 to 30%. This is shown in table 24.

Table 24 - Inhibition of human platelet aggregation by Coriander and Cumin mix (roasted) extract

Extract	IC_{50} in mg *	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.210	3.94 ± 0.11	19.2 ± 2.8
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.096	1.07 ± 0.03	23.5 ± 2.4
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.120	4.48 ± 0.13	20.0 ± 2.9
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.364	4.75 ± 0.14	18.2 ± 3.0
+ Ristocetin	0.0	7.04 ± 0.21	0.0
″	0.350	5.75 ± 0.15	18.3 ± 2.8

Values are Mean ± S.D of triplicates.
Coriander and cumin seeds were roasted for 8 min at 120.5 and 125.5 °C respectively.

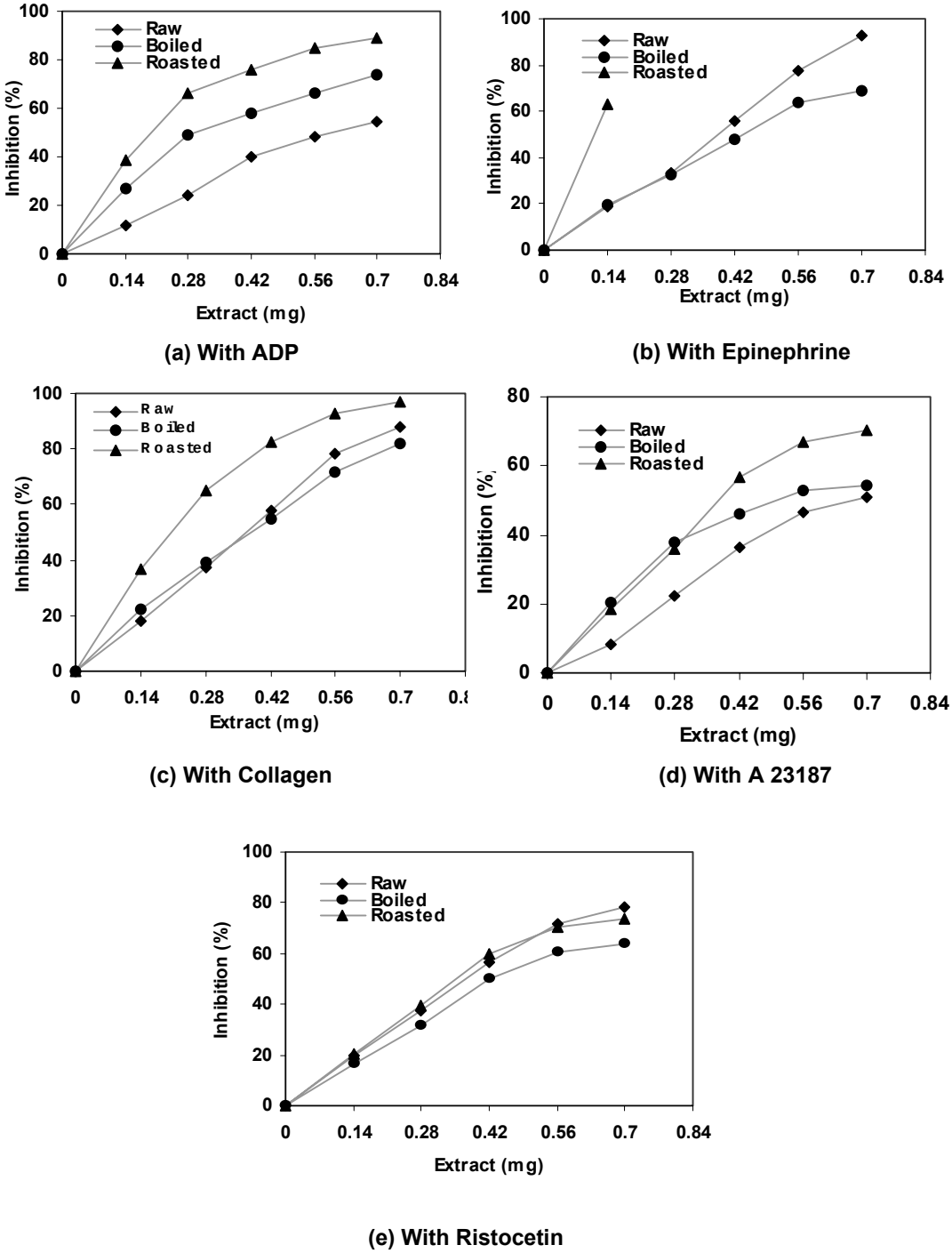


Figure 12 – Effect of raw and processed cumin extracts on human platelet aggregation with various agonists

The combination of coriander and cumin which were used in the ratio of 1:1 to see their synergistic action on platelet aggregation has shown good linearity in percent inhibition when plotted into graphs as shown in figure 12. On ADP and collagen induced aggregation, roasted extract of coriander and cumin mix showed better inhibition as shown in figures 12 (a) and (c). Very low concentrations ranging between 0.03 to 0.14 mg of roasted extract was required to inhibit platelet aggregation as shown in table 22. Similarly for A 23187 induced aggregation was inhibited better by the roasted extract of coriander and cumin mix as shown in figure 12 (d) where as for ristocetin induced aggregation the three extracts had more or less similar effect on aggregation as shown in figure 12 (e).

Coriander is also consumed in the form of processed dhal. Coriander dhal supari is used as a mouth freshener in combination with turmeric and salt. It is also used in paans as such. This extract inhibited human platelet aggregation with ADP (20 to 83%), epinephrine (22 to 81%), collagen (38 to 91%) and A 23187 (13 to 58%). No significant inhibition was seen with ristocetin induced aggregation at concentrations varying between 0.08 to 0.4 mg. The percent inhibition varied between 6 to 23% as shown in table 25. IC_{50} was determined for each of the above agonists.

Table 25 - Inhibition of human platelet aggregation by Coriander dhal extract

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.04	3.88 ± 0.16	20.4 ± 4.1
″	0.08	2.82 ± 0.12	42.3 ± 4.3
″	0.12	1.97 ± 0.07	59.6 ± 3.6
″	0.16	1.17 ± 0.03	76.0 ± 2.6
″	0.20	0.83 ± 0.02	82.9 ± 2.4
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.04	1.09 ± 0.05	22.0 ± 4.8
″	0.08	0.81 ± 0.03	42.4 ± 3.8
″	0.12	0.47 ± 0.01	66.1 ± 2.6
″	0.16	0.38 ± 0.01	72.9 ± 2.1
″	0.20	0.27 ± 0.01	80.7 ± 1.5
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.08	3.48 ± 0.17	37.8 ± 4.9
″	0.16	1.86 ± 0.08	66.7 ± 4.3
″	0.24	1.25 ± 0.03	77.6 ± 2.4
″	0.32	0.74 ± 0.02	86.7 ± 2.9
″	0.40	0.50 ± 0.01	91.1 ± 2.0
+ A 23187	0.0	5.84 ± 0.18	0.0
″	0.08	5.11 ± 0.18	12.5 ± 3.5
″	0.16	4.20 ± 0.15	28.1 ± 3.6
″	0.24	3.5 ± 0.10	40.0 ± 2.9
″	0.32	2.66 ± 0.06	54.4 ± 2.3
″	0.40	2.45 ± 0.04	58.1 ± 1.6
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	0.08	6.60	6.2
″	0.16	6.31	10.4
″	0.24	6.0	14.8
″	0.32	5.72	18.7
″	0.40	5.43	22.9

Values are Mean ± S.D of triplicates.

The IC_{50} of Coriander dhal extract is seen in table 26 with the respective agonists.

Table 26 - IC_{50} for inhibition of human platelet aggregation by Coriander dhal extract

Agonist	IC_{50}
ADP	0.096
Epinephrine	0.096
Collagen	0.120
A 23187	0.304
Ristocetin	NS

Values are expressed in mg.
Values are mean of triplicates.
NS – Not significant.

The IC_{50} of coriander dhal extract were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 70.3, 100.0, 68.1 and 67.8% were seen with ADP, epinephrine, collagen and A 23187 respectively. Ristocetin induced aggregation was inhibited to only 32.1% at 8 min incubation and a concentration of 0.4 mg, which was not very significant, compared with other as shown in Figure 13. A maximum inhibition of 100% was seen with epinephrine-induced aggregation with 8 min incubation at its IC_{50}.

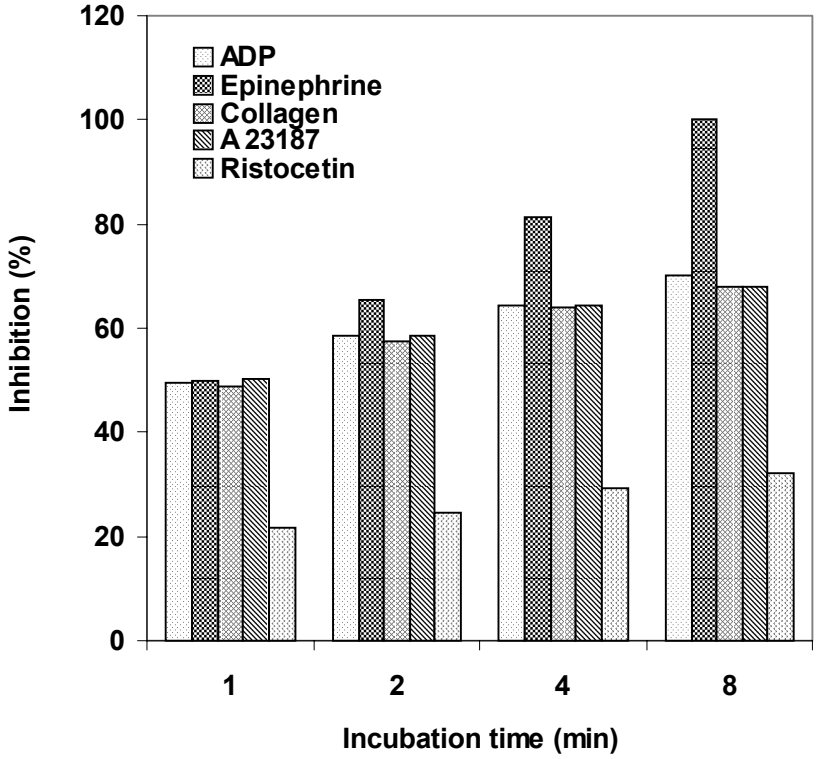


Figure 13 - Effect of Coriander dhal extract on human platelet aggregation at IC_{50}

The importance of spices is attributed to the active and aromatic principles present in them, which are reported to possess a variety of health benefits in addition to their property to improve the sensory attributes of food preparations. A number of active principles and essential oil components have been isolated and reported from various spices. Coriander and cumin have been reported to contain several active principles.

From coriander, α-Linalool is the major constituent isolated which constitutes about 60 to 70% of the total essential oil components (0.2% to 2%). This was used on human platelets to see its effect on their aggregation. With ADP (16 to 65%), epinephrine (26 to 83%), collagen (19

to 90%) and A 23187 (20 to 86%) inhibitions were observed as shown in table 17. IC_{50} were computed from the data of inhibitions of platelet aggregation with concentration range of 0.25 to 5 μl. The inhibition was between 8 to 23% for concentrations between 1 to 5 μl of α-Linalool with ristocetin, which was not significant.

In the case of cumin, α- Terpinene was tested for its effect on human platelet aggregation since it accounts to 29.5% of the total essential oil content of 2.5 to 4.5 %. Human platelet aggregation with ADP (12 to 60%), epinephrine (22 to 90%), collagen (2 to 56%) and A 23187 (20 to 83%) was inhibited and the results were shown in table 18. Varying concentrations between 0.25 to 5 μl resulted in linear response to inhibitions from which IC_{50} were calculated. The response towards ristocetin induced aggregation varied between 2 to 24% at concentrations of 1 to 5 μl of α-Terpinene and it was not significant.

Table 27 - Inhibition of human platelet aggregation by α-Linalool from coriander

Extract	Amount (μl)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.25	4.08 ± 0.16	16.4 ± 3.9
″	0.50	3.42 ± 0.13	29.9 ± 3.8
″	0.75	2.44 ± 0.09	50.0 ± 3.7
″	0.825	2.03 ± 0.07	58.4 ± 3.5
″	1.0	1.71 ± 0.04	64.9 ± 2.4
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.25	1.04 ± 0.04	25.9 ± 3.9
″	0.50	0.78 ± 0.03	44.4 ± 3.7
″	0.75	0.57 ± 0.02	59.3 ± 3.2
″	1.0	0.27 ± 0.01	79.6 ± 1.9
″	1.25	0.23 ± 0.01	83.2 ± 1.3
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.25	4.53 ± 0.17	19.1 ± 3.8
″	0.50	3.36 ± 0.13	40.0 ± 3.9
″	0.75	2.22 ± 0.09	60.3 ± 4.1
″	1.0	1.11 ± 0.04	80.2 ± 3.6
″	1.25	0.54 ± 0.02	90.4 ± 3.7
+ A 23187	0.0	5.84 ± 0.18	0.0
″	1.0	4.66 ± 0.18	20.2 ± 3.9
″	2.0	3.51 ± 0.15	39.9 ± 4.8
″	3.0	2.36 ± 0.11	59.6 ± 4.7
″	4.0	1.18 ± 0.04	79.8 ± 3.4
″	5.0	0.84 ± 0.02	85.7 ± 2.4
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	1.0	6.47	8.1
″	2.0	6.07	13.8
″	3.0	5.74	18.4
″	4.0	5.53	21.5
″	5.0	5.42	23.0

Values are Mean ± S.D of triplicates.

Table 28 - Inhibition of human platelet aggregation by α-Terpinene from cumin

Extract	Amount (mg)	Slope	Inhibition (%)
+ ADP	0.0	4.88 ± 0.16	0.0
″	0.25	4.28 ± 0.16	12.2 ± 3.7
″	0.50	3.60 ± 0.13	26.3 ± 3.6
″	0.75	3.07 ± 0.09	37.0 ± 2.9
″	1.0	2.55 ± 0.06	47.8 ± 2.4
″	1.25	2.12 ± 0.04	56.5 ± 1.9
″	1.50	1.98 ± 0.02	59.4 ± 1.0
+ Epinephrine	0.0	1.40 ± 0.10	0.0
″	0.25	1.09 ± 0.04	22.0 ± 4.5
″	0.50	0.82 ± 0.04	41.5 ± 4.3
″	0.75	0.51 ± 0.02	63.5 ± 4.3
″	1.0	0.17 ± 0.01	87.8 ± 1.8
″	1.25	0.14 ± 0.01	90.1 ± 1.4
+ Collagen	0.0	5.60 ± 0.18	0.0
″	0.25	5.47 ± 0.15	2.4 ± 2.7
″	0.50	4.83 ± 0.13	13.8 ± 2.7
″	0.75	4.13 ± 0.10	26.3 ± 2.4
″	1.0	3.36 ± 0.08	40.0 ± 2.4
″	1.25	2.66 ± 0.05	52.5 ± 1.5
″	1.50	2.45 ± 0.03	56.2 ± 1.2
+ A 23187	0.0	5.84 ± 0.18	0.0
″	1.0	4.66 ± 0.17	20.2 ± 3.7
″	2.0	3.51 ± 0.14	39.9 ± 4.0
″	3.0	2.36 ± 0.09	59.6 ± 3.8
″	4.0	1.18 ± 0.02	79.8 ± 1.7
″	5.0	0.97 ± 0.01	83.3 ± 1.0
+ Ristocetin	0.0	7.04 ± 0.24	0.0
″	1.0	6.89	2.1
″	2.0	6.45	8.4
″	3.0	6.0	14.8
″	4.0	5.61	20.3
″	5.0	5.34	24.2

Values are Mean ± S.D of triplicates.

The IC_{50} of α-Linalool from coriander is given in table 29 with the respective agonists.

Table 29 - IC_{50} for inhibition of human platelet aggregation by α- Linalool from coriander

Agonist	IC_{50}
ADP	0.75
Epinephrine	0.63
Collagen	0.63
A 23187	2.50
Ristocetin	NS

Values are expressed in μl.
Values are mean of triplicates.
NS – Not significant.

The IC_{50} of α-Terpinene from cumin is given in table 30 with the respective agonists.

Table 30 - IC_{50} for inhibition of human platelet aggregation by α-Terpinene from cumin

Agonist	IC_{50}
ADP	1.05
Epinephrine	0.58
Collagen	1.23
A 23187	2.30
Ristocetin	NS

Values are expressed in μl.
Values are mean of triplicates.
NS – Not significant.

Human platelet aggregation is followed with the inhibitors being incubated for one min in the normal course. It was of interest to determine the effect of longer duration of incubation of platelet aggregation. Hence the platelets were pre incubated for 2, 4 and 8 min at the IC_{50} of the inhibitors followed by the addition of respective agonists. The results for α-Linalool and α-Terpinene are depicted below in the figures 14 and 15.

The IC_{50} of α-Linalool were used to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 82.0, 65.9, 67.8 and 63.0% were seen with ADP, epinephrine, collagen and A 23187 respectively. Ristocetin induced aggregation was inhibited to only 29.9% at 8 min incubation and a concentration of 5.0 μl, which was not very significant, compared with others as shown in Figure 14.

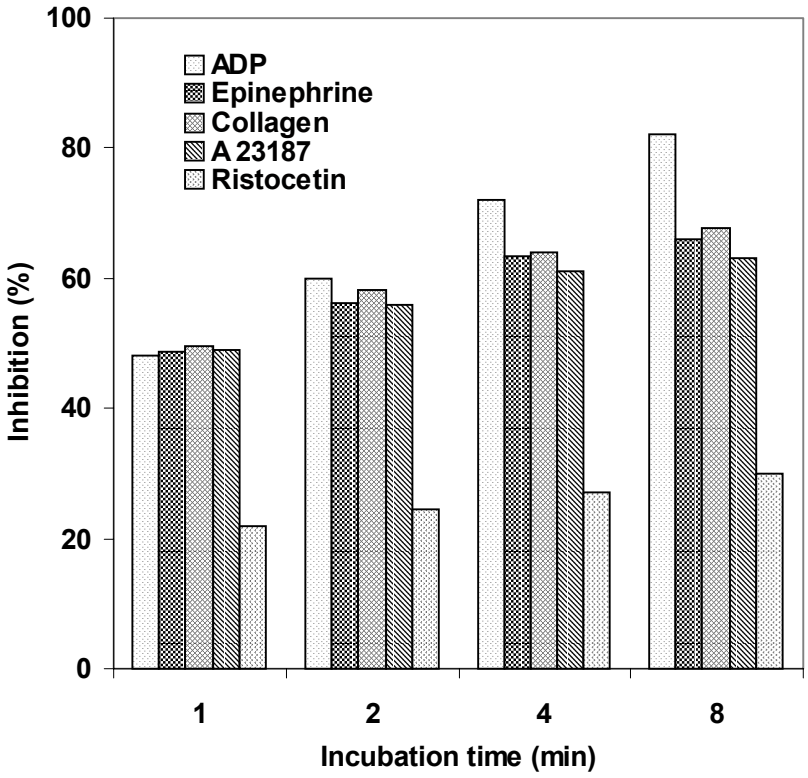


Figure 14 - Effect of α- Linalool from coriander on human platelet aggregation at IC_{50}

Similarly experiments were done with α-Terpinene to determine the effect of increase in the duration of incubation to 2, 4 and 8 min on platelet aggregation. A maximum inhibition of 100.0, 100.0, 75.3 and 62.0% were seen with ADP, epinephrine, collagen and A 23187 respectively. Ristocetin induced aggregation was inhibited to only 35.1% at 8 min incubation and a concentration of 5.0 μl, which was not very significant, compared with other as shown in Figure 15.

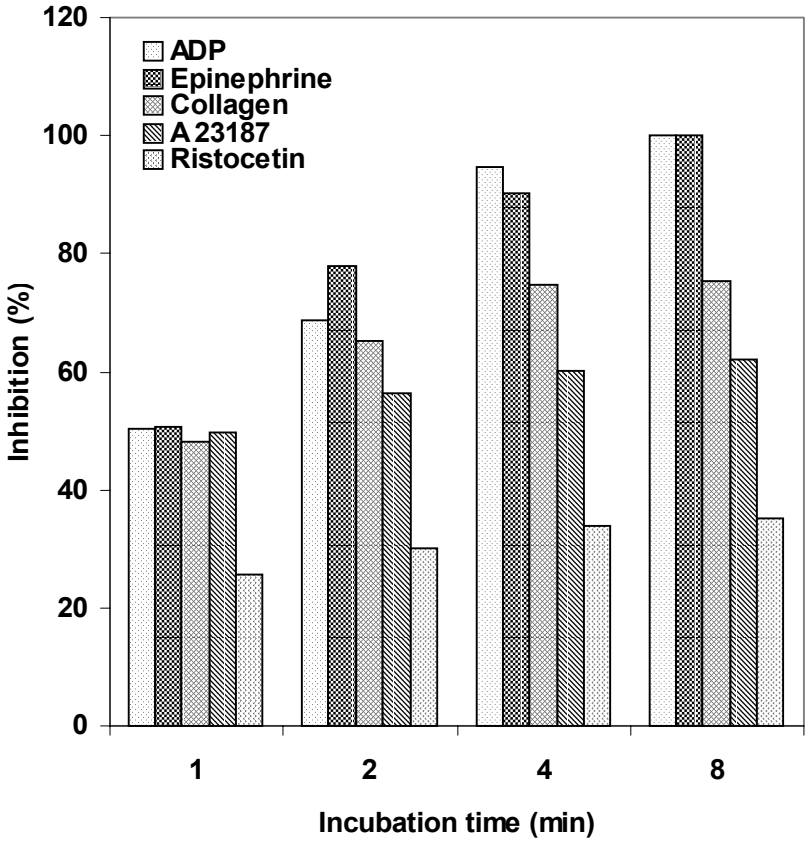


Figure 15 - Effect of α- Terpinene from cumin on human platelet aggregation at IC_{50}

Table 31 - IC_{50} for inhibition of human platelet aggregation by raw and processed spice extracts

Extract	ADP	Epinephrine	Collagen	A 23187	Ristocetin
Coriander (raw)	0.360	0.198	0.306	0.324	0.372
Coriander (boiled)	0.48	0.168	0.372	0.456	0.456
Coriander (roasted)	0.48	0.156	0.336	0.528	0.384
Cumin (raw)	0.512	0.320	0.416	0.608	0.528
Cumin (boiled)	0.608	0.182	0.344	0.80	0.416
Cumin (roasted)	0.656	0.061	0.400	0.720	0.576
Coriander + Cumin mix (raw)	0.546	0.364	0.392	0.644	0.378
Coriander + Cumin mix (boiled)	0.147	0.217	0.189	0.392	0.448
Coriander + Cumin mix (roasted)	0.210	0.092	0.210	0.364	0.350
Coriander dhal	0.096	0.096	0.120	0.304	NS
α - Linalool*	0.75	0.63	0.63	2.5	NS
α - Terpinene*	1.05	0.58	1.23	2.3	NS

Values are expressed in mg.
* Values are expressed in μl.
Values are mean of triplicates.
NS – Not significant.

Aqueous extracts of coriander and cumin and their processed extracts inhibited ADP, epinephrine, collagen, A 23187 and ristocetin induced platelet aggregation. For all these extracts it was seen that epinephrine induced aggregation was most sensitive.

Least sensitivity to these extracts was seen with calcium ionophore A 23187 to a greater extent where as to a certain extent their response was marginal with ristocetin as shown in table 31.

Among the raw extracts it is seen that coriander inhibited better than cumin and the combination of these two. The decrease in the IC_{50} of the combination of coriander and cumin may be due their synergistic effect and also to any other more sensitive components present in these spices. In case of the boiled extracts, ADP and epinephrine induced aggregation was sensitive to coriander extract where as with collagen and A 23187, the combination of coriander and cumin mix was effective and all of them had similar IC_{50} for ristocetin induced aggregation.

With roasted extracts it was observed that roasted cumin inhibited epinephrine induced aggregation very effectively with a IC_{50} of 0.061 mg which is the lowest for all the extracts followed by the roasted extract of mixture of coriander and cumin with a IC_{50} of 0.092 mg. But with other agonists mixture of roasted coriander and cumin was more effective in inhibiting the platelet aggregation with low IC_{50}.

It is interesting to note that coriander dhal inhibited the platelet aggregation with IC_{50} of 0.096, 0.096, 0.12 and 0.304 mg respectively with ADP, epinephrine, collagen and A 23187 where as there is no significant inhibition of aggregation with ristocetin.

This may be due the polishing given to coriander seeds where by the outer unwanted layers are removed and to some extent due to unidentified components present in them. α-Linalool from coriander and α-

Terpinene from cumin inhibited platelet aggregation effectively with agonists ADP, epinephrine, collagen and A 23187 where as there is no significant inhibition on ristocetin-induced aggregation.

Platelet aggregation:

Platelet aggregation consists of a series of exquisitely co-ordinated responses. The membrane surface of human platelets is responsible for a wide variety of reactions. This surface has receptors for various agonists like thrombin, ADP, collagen, serotonin and antagonists like prostacyclin and prostaglandins that effect platelet functions. It also has receptors, which enhance the rate of coagulation (Jennings and Phillips, 1982). Resting unstimulated platelets do not bind to fibrinogen but following ADP or epinephrine stimulation, binding ensues. But, one essential feature is that fibrinogen binding takes place in the presence of divalent calcium and magnesium ions (Flow and Marguerie, 1980). The activation of platelets by exogenous inducers like ADP, epinephrine and collagen is through the surface glycoprotein receptors like glycoproteins IIb-IIIa (GP IIb-IIIa) coupled with an increase in the free calcium levels in the platelets (Bloekmans, *et al.*, 1995).

The GP IIb-IIIa complex is an integral component of platelet membranes whose function is essential for normal adhesion and aggregation. It belongs to a family of widely distributed and structurally related adhesion receptors named collectively integrins or cytoadhesins. One of the functional features of platelet GP IIb-IIIa receptor is the ability to interact with at least four different ligands like fibrinogen, von Willebrand

factor, fibrinectin and vitronectin where as other integrins exhibit more restricted substrate specificity (Berliner, *et al.*, 1988). Fibrinectin influences the platelet spreading on collagen and sub endothelial matrices (Plow, *et al.*, 1984).

The formation of the GP IIb-IIIa fibrinogen complex is calcium dependent, fibrinogen specific, saturable and inhibited by specific amino acids suggesting that GP IIb and GP IIIa, which probably exist on the platelet membranes as a macromolecular complex, act under the proper physiological circumstances as the fibrinogen binding site is required for normal platelet aggregation (Nachman and Leung, 1982). The role of GP IIb-IIIa complex in calcium influx is through its binding to fibrinogen which in turn helps in incorporating it into the cytoskeleton of aggregating platelets (Yamaguchi, *et al.*, 1987).

Glycoprotein IIb is composed of two disulfide linked sub-units of M_r 116000 and 23000. Glycoprotein IIIa is a M_r 95000 polypeptide containing intramolecular disulfide bonds (Shattil *et al.*, 1985). The integrin GP IIb-IIIa is the most abundant platelet receptor containing approximately 50-80000 molecules and each sub-unit consists of a large extracellular domain (~700-1000 amino acids and short transmembrane and cytoplasmic domains (Phillips *et al.*, 1988).

Glycoproteins on platelet membranes become a fibrinogen (Fgn) receptor and mediate platelet plug formation. The proposed site on the Fgn molecule that interacts with the GP IIb-IIIa complex is a dodecapeptide, His-His-Leu-Gly-Gly-Ala-Lys-Gly-Al-Gly-Asp-Val (His-10-

Val), residues 400-411 of the carboxyl terminus of the fibrinogen γ chain and a tetrapeptide near the carboxyl terminus of the α chain or a tripeptide near the amino terminus of the α chain (Rybak and Renzulli, 1989).

GP IIb-IIIa complex interacts with the cytoskeleton in resting and activated platelets and serves as a transmembrane link between extracellular ligands and the cytoskeleton (Padoin *et al.*, 1996). Platelet cytoskeleton consists of separable pseudopodial and contractile assemblies and a membrane skeleton, which may serve to stabilize the plasma membrane of unactivated platelets. The pseudopodial cytoskeleton is composed primarily to ABP and α-actinin in association with actin filaments while the contractile gel is composed of myosin associated filamentous actin (Kouns, *et al*., 1991).

The platelet cytoskeleton is not merely a contractile structure that regulates platelet shape change and spreading, but it also represents an intracellular network connecting and organizing several molecules involved in signal transduction (Canobbio *et al.*, 2004).

von Willebrand factor (vWF) is a large multimeric protein synthesized by megakaryocytes and endothelial cells and is stored in the Weibel-Palade bodies of endothelial cells and in the α-granules of platelets from where it can be released on cell activation and enables platelets to adhere to exposed sub-endothelium. Two main glycoproteins, GP Ib-IX-V and GP IIb-IIIa are involved in platelet-vWF interactions. GP IIb-IIIa belonging to the integrin family of adhesion receptors has a unique α sub-unit and β-3 subunit that is shared by other integrins. The GP Ib-IX-V is a

unique platelet adhesion receptor and this complex consists of four transmembrane subunits, GP Ibα, GP Ibβ, GP IX and GP V, each of which is a member of the leucine rich repeat protein superfamily. A binding interaction between vWF and GP Ibα involves the N-terminal 282 amino acids of GP Ibα, C-terminal flanking region and the N-terminal flanking sequence and the leucine rich repeat region. The integrin GP IIb-IIIa required for ligand binding occurs in response to chemical agonists of platelets as well as to VWF binding to GP Ibα. Studies show that GP IIb-IIIa integrin functions as a mediator of bi-directional signaling i.e. 'inside-out' and outside-in' signaling.

During 'inside-out' signaling, primary platelet agonists stimulate signal transduction pathways and a change in cytoskeletal proteins, resulting in the activation of numerous platelet functions. 'Inside-out' signaling specifically refers to the activation of the receptor function for soluble adhesive proteins, which allows them to crosslink platelets upon binding to activated GP IIb-IIIa on stimulated platelets to initiate platelet aggregation.

'Outside-in' signaling occurs during the platelet aggregation and adhesion and is induced by the binding of vWF and fibrinogen to the extracellular domain of GP IIIa. 'Outside-in' GP IIb-IIIa signaling induces diverse functions that are critical to platelet physiology, platelet aggregation and responses to vessel walls like the generation of thromboxane A_2, increases in the cytoplasmic Ca^{2+}, calpain activation, phosphorylation of

platelet proteins and secretion of P-selectin and α-granule proteins (Schmugge et al., 2003).

The 'Outside-in' signaling is triggered by ligand-induced oligomerisation of GP IIb-IIIa, followed by cytoskeletal rearrangement and a characteristic wave of tyrosine phosphorylation and dephosphorylation of a number of platelet molecules. These events lead to a process commonly referred to as clot retraction. The *in vivo* significance of clot retraction for haemostasis may be to improve the mechanical stability of thrombi under shear stress, thus providing less surface area for fibrinolytic proteins and reduce the susceptibility of fibrinolysis (Seiffert, *et al.*, 2003).

Next important feature is the cyclic AMP, a rise in its concentration results in the inhibition of secretion in human platelets. It can be seen that platelet myosin kinase is the substrate for the catalytic subunit of protein kinase and that phosphorylation of this myosin kinase reduces its activity where as dephosphorylation causes an increase in its activity. The phosphorylation of myosin kinase can be correlated with the secretion by platelets resulting from contraction of platelet actomyosin. The protein kinase enzyme is dependent on the cyclic AMP (Hathaway *et al.*, 1981).

ADP induced aggregation inhibits the increase in the intraplatelet cyclic AMP levels and stimulation of adenyl cyclase activity. This decrease in the cyclic AMP levels will help in the mobilisation of fibrinogen binding sites that are required for the formation of platelet plug (Hawiger *et al.*, 1980). Not only that, epinephrine, thrombin and serotonin which induce platelet aggregation inhibit the adenyl cyclase activity (Zieve and

Greenough, 1969). Calcium is known to inhibit adenyl cyclase activity where as removal of calcium increases PGE_1 stimulation of adenyl cyclase activity (Wolfe and Shulman, 1969).

Adenosine diphosphate (ADP), a low molecular weight agent is stored in the dense granules of platelets having its own defined sequence of activation. The effect of ADP is mediated by a specific receptor, which is designated as P2T consisting of $P2T_{AC}$ and $P2T_{PLC}$. Calcium influx is mediated through receptor operated Ca^{2+} channel designated as P2X1 where as adenyl cyclase inhibition and intracellular Ca^{2+} mobilisation are modulated by the $P2T_{AC}$ receptor thorough multiple G-proteins. On the other hand $P2T_{PLC}$ receptor is coupled with the activation of phospholipase C and an increase in inositol triphosphate (IP_3) levels (Daniel *et al.*, 1998).

Epinephrine induced aggregation potentiates ADP mediated aggregation in human platelets (Marquis *et al.*, 1970). The receptor for epinephrine on platelets has been designated as α_2-adrenergic site. Epinephrine possibly mediates an increase in the number of available ADP receptors on cell surface (Figures *et al.*, 1986).

Collagen is another important platelet agonist, which is associated with a burst in hydrogen peroxide, a pro-oxidant species that contributes to activation of platelets (Pignatelli *et al.*, 1998). This hydrogen peroxide acts as a secondary messenger in stimulating the arachidonic acid metabolism by contributing to platelet production of thromboxane A_2, stimulation of IP_3 and calcium mobilisation (Pignatelli *et al.*, 1999). Collagen enhances tissue factor activity rapidly on the platelet surface bound to monocytes and

neutrophils. Intravascular tissue factor of the platelet-leukocyte complexes could play a role during the physiologic haemostasis (Zillmann *et al.*, 2001). Glycoprotein IV (CD 36) present on platelets play an important role in the early stages of the platelet aggregation mediated through collagen acting as its specific receptor (Tandon *et al.*, 1989).

Apart from that another receptor present on the platelet membranes for collagen-induced aggregation under flow conditions is Glycoprotein VI, which has a molecular weight of 62 kDa. It also induces tyrosine phosphorylation of some platelet proteins, especially phospholipase Cγ2 (PLCγ2) and the tyrosine kinase Syk (Ichinohe *et al.*, 1997; Moroi and Jung, 2004).

Human platelets maintain a low cytosolic free calcium concentration by controlling the plasma membrane calcium transport. For normal calcium haemostasis, an intact GP IIb-IIIa complex is necessary in platelets (Brass, 1985). So for calcium mobilisation an ionophore A 23187, which is a carboxylic antibiotic and a lipid soluble molecule, forms complex with alkali metal cations and transports them across a variety of membranes. It has a molecular weight of 523 and elemental analysis of $C_{29}H_{37}N_3O_6$ and acts as a free mobile carrier for endogenous calcium, magnesium and inorganic phosphate (Reed and Lardy, 1972). A 23187 acts as a carrier of calcium ions across lipid bilayers in the cell membranes, which in turn help to increase the cytosolic calcium levels. A rise in the cytosolic calcium levels brings about the activation of the platelets through the stimulation of the

enzymes, which are not fully functional at low levels of calcium as present in resting platelets (Heemskerk, 1994).

Calcium antagonists usually may not bind to the specific receptor sites but may cause the thickening of membranes by their insertion into the membrane bilayers thus affecting calcium influx and modify the membrane permeability to calcium ions (Blache, *et al.*, 1987). The intracellular free calcium in activated platelets undergoes steric hindrance due to the damage of the calcium channels near the GP IIb-IIIa complex in the presence of anti IIb-IIIa monoclonal antibodies. This also decreases agonist induced cytoskeleton reorganization of platelets (Sinigaglia *et al.*, 1988).

On the other hand the presence of extracellular calcium is required for platelet aggregation and secretion in response to ADP and epinephrine. In the absence of extracellular calcium both platelet aggregation and serotonin release were greatly affected though magnesium ions were present. Though GP IIb-IIIa complex is the major calcium binding glycoproteins in unstimulated platelets but neither GP IIb-IIIa complex nor GP-Ib represents the new calcium ion binding sites on stimulated platelets. Upon stimulation with ADP or epinephrine, normal platelets developed new calcium binding sites indicating that specific calcium binding sites are present on the platelet membranes which have high affinity to extracellular calcium ions (Brass and Shattil, 1983). But the calcium channel blockers reduce the entry of extracellular calcium ions into the cells which inturn prevents the contraction of dense granules affecting the release of their

contents. This leads to an inhibition in platelet aggregation (Salam, et al., 1991).

Ristocetin, a glycoprotein synthesized by the actinomycete *Nocardia lurida* possess antibacterial and antibiotic activity (Ruggeri, *et al.*, 1983). This ristocetin induces platelet agglutination through glycoprotein Ib (GP-Ib) (Kao, et al., 1979). Also it is seen that vWF coflocculates on the surface of the platelets with the vWF receptor, the α-chain of platelet GP-Ib. Subsequently ristocetin dependent 'tethers' of vWF emanating from GP-Ib at the surface of the platelet would complex with similarly disposed tethers on the neighbouring platelets. In support of such a mechanism, the reported ristocetin-dependent binding domains of both vWF and GP-Ib contain X-Pro-Gly-X' sequence (Scott, et al., 1991).

Next the important aspect is the release reactions from platelets after aggregation is completed with a variety of agonists. The released products estimated were malondialdehyde and serotonin.

Malondialdehyde (*MDA*) formation:

MDA is one of the end products of cyclooxygenase pathway of arachidonic acid metabolism (Rathan, 1988). Lipid bilayers of cell membranes are highly susceptible to peroxidation due to their polyunsaturated fatty acids. Free radical reactions in the lipid domain also results in damage to proteins thus leading to alteration and impairment of membrane functions (Wiseman, 1996) resulting in adverse effects on the biological system, which may include atherosclerosis and cancer (Cook and Samman, 1996). These free radicals also interfere with membrane

surface receptors such as GP IIb/IIIa (Koerner *et al.*, 1992). But, the presence of GP IIb/IIIa inhibitors significantly decreased the reactive oxygen species formation in activated platelets (Chakrabarti *et al.*, 2004).

The fatty acids from marine origin have been recognised to inhibit platelet aggregation by altering the redox status of the cell due to the increase in platelet glutathione peroxidase (GPx) activity, which in turn degrades the lipid hydroperoxides formed. Otherwise these lipid hydroperoxides induce platelet activation through cyclooxygenation of arachidonic acid (Lagarde *et al.*, 1997).

Table 32 – Effect of raw and processed spice extracts on MDA released during human platelet aggregation

Extract	ADP	Epinephrine	Collagen	A 23187
Control	250.6 ± 22.5	138.9 ± 11.7	288.4 ± 20.2	218.9 ± 25.3
Coriander (raw)	142.8 ± 17.8 (43.0 %)	68.6 ± 9.2 (50.6 %)	154.0 ± 18.9 (46.6 %)	152.6 ± 21.2 (30.3 %)
Cumin (raw)	157.7 ± 21.4 (37.1 %)	64.5 ± 8.1 (53.6 %)	156.7 ± 19.1 (45.7 %)	156.2 ± 23.0 (28.6 %)
Coriander (boiled)	155.2 ± 20.0 (38.1%)	75.7 ± 9.9 (45.5 %)	169.4 ± 17.8 (41.3 %)	160.7 ± 19.4 (26.6 %)
Cumin (boiled)	166.1 ± 20.8 (33.7 %)	71.6 ± 10.9 (48.5 %)	168.2 ± 19.8 (41.7 %)	168.4 ± 22.4 (23.1 %)
Coriander (roasted)	164.9 ± 18.6 (34.2 %)	68.4 ± 8.7 (50.8 %)	172.8 ± 20.0 (40.1 %)	167.2 ± 24.1 (23.6 %)
Cumin (roasted)	154.7 ± 19.2 (38.3 %)	62.6 ± 8.5 (54.9 %)	164.1 ± 19.8 (43.1 %)	172.1 ± 20.8 (21.4 %)
Coriander + Cumin mix (raw)	152.8 ± 17.4 (39.0 %)	80.4 ± 7.2 (42.1 %)	170.2 ± 21.4 (47.8 %)	170.5 ± 21.7 (22.1 %)
Coriander + Cumin mix (boiled)	149.1 ± 16.1 (40.5 %)	70.9 ± 8.8 (49.0 %)	150.6 ± 17.2 (41.7 %)	162.2 ± 23.4 (25.9 %)
Coriander + Cumin mix (roasted)	150.2 ± 15.7 (40.1 %)	69.4 ± 6.8 (50.1 %)	158.2 ± 18.1 (45.2 %)	165.0 ± 22.2 (24.6 %)
Coriander dhal	159.8 ± 14.2 (36.2 %)	79.6 ± 6.8 (42.7 %)	163.7 ± 15.1 (43.3 %)	164.3 ± 18.4 (24.9 %)
α-Linalool	144.2 ± 18.9 (42.5 %)	70.2 ± 7.8 (49.2 %)	157.8 ± 17.8 (45.3 %)	159.2 ± 20.7 (27.3 %)
α-Terpinene	149.8 ± 16.5 (40.2 %)	78.1 ± 9.4 (43.8 %)	165.6 ± 16.4 (42.6 %)	166.7 ± 21.7 (23.9 %)

Values are expressed in nmoles of MDA released/mg protein/hr.
Values are Mean ± S.D of triplicates at IC_{50}.
Values in parenthesis are percent inhibition of MDA formed during platelet aggregation in the presence of these extracts at IC_{50}.

All the spice extracts have inhibited the release of MDA after platelet aggregation with ADP, epinephrine, collagen and A 23187 at their IC_{50} as shown in table 32 indicating that arachidonic acid metabolism was being affected.

A reduction in the MDA released varied between 20 to 50%. The maximum inhibition (54.9%) was seen with roasted cumin extract on epinephrine-induced aggregation where as the minimum inhibition was seen with the same extract on A 23187 induced aggregation (21.4%). The reduction for ADP induced aggregation varied between 34 to 43%, epinephrine induced aggregation varied between 42 to 54%, collagen induced aggregation varied between 41 to 48% and for A 23187 induced aggregation it was between 21 to 30%. The lipid peroxides (MDA is one of the end products) increase the platelet sensitivity for different agonists thus can be a causative factor in the development of coronary heart diseases (Neiva, *et al.*, 1999). These spice extracts hence possess component(s), which may be protective to the platelets by inhibiting the formation of release products through arachidonic acid degradation.

Serotonin secretion:

Serotonin or 5-hydroxy tryptamine is an important biogenic amine and a neurotransmitter (Anderson, et al., 1987). It has been implicated as having an important physiological role and is suggested to be involved in many human diseases (Peskar and Spector, 1973). Serotonin is usually stored in the dense granules of platelets (Moncada and Vane, 1978).

During aggregation by any agonist, serotonin liberated enhances the retention or binding of procoagulant proteins on cell surface of stimulated platelets. Presence of calcium ions is essential for the secretion of serotonin from intact platelets (Dale, *et al.*, 2002).

Table 33 - Effect of raw and processed spice extracts on Serotonin formed during human platelet aggregation

Extract	ADP	Epinephrine	Collagen	A 23187
Control	243.3 ± 16.1	176.5 ± 14.8	257.0 ± 19.7	234.2 ± 21.3
Coriander (raw)	144.2 ± 15.9 (40.7 %)	98.2 ± 14.3 (44.4 %)	144.2 ± 18.9 (43.9 %)	145.6 ± 20.2 (34.5 %)
Cumin (raw)	140.9 ± 15.2 (42.1 %)	100.2 ± 14.7 (43.2 %)	149.8 ± 19.2 (41.7 %)	150.1 ± 20.9 (41.6 %)
Coriander (boiled)	158.4 ± 14.7 (34.9 %)	104.4 ± 13.0 (40.9 %)	150.6 ± 18.1 (41.4 %)	154.6 ± 19.8 (39.8 %)
Cumin (boiled)	152.7 ± 13.6 (34.9 %)	109.0 ± 14.6 (38.2 %)	146.7 ± 18.5 (42.9 %)	162.2 ± 18.7 (36.9 %)
Coriander (roasted)	154.8 ± 14.8 (36.4 %)	102.7 ± 13.9 (41.8 %)	154.5 ± 19.0 (39.9 %)	151.3 ± 19.4 (41.1 %)
Cumin (roasted)	155.5 ± 16.0 (36.1 %)	95.5 ± 12.1 (45.9 %)	153.6 ± 18.6 (40.2 %)	160.8 ± 19.0 (37.4 %)
Coriander + Cumin mix (raw)	145.4 ± 14.9 (40.2 %)	108.1 ± 13.6 (38.8 %)	148.7 ± 19.3 (42.1 %)	153.7 ± 18.5 (40.2 %)
Coriander + Cumin mix (boiled)	149.2 ± 14.1 (38.7 %)	107.3 ± 14.5 (39.2 %)	145.6 ± 17.8 (43.4 %)	147.9 ± 18.0 (42.5 %)
Coriander + Cumin mix (roasted)	147.6 ± 15.1 (39.3)	103.8 ± 13.4 (41.2 %)	147.4 ± 18.3 (42.7 %)	149.5 ± 19.3 (41.8 %)
Coriander dhal	140.3 ± 15.6 (42.3 %)	96.6 ± 15.0 (39.6 %)	142.9 ±18.0 (44.4 %)	143.4 ± 21.1 (44.2 %)
α - Linalool	150.1 ± 15.4 (38.3 %)	102.2 ± 13.7 (45.3 %)	152.6 ± 17.4 (40.6 %)	156.2 ± 20.7 (39.2 %)
α-Terpinene	156.2 ± 14.7 (35.8 %)	110.9 ± 14.2 (37.2 %)	154.7 ± 17.0 (39.8 %)	152.5 ± 19.9 (40.7 %)

Values are expressed in nmoles of serotonin released/mg protein/hr.
Values are Mean ± S.D of triplicates at IC_{50}.
Values in parenthesis are percent inhibition of serotonin released during platelet aggregation in the presence of these extracts at IC_{50}.

A decrease in the serotonin liberated from platelets after aggregation with ADP, epinephrine, collagen and A 23187 may be seen in Table 33. The decrease varied between 30 to 45%. All these extracts, which inhibited serotonin release from dense granules, were more or less similar in their action.

Effect of coriander and cumin extracts on washed human platelets:

The IC_{50} of each of the extracts of coriander, cumin, the combination of 1:1 of coriander plus cumin and coriander dhal were used on washed platelets as shown in Tables 34 to 36.

Table 34 - Effect of coriander and its processed extracts on washed human platelets

Extract	IC_{50}	Slope	Inhibition (%)
ADP only	0.0	4.48	0.0
Coriander (raw) extract + ADP	0.36	2.12	52.6
Coriander (boiled) extract + ADP	0.48	2.02	54.9
Coriander (roasted) extract + ADP	0.48	2.08	53.7
Coriander dhal extract + ADP	0.096	2.20	50.8
Collagen only	0.0	5.20	0.0
Coriander (raw) extract + collagen	0.306	2.36	54.6
Coriander (boiled) extract + collagen	0.372	2.52	51.5
Coriander (roasted) extract+ collagen	0.336	2.46	52.7
Coriander dhal extract + collagen	0.120	2.48	52.3
A 23187 only	0.0	5.20	0.0
Coriander (raw) extract + A 23187	0.324	2.37	54.4
Coriander (boiled) extract + A 23187	0.456	2.52	51.6
Coriander (roasted) extract + A 23187	0.528	2.47	52.5
Coriander dhal extract + A 23187	0.304	2.42	53.6

IC_{50} in mg is done of PRP with various extracts and agonists.

Table 35 - Effect of cumin and its processed extracts on washed human platelets

Extract	IC_{50}	Slope	Inhibition (%)
ADP only	0.0	4.48	0.0
Cumin (raw) extract + ADP	0.512	2.16	51.8
Cumin (boiled) extract + ADP	0.608	2.06	54.0
Cumin (roasted) extract + ADP	0.656	2.14	52.2
Collagen only	0.0	5.20	0.0
Cumin (raw) extract + collagen	0.416	2.34	55.0
Cumin (boiled) extract + collagen	0.344	2.48	52.3
Cumin (roasted) extract + collagen	0.40	2.40	53.9
A 23187 only	0.0	5.20	0.0
Cumin (raw) extract + A 23187	0.608	2.30	55.8
Cumin (boiled) extract + A 23187	0.80	2.44	53.1
Cumin (roasted) extract + A 23187	0.72	2.38	54.2

IC_{50} in mg is done of PRP with various extracts and agonists.

Table 36 - Effect of coriander and cumin mix and its processed extracts on washed human platelets

Extract	IC_{50}	Slope	Inhibition (%)
ADP only	0.0	4.48	0.0
Coriander + cumin mix (raw) extract + ADP	0.546	2.12	52.6
Coriander + cumin mix (boiled) extract + ADP	0.147	2.37	47.1
Coriander + cumin mix (roasted) extract + ADP	0.210	2.4	46.4
Collagen only	0.0	5.20	0.0
Coriander + cumin mix (raw) extract + collagen	0.392	2.36	54.6
Coriander + cumin mix (boiled) extract + collagen	0.189	2.50	51.9
Coriander + cumin mix (roasted) extract + collagen	0.210	2.45	52.9
A 23187 only	0.0	5.20	0.0
Coriander + cumin mix (raw) extract + A 23187	0.644	2.48	52.2
Coriander + cumin mix (boiled) extract + A 23187	0.392	2.60	50.0
Coriander + cumin mix (roasted) extract + A 23187	0.364	2.57	50.6

IC_{50} in mg is done of PRP with various extracts and agonists.

Washed platelets in the absence of normal plasma constituents, which may interfere with the component(s) of spice extracts that were causing inhibition of platelet aggregation, major changes were not observed. The inhibitions varied between 46.0 to 56.0 %. The changes in the IC_{50} were not that significant in the presence or absence of the plasma constituents.

Discussion:

Platelet aggregation is one of the least understood mechanisms in blood coagulation. But recent advances have helped in atleast understanding this mechanism to a certain extent. Platelet surface consists of a battery of receptors, which respond to a variety of stimulations. But, in the presence of these spice extracts inhibition of platelet aggregation may be seen due a variety of reasons.

Spice extracts inhibit platelet aggregation by exogenous inducers like ADP, epinephrine and collagen indicating that it is likely to not only compete with the GP IIb-IIIa receptors along with these agonists but also reduces the interaction of these agonists with the receptors. Further these agonists along with calcium ionophore A 23187 increases the cytosolic calcium levels by the binding of GP IIb-IIIa complex to fibrinogen which in turn helps in its incorporation into the cytoskeleton of aggregating platelets. These extracts may interfere with the calcium signaling influx in the activated platelets by blocking the formation of GP IIb-IIIa fibrinogen complex. This inturn blocks the pathways of platelet aggregation due to the

lack of stimulation of enzymes in the absence of the rise in cytosolic levels of calcium.

Calcium antagonists usually bring about inhibition of platelet aggregation by either causing the thickening of membrane bilayers or by binding to the specific receptors. In a similar way these extracts may also modify the membrane structure and thus prevent the permeability of the calcium ions. Also the intracellular free calcium in activated platelets may undergo steric hindrance in the presence of these spice extracts resulting in damage of the calcium channels near the GP IIb-IIIa complex and a decrease in the cytoskeletal reorganisation of platelets.

Under normal conditions, during platelet aggregation agonists like ADP and epinephrine, new calcium binding sites develop. But, in the presence of these spice extracts the interaction of ADP and epinephrine itself was altered and new binding sites were not available resulting in the inhibition of platelet aggregation.

A rise in the cyclic AMP levels results in the inhibition of secretions from human platelets. Generally it is observed that activation of adenyl cyclase activity inhibits the cyclic AMP levels thus mobilizing the fibrinogen binding to platelets. But, these spice extracts may interfere with the activation of adenyl cyclase activity, resulting in the increase in cyclic AMP levels and in turn contributes to the inhibition of platelet aggregation.

During early stages of aggregation by collagen, a strong agonist, GP IV (CD 36) and GP VI play an important role. These spice extracts may

bind with these glycoproteins and thus also prevent the activation of platelet release mechanisms.

Collagen also brings about a burst in hydrogen peroxide levels, which is a pro-oxidant species contributing to the activation of platelets by acting as a secondary messenger that helps in stimulating the arachidonic acid pathway. But in the presence of these spice extracts, it may be said that release of hydrogen peroxide during aggregation was hindered as MDA, which is one of the end products of cyclo-oxygenase pathway of arachidonic acid metabolism was decreased significantly. An increase in the platelet glutathione peroxidase (GPx) activity adversely affects the hydroperoxide formation. These spice extracts may inturn possess(s) components, which have the ability to increase the GPx activity thus countering the hydroperoxide formation.

Serotonin present in the dense granules of platelets is released due to cytoskeletal reorganisation, which brings about an increase in the cytosolic calcium levels and inhibits the adenyl cyclase activity. The released serotonin enhances the retention or binding of procoagulant proteins on the cell surface of stimulated platelets. But, these spices extracts were inhibiting the release of serotonin from platelets indicating that calcium mobilisation and adenyl cyclase activity were being affected.

Also the retention or binding of these procoagulant proteins on the stimulated platelets may be obstructed because the component(s) present in these spices extracts were present on platelet surface.

Ristocetin induces platelet agglutination through GP Ib by coflocculating with vWF receptor. These spice extracts were also interacting with the GP Ib receptor present on the platelet membrane surface and thus were bringing about the inhibition of ristocetin induced aggregation. But, if the spice extracts do not bind to GP Ib receptor, then the inhibition of human platelet aggregation will not be taking place.

Hence in conclusion it may be said that, these spice extracts (raw and processed) possess component(s), which have the ability to inhibit platelet aggregation through the series of exquisitely co-ordinated events.

2. EFFECT OF CORIANDER AND CUMIN EXTRACTS ON HUMAN PLATELET MEMBRANE LIPID PEROXIDATION AND MEMEBRANE FLUIDITY:

Lipid peroxidation is an important phenomenon, which brings about undesirable changes in the lipids, proteins and nucleic acids, thus adversely affecting the biosystems. Mislead by the name, lipid peroxidation is sometimes considered to happen by the attack of peroxide or hydroxyl radical. But it is initiated by the abstraction of an electron and a hydrogen atom from a carbon atom of an unsaturated fatty acid (RH) by an active iron species (Halliwell and Gutteridge, 1985). But, this phenomenon is brought about by adding or removing electrons.

Oxygen, an effective electron sink in biosystems, is essential for aerobic life, but is toxic in excess amounts. With all its great features, the diradical oxygen is chemically inert. Only a metal center has the ability to place an electron in its anti-bonding orbital. Nature devised the exquisite cytochrome oxidase complex with copper centers for the four electron

reduction of O_2 to water. The intermediate reduced forms with increased number of electrons per dioxygen molecule (e/O_2 ratio) are known to be formed: superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxy radical (OH). Added to these is the singlet oxygen ($^1O_2$) with spin reversal formed from peroxo groups on oxidation by hypohalous acids. Known as reactive oxygen species (ROS), these are responsible for many of the essential actions and also the damages attributed to oxygen (Khan and Kasha, 1994).

The other biological electron sink that remains backstage is CO_2. Electrons from H_2O oxidation find their way through photosystems and NADPH to reduce CO_2 and reside in carbohydrates. Some important reactions of carbon compounds occur through initial formation of carbon radicals. But little appreciation is shown to carbon radical species even in the better-known reactions (Ramasarma, 2000).

Lipid peroxidation is an auto-oxidative process initiated by a variety of free radicals to which the polyunsaturated fatty acids present in the cell membranes are susceptible due to formation of $LOO^{\bullet}$, endoperoxides and hydroxyperoxides. Once formed, unstable $LOO^{\bullet}$ are able to propagate a chain reaction of the lipid peroxidation process. Along with the changes in the membranes, an increase in phospholipid bilayer rigidity is seen. Modification of the physical properties of membrane resides in their relation to a number of cellular functions, including the activity of membrane-associated enzymes (Garcia, *et al.*, 1997).

Oxygen radicals and other oxygen-derived species are important causative agents of aging and several other human diseases including cancer, multiple sclerosis, Parkinson's disease, autoimmune diseases and senile dementia. Increased oxygen radical generation and lipid peroxidation have been suggested to be responsible for toxic actions of a wide range of compounds (Halliwell and Gutteridge, 1986).

But on the other hand, these reactive oxygen species play an essential role in controlling cell functions like formation of intermediate metabolites involved in several enzymic reactions, in post translational protein turnover and in the control of vascular tone. Activated neutrophils and macrophages release superoxide anion radicals, which are important in host defense mechanisms by killing bacteria. Also platelets have been reported to release superoxide anion radicals under appropriate conditions and this has been suggested to have a comparable function on the killing of parasites. Platelets primed by exposure to sub-threshold concentrations of arachidonic acid or collagen are known to be activated by nanomolar levels of hydrogen peroxide and that these reactive oxygen species act as 'second messengers' during the initial phase of the platelet activation (Iuliano *et al.*, 1994).

Apoptosis is a form of cell death in which an individual cell undergoes an internally controlled transition from an intact metabolically active state into a number of shrunken remnants of its membrane bound integrity. Lysis of internal organelles apparently does not occur during this process, and little external leakage of the contents of the dying cells can be

detected. Apoptotic cells do not induce an inflammatory response *in vivo*, but the shrunken apoptotic bodies are phagocytosed by macrophages and their contents recycled. Exposure of cells to oxidants such as hydrogen peroxide or various redox-active quinones causes multiple intracellular alterations, including elevation of cytosolic Ca^{2+}, energy depletion and oxidation of glutathione, NADPH, protein thiols and lipids (Slater *et al.*, 1995).

Accumulation of lipid-laden foam cells in the subendothelial region of arterial walls is an early event in the development of atherosclerosis. Many foam cells arise from macrophages, probably by the scavenger receptor-mediated uptake of low-density lipoproteins (LDLs) that have undergone chemical and structural modification. Peroxidation of LDL lipids and covalent modification of apoprotein B are important events in atherogenesis. The chain breaking antioxidant inhibitors of lipid peroxidation appear to have an anti-atherogenic action *in vivo* (Smith *et al.*, 1992).

Platelets have the capacity to generate oxygen-derived free radicals and are often present at the inflammatory foci with other free radical generating cells such as white blood cells. Free radicals can modify platelet adhesion and aggregation directly or through effects on the vascular endothelium, which generates prostacyclin and nitric oxide. Any molecule that reacts with a free radical is referred to here to as a 'scavenger'. These may be substances of low molecular weight such as vitamins E or C, complex enzyme systems such as superoxide dismutase

(SOD) or simple molecules such as nitric oxide (NO) (Salvemini and Botting, 1993).

The presence of superoxide radicals in the surrounding medium of platelets may have significant impact on the haemostasis, coagulation and thrombosis. These superoxide radials could be important determinants of platelet viability during processing, concentration and storage for clinical transfusion. The non-superoxide related reducing activities might represent a biochemical basis for platelet-blood vessel interactions with particular reference to blood vessel integrity (Marcus *et al.*, 1977). The superoxide radicals have detrimental effect on the blood vessel integrity causing atherosclerosis (Salvemini *et al.*, 1989).

Ascorbic acid stimulates collagen synthesis in cultured dermal fibroblasts through stimulation of the expression of collagen genes. This process has recently been shown to involve the induction of lipid peroxidation by malondialdehyde formation and modification of protein or DNA. Also it may involve the regulation of membrane bound molecules involved in signal transduction (Geesin *et al*., 1991).

Reactive oxygen and nitrogen species (ROS/RNS) are essential to energy supply, chemical signaling, detoxification and immune functions and, as a consequence, continuously produced in the human body. For most part they are exquisitely controlled but over-production, exposure to external oxidants or the failure of antioxidant defense mechanisms is implicated in damage to DNA, lipids and proteins. In turn, damage to these

biomolecules is associated with increased risk of chronic diseases including cancer and cardiovascular diseases (Gutteridge, 1993).

The term 'antioxidant' which is very frequently used in the biomedical literature is rarely defined. But in a broader view it may be defined as 'any substance that, when present at low concentrations compared to those of an oxidisable substrate, significantly delays or inhibits oxidation of that substrate'. These antioxidants act at different levels in an oxidative sequence. Antioxidants could act against lipid peroxidation by:

(a) Decreasing localized O_2 concentrations by combining with O_2 or displacing it.

(b) Preventing initiation of lipid peroxidation by scavenging species capable of abstracting hydrogen atoms like hydroxyl radicals.

(c) Quenching or scavenging singlet O_2 that can react directly with membrane lipids to produce peroxides. For example, lycopene has been reported to be the best lipid soluble quencher of singlet O_2 in human plasma.

(d) Binding metal ions in forms that will not generate reactive species such as OH, ferryl, or Fe^{2+} / Fe^{3+} / O_2 complexes, which will not decompose lipid peroxides to their respective peroxyl and alkoxyl radicals.

(e) Removing peroxides by converting them into non-radical products such as alcohols.

(f) Chain breaking i.e., reacting with chain-propagating radicals (peroxyl and possibly alkoxyl) and preventing continued hydrogen abstraction

from fatty acid side chains. Chain breaking antioxidants are often phenols or aromatic amines.

Antioxidants inhibiting lipid peroxidation by mechanisms a, b, c or d can be called “preventive antioxidants”. Those acting by mechanism ‘d’ would not be expected to be consumed during the course of the reactions. Antioxidants of the fifth type are also preventive antioxidants, but they may or may not be consumed during the reaction depending on the chemical behavior. Chain breaking antioxidants, acting by combining with chain propagating radicals, will be consumed as also antioxidants acting by scavenging singlet O_2.

The antioxidants in the human plasma include ceruloplasmin, albumin (the protein itself and possibly also albumin bound bilirubin), ascorbic acid, transferrin, haptoglobin and hemopexin (Halliwell and Gutteridge, 1990).

The antioxidant hypothesis suggests that reducing agents (i.e. antioxidants) have the capacity to prevent oxidative damage and thus increased levels will also reduce the risk of chronic diseases. Dietary compounds, which are capable of acting as chemical antioxidants, are likely to be of benefit by augmenting cellular defenses and helping to protect components of the cell from oxidative damage. Compelling epidemiological evidence links consumption of diets rich in antioxidants, direct or indirect, with reduced risk of degenerative diseases. Human studies have shown that high intake of foods rich in dietary antioxidants like fruits, vegetables, cereals, grains, and some specific oils and fats and

associated high plasma anti-oxidant levels reduced the morbidity and mortality rates (Doll and Peto, 1981; Astley, 2003).

Therapeutically, antioxidants, which are inhibitors of lipid peroxidation, are unlikely to succeed in protecting against oxidant stress in disease and toxicology. But if the source of the oxidants is identified an approach can be made to block them using the known antioxidants. The inhibitors of arachidonic acid mechanism, Ca^{2+} chelators and antioxidants offered some protection against reoxygenation injury in the heart (Halliwell, 1987).

Lipid peroxidation induced by iron-ascorbic acid system was effectively inhibited by various extracts of coriander and cumin. Malondialdehyde (MDA) formed was estimated. Experimental evidences indicate that the metabolites of lipid peroxidation adversely affect the biosystems bringing about undesirable changes. Spices have been known to possess component(s) which act as antioxidants and protect the various membrane bilayers by either scavenging the reactive oxygen spices or by chelating the transition metal ions required for initiating lipid peroxidation (Tyler, 1975; Halliwell and Gutteridge, 1984).

Oral administration of turmeric in nutritionally relevant doses to rabbits significantly reduced the susceptibility to oxidation of cellular and subcellular membranes of erythrocytes and liver microsomes in an atherosclerotic process (Mesa *et al.*, 2003). Retinol helps in the maintenance of membrane integrity and stability but its deficiency can provoke a general alteration in the physical characteristics of the

membranes thus increasing the chances of lipid peroxidation in the tissue microsomes. The feeding of curcumin / turmeric to retinol deficient rats have corrected the changes brought about in the membranes due to lipid peroxidation (Kaul and Krishnakantha, 1997).

Similarly, eugenol (25-150 μM) from cloves, capsaicin (25-150 μM) from red chillies, and zingerone (> 150 μM) from ginger inhibited lipid peroxidation in rat liver microsomes. On the other hand linalool from coriander, cuminaldehyde from cumin and piperine from black pepper marginally inhibited lipid peroxidation in rat liver microsomes at concentrations as high as 600 μM (Reddy and Lokesh, 1992).

Spice active principles like curcumin, quercetin and capsaicin at 10 μM produced inhibition of 40-85 % of LDL (low density lipoprotein) oxidation at different incubation times. Spice active principles constitute about 1-4 % of the respective spices and are effectively offering antioxidant protection against oxidation of human LDL (Naidu and Thippeswamy, 2002).

A study with diallyl sulphide (DAS), an active principle of garlic provides protection against radiation injury in mouse colon. Also tissue cultured human lymphocytes were protected from radiation with garlic (Jaiswal and Bordia, 1996). Certain phenolic substances present in red wine bind to plasma LDL and offer protection against lipid peroxidation to this lipoprotein (Fuhrman *et al.*, 1995).

Flavanoids like catechins and epicatechin protect platelets against peroxidative stress and inhibit platelet aggregation (Neiva *et al.*, 1999).

Dried and minced leaves of Ilex inhibited copper induced LDP (low density lipoprotein) oxidation *in vivo* (Schinella *et al.*, 2000).

Turmerin, a water-soluble antioxidant peptide from turmeric effectively protected against reactive oxygen species induced lipid peroxidation of human erythrocyte ghosts and DNA of human lymphocytes and calf thymus, thus acting as a DNA protectant (Srinivas *et al.*, 1992). Also ingestion of coriander seeds brought about changes in the lipid peroxide levels in rats (Chithra and Leelamma, 1999).

Coriander (raw) extract inhibited iron-ascorbic acid induced lipid peroxidation in human platelet membranes between 17 to 83 %. On the other hand, boiled coriander extract inhibited between 19 to 74 % where as roasted coriander extract inhibited between 20 to 84 % as shown in figure 16. The inhibition is linear around 0.42 mg and no further significant increase is seen beyond this concentration. The inhibition with raw and processed coriander extract did not show much difference. This suggests that the antioxidant potency is not altered even after boiling or roasting of coriander.

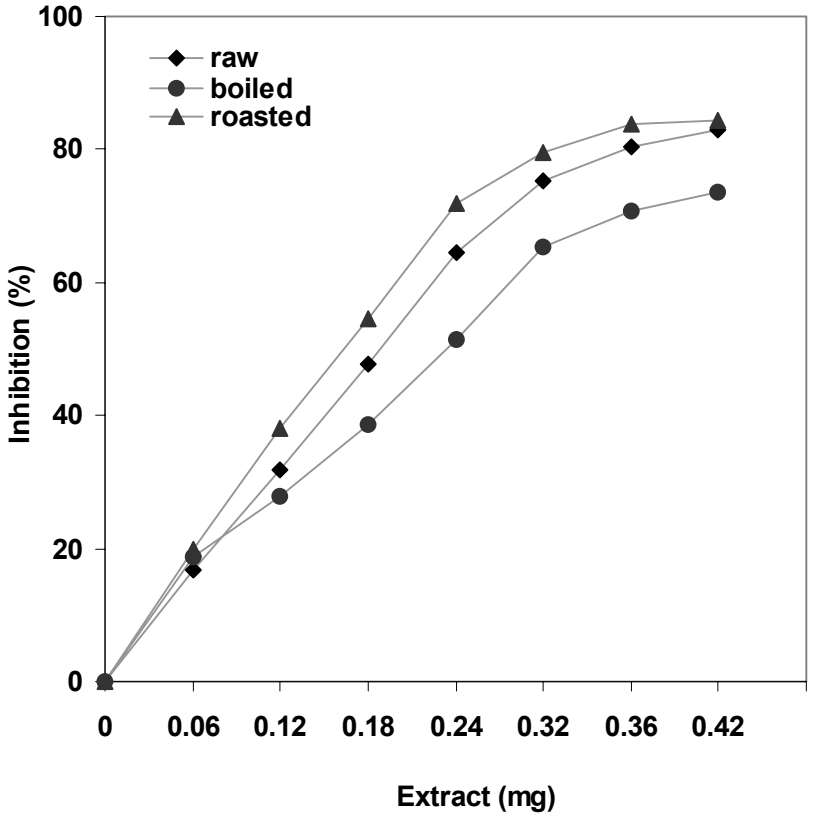


Figure 16 - Inhibition of Lipid peroxidation (MDA formation) in human platelet membranes by coriander extracts

Cumin (raw) extract also inhibited iron-ascorbic acid induced lipid peroxidation in human platelet membranes between 18 to 78 %. On the other hand, boiled cumin extract inhibited between 18 to 75 % where as roasted cumin extract inhibited between 14 to 68 % as shown in figure 17. The inhibition is linear around 0.48 mg of the extract and not further significant increase was seen beyond this concentration.

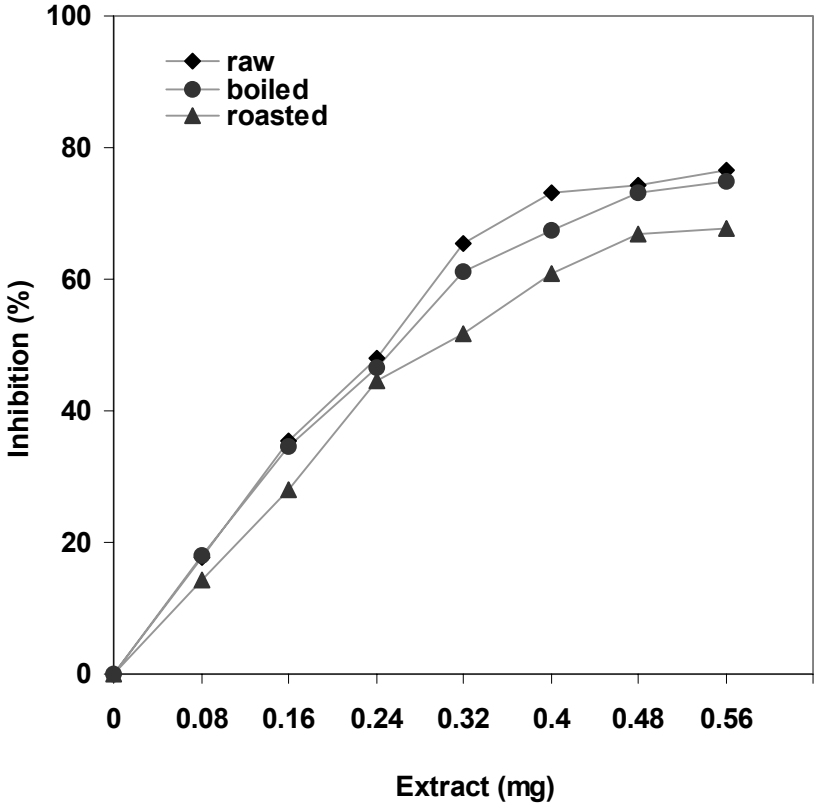


Figure 17 - Inhibition of Lipid peroxidation (MDA formation) in human platelet membranes by cumin extracts

A combination of coriander and cumin (raw) extract inhibited iron-ascorbic acid induced lipid peroxidation in human platelet membranes between 14 to 58 %. On the other hand, boiled coriander and cumin extract inhibited between 13 to 65 % where as roasted coriander and cumin extract inhibited between 11 to 66 % as shown in figure 18. The inhibition is linear around 0.48 mg of the extract and not further significant increase was seen beyond this concentration.

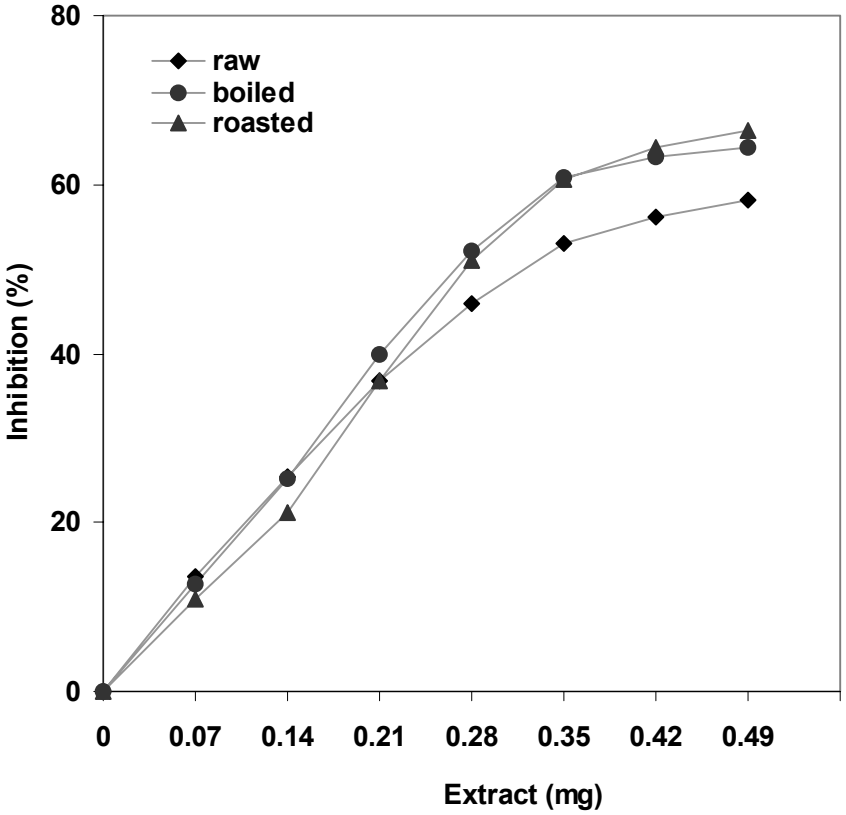


Figure 18 - Inhibition of Lipid peroxidation (MDA formation) in human platelet membranes by coriander + cumin mix extracts

Processed coriander dhal extract inhibited iron-ascorbic acid induced lipid peroxidation in human platelet membranes between 24 to 64 % as shown in figure 19. The inhibition shown by coriander dhal is not as affective as that shown by coriander or cumin. The inhibition was not linear but hyperbolic.

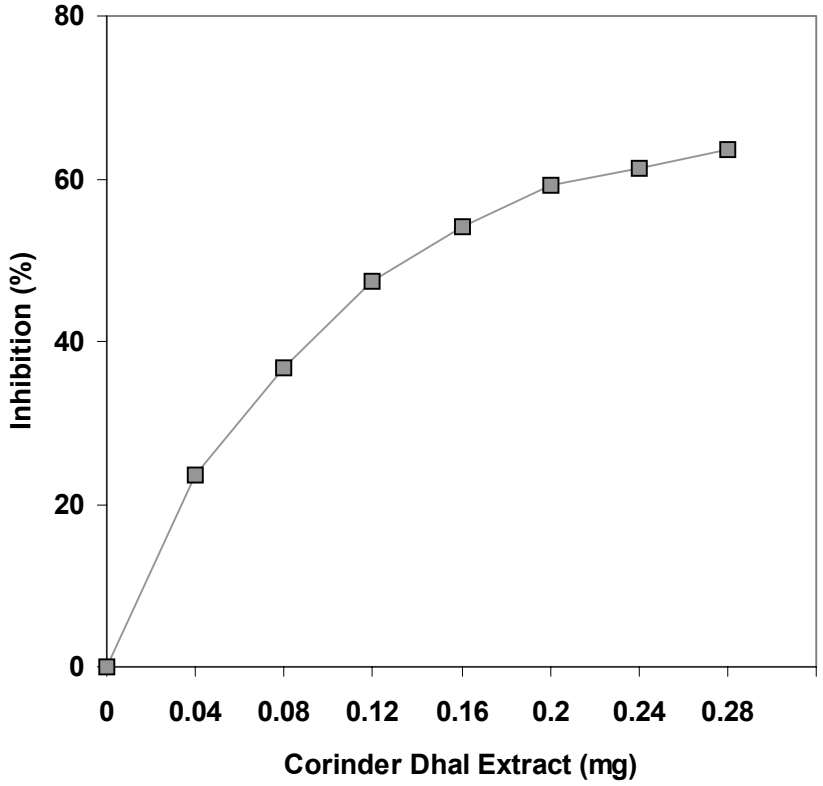


Figure 19 - Inhibition of Lipid peroxidation (MDA formation) in human platelet membranes by processed coriander dhal extract

α-Linalool from coriander inhibited iron-ascorbic acid induced lipid peroxidation in human platelet membranes between 17 to 76 % where as α-Terpinene from cumin inhibited between 15 to 68 % as shown in figure 20.

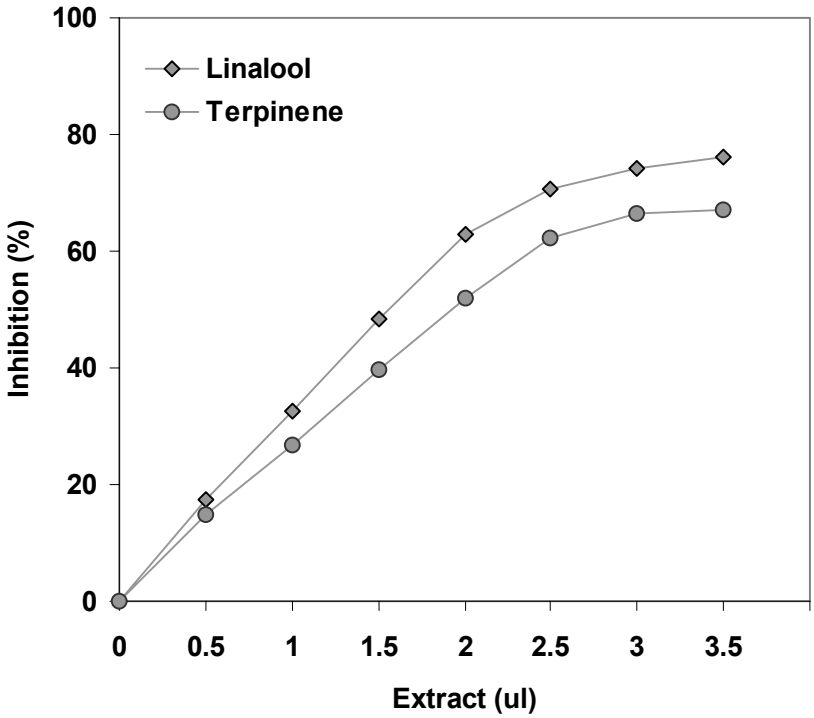


Figure 20 - Inhibition of Lipid peroxidation (MDA formation) in human platelet membranes by α - Linalool from coriander and α - Terpinene from cumin

Varying concentrations of spice extracts were used to determine the IC_{50}. Table 37 shows the IC_{50} of the respective extracts of raw and processed coriander and cumin. Processed coriander dhal was required in very low quantity (0.136 mg) to inhibit the lipid peroxidation followed by roasted coriander (0.162 mg) and raw coriander (0.186 mg).

Other extracts had more or less similar IC_{50} where as least sensitivity was shown by roasted extract of cumin (0.312 mg) and raw extract of coriander and cumin mix (0.308 mg).

Table 37 - Inhibition of human platelet membrane lipid peroxidation (MDA formation) by raw and processed spice extracts

Inhibitor	IC_{50}
Coriander (raw)	0.186
Coriander (boiled)	0.234
Coriander (roasted)	0.162
Cumin (raw)	0.224
Cumin (boiled)	0.256
Cumin (roasted)	0.312
Coriander + Cumin mix (raw)	0.308
Coriander + Cumin mix (boiled)	0.259
Coriander + Cumin mix (roasted)	0.287
Coriander dhal	0.136
α - Linalool *	1.60
α-Terpinene *	1.95

Values are expressed in mg.
* Values are expressed in μl.
Values are Mean $\pm$ S.D of triplicates.

During lipid peroxidation in addition to malondialdehyde other metabolites like conjugated dienes are formed. Using the IC_{50} of the different extracts, which inhibited malondialdehyde formation, the inhibition of conjugated dienes formation was also assayed as shown in Table 38. It may be observed that the percent inhibition ranged between 40 to 65%.

The best inhibition was seen with raw extract of cumin (64.9%) where as least inhibition was shown by processed coriander dhal (40.4%). Hence it may be mentioned that apart from inhibiting the MDA formation, the spice extracts have component(s), which can actively inhibit the conjugated dienes formation.

Table 38 - Inhibition of human platelet membrane lipid peroxidation (conjugated dienes formation) by raw and processed spice extracts at IC_{50}

Inhibitor	IC_{50} (mg)	Amount of 5-HPETE formed	Inhibition (%)
Control	0.0	7.20 ± 0.0	0.0
Coriander (raw)	0.186	3.51 ± 0.05	51.3 ± 0.65
Cumin (raw)	0.224	2.53 ± 0.09	64.9 ± 1.3
Coriander (boiled)	0.234	4.15 ± 0.15	42.1 ± 2.0
Cumin (boiled)	0.256	3.91 ± 0.05	45.7 ± 0.7
Coriander (roasted)	0.162	2.82 ± 0.13	60.8 ± 1.8
Cumin (roasted)	0.312	3.15 ± 0.13	56.4 ± 1.8
Coriander + Cumin mix (raw)	0.308	3.17 ± 0.11	56.1 ± 1.5
Coriander + Cumin mix (boiled)	0.259	3.10 ± 0.10	57.0 ± 1.6
Coriander + Cumin mix (roasted)	0.287	3.88 ± 0.11	46.2 ± 1.5
Coriander dhal	0.136	4.29 ± 0.12	40.4 ± 1.5
α - Linalool *	1.60	3.41 ± 0.09	52.7 ± 1.2
α-Terpinene *	1.95	3.72 ± 0.08	50.5 ± 1.1

Values of 5-HPETE are expressed as μmoles released/mg protein/hr at IC_{50} of MDA formation.
* Values of IC_{50} are expressed in μl.
Values are Mean ± S.D of triplicates.

Another interesting aspect, which has been dealt with, is the inhibition of MDA formation in platelets, which are first treated with the respective spice extracts at IC_{50} and followed by agonists induced platelet aggregation.

From this, the platelet membranes were prepared and subjected to lipid peroxidation by iron-ascorbic acid system (Miller and Aust, 1989). The basis for this study was to see if the component(s) in these spices are

interacting with the platelet membranes. Table 39 shows results of such a study indicating the percentage inhibition of MDA formed.

Table 39 - Inhibition of lipid peroxidation by spice extracts in agonist challenged human platelet membranes at IC_{50}

Spice / Agonist	MDA formed	Inhibition (%)
ADP only	2.14 ± 0.02	0.0
Epinephrine only	1.54 ± 0.01	0.0
Collagen only	2.35 ± 0.02	0.0
A 23187 only	2.25 ± 0.03	0.0
Coriander (raw) extract:		
+ ADP	1.38 ± 0.01	35.4 ± 0.0
+ epinephrine	1.07 ± 0.02	30.9 ± 1.5
+ collagen	1.21 ± 0.03	48.6 ± 0.8
+ A 23187	1.47 ± 0.03	34.6 ± 1.0
Coriander (boiled) extract:		
+ ADP	1.43 ± 0.02	33.1 ± 0.5
+ epinephrine	1.16 ± 0.02	25.0 ± 1.5
+ collagen	1.32 ± 0.02	43.7 ± 0.5
+ A 23187	1.55 ± 0.01	31.0 ± 0.9
Coriander (roasted) extract:		
+ ADP	1.49 ± 0.01	30.5 ± 0.3
+ epinephrine	1.14 ± 0.01	26.3 ± 0.2
+ collagen	1.35 ± 0.03	42.4 ± 0.9
+ A 23187	1.59 ± 0.01	30.7 ± 0.7
Cumin (raw) extract:		
+ ADP	1.25 ± 0.02	41.7 ± 1.1
+ epinephrine	1.02 ± 0.01	33.8 ± 0.2
+ collagen	1.36 ± 0.01	48.6 ± 0.8
+ A 23187	1.50 ± 0.03	33.2 ± 1.8
Cumin (boiled) extract:		
+ ADP	1.38 ± 0.01	35.3 ± 1.0
+ epinephrine	1.09 ± 0.01	29.2 ± 0.2
+ collagen	1.43 ± 0.02	39.3 ± 1.1
+ A 23187	1.56 ± 0.01	30.8 ± 1.1
Cumin (roasted) extract:		
+ ADP	1.41 ± 0.01	34.2 ± 0.2
+ epinephrine	1.14 ± 0.01	23.0 ± 0.2
+ collagen	1.46 ± 0.01	38.0 ± 0.7
+ A 23187	1.57 ± 0.01	30.1 ± 0.0
Coriander + cumin (raw) mix extract:		
+ ADP	1.17 ± 0.02	44.6 ± 1.3

+ epinephrine	0.93 ± 0.02	40.0 ± 0.6
+ collagen	1.12 ± 0.02	52.3 ± 1.2
+ A 23187	1.38 ± 0.03	38.8 ± 0.7
Coriander + cumin (boiled) mix extract:		
+ ADP	1.21 ± 0.02	43.1 ± 1.2
+ epinephrine	0.99 ± 0.01	35.9 ± 1.3
+ collagen	1.20 ± 0.02	48.8 ± 1.5
+ A 23187	1.41 ± 0.02	37.0 ± 1.4
Coriander + cumin (roasted) mix extract:		
+ ADP	1.22 ± 0.02	42.9 ± 1.2
+ epinephrine	1.02 ± 0.01	33.8 ± 0.9
+ collagen	1.18 ± 0.01	49.7 ± 0.4
+ A 23187	1.46 ± 0.02	35.0 ± 1.4
Coriander dhal extract:		
+ ADP	1.37 ± 0.02	37.7 ± 1.4
+ epinephrine	0.98 ± 0.01	36.4 ±0.3
+ collagen	1.10 ± 0.01	53.1 ± 0.7
+ A 23187	1.44 ± 0.02	35.9 ± 1.4
α-Linalool:		
+ ADP	1.97 ± 0.02	7.7 ± 1.6
+ epinephrine	1.39 ± 0.01	9.8 ± 1.3
+ collagen	1.88 ± 0.02	20.0 ± 1.2
+ A 23187	2.04 ± 0.02	9.4 ± 0.1
α-Terpinene:		
+ ADP	2.0 ± 0.01	6.3 ± 0.2
+ epinephrine	1.40 ± 0.01	9.4 ± 0.3
+ collagen	1.94 ± 0.02	17.3 ± 0.3
+ A 23187	1.98 ± 0.0	11.8 ± 0.6
Unchallenged platelet membranes	2.15 ± 0.03	0.0

Values are mean ± S.D of duplicates.
Values are expressed as μmoles of MDA formed/mg protein/hr.

The inhibition varied between 23 to 49% (table 39) with highest inhibition revealed by boiled extract of coriander and cumin mix with collagen (48.8%) followed by roasted cumin extract with epinephrine (48.6%) where as least inhibition was shown by roasted cumin extract with epinephrine (23.0%). It suggests that the component(s) tend to bind to

platelets irreversibly and thus prevent the lipid peroxidation in platelet membranes. Also the component(s) of the spice extracts, which were embedded into the platelet membranes, have protective effect lasting even when challenged by iron-ascorbic acid system, thus resulting in the decrease in MDA formation compared with the control.

Discussion:

The reactive oxygen species produced in limited amounts that the human body can control have beneficial affects like acting as secondary messengers to platelets during the initial phase of their activation or they play an important role in other cell functions.

The excess formation of these reactive oxygen species results in lipid peroxidation. This lipid peroxidation adversely affects the biological systems by damaging the membrane proteins leading to the alteration or impairment in the membrane functions. Also lipid bilayers of cell membranes are highly susceptible to peroxidation due to their poly unsaturated fatty acids.

Although the human body has its own antioxidant mechanism to scavenge the reactive oxygen species to overcome fight lipid peroxidation, exposure to external oxidants or failure in the antioxidant mechanisms, increase the risk of chronic diseases including cancer and cardiovascular diseases, multiple sclerosis, autoimmune diseases, parkinson's disease to name a few. It is here where in the ancient knowledge was used, as spices are the known antioxidants that are likely to exert their effect on lipid

peroxidation by scavenging the reactive oxygen species, which may other wise initiate lipid peroxidation.

Lipid peroxidation initiated by the iron-ascorbic acid system *in vitro* was inhibited in human platelet membranes by extracts of raw and processed coriander and cumin. The MDA and conjugated dienes formed were considerably inhibited by these extracts. This may be possible because these spice extracts may possess component(s), which may be able to chelate the metal ions or scavenge / quench the reactive oxygen species formed. They may also remove the peroxides from circulation by converting them into non-radical products like alcohol. These spice extracts may be chain-breaking antioxidants or may decrease the availability of localized O_2 concentration, as it is an effective electron sink.

Lipid peroxidation was induced in platelet membranes, which were first incubated with the respective spice extracts at their IC_{50}, then induced with aggregation and platelet membranes prepared.

Here also a decrease in the MDA formed was observed indicating that, the component(s) in these spices tend to bind strongly to the platelet membranes and thus provide protection on agonist induced peroxidation.

Hence in conclusion it may be said that these spice extracts do possess antioxidant properties, which protect the human platelet membranes from undergoing irreversible damage.

Membrane Fluidity:

All membrane functions are influenced by membrane fluidity, which inturn is determined by lipid profile of the plasma membranes (Stubbs and

Smith, 1984). Membrane fluidity can be influenced by a variety of factors, physical and chemical. Of the physical effectors, temperature, pressure, pH and calcium ions are the most important where as the chemical effectors include protein: lipid, sphingomyelin: phosphatidyl choline, acyl chain length and the degree of unsaturation (Gibney and Bolton-Smith, 1988).

The composition and organisation of biological membranes are important factors that determine the fluidity, and thereby the activity and functions of membrane bound proteins such as enzymes, receptors, carrier or ion channels. Because membrane phospholipids are composed largely of unsaturated fatty acids and are located in an oxygen-containing environment, membranes are susceptible to lipid peroxidation. This process is a free radical chain reaction initiated by oxygen reactive species such as superoxide, hydroxyl radical and singlet oxygen and can significantly damage the polyunsaturated fatty acids and proteins in the membranes. Thus peroxidation is known to alter membrane lipid fluidity and affect the function of internal proteins and of membranes as a whole (Sevanian and Hochstein, 1985; Levin *et al*., 1990).

Platelets can generate reactive oxygen species and the platelet lipid peroxidation *in vivo* may be an important factor in determining the age-associated atherosclerosis reactions (Hossain *et al*., 1999). Malondialdehyde is a bifunctional compound that can react with free amino groups of proteins and lipids to form fluorescent cross-linked products

between various membrane components thus causing a decrease in membrane fluidity (Jain, 1987; Chu *et al.*, 1982).

Calcium ions exert inhibitory effects by directly interacting with protein components of the adenylate cyclase complex, which is noted to have divalent cation binding sites that perform regulatory roles (Gordon *et al*., 1983). The increase in bilayer fluidity stimulates adenylate cyclase activity where as the decrease in fluidity causes a reduction in its activity (Needham *et al*., 1987).

Human platelet membranes were incubated with 1,6-Diphenyl-1,3,5-Hexatriene (DPH) at 37 °C with constant stirring for 30 min. In a similar way platelet membranes were pre incubated with spice extracts and the anisotropy parameter determined are shown in table 40.

Table 40 – Anisotropy parameter of DPH at 37 °C in human platelets with spice extracts

Extract	Amount (mg)	Anisotropy parameter *
Control	0.0	1.18 ± 0.012
Coriander (raw)	0.360	1.23 ± 0.009
Coriander (boiled)	0.480	1.28 ± 0.010
Coriander (roasted)	0.480	1.21 ± 0.014
Cumin (raw)	0.512	1.41 ± 0.011
Cumin (boiled)	0.608	1.35 ± 0.016
Cumin (roasted)	0.656	1.39 ± 0.012
Coriander + Cumin mix (raw)	0.546	1.32 ± 0.015
Coriander + Cumin mix (boiled)	0.147	1.30 ± 0.017
Coriander + Cumin mix (roasted)	0.210	1.28 ± 0.010
Coriander dhal	0.096	1.39 ± 0.013

* Values are mean ± S.D of triplicates.

It may be observed that there was a slight increase in the anisotropy parameter in the presence of spice extracts with the maximum being for roasted cumin and coriander dhal extract where as the least was seen for roasted coriander extract.

Human platelet membranes were incubated with extracts of coriander and cumin and then lipid peroxidation was induced by iron-ascorbic system. The changes in the anisotropy parameters due to induction of lipid peroxidation in the presence and absence of spice extracts were studied and the results are shown in table 41.

Table 41 – The effects of incubation with spice extracts prior to lipid peroxidation on the anisotropy parameter of DPH at 37 °C in human platelets

Extract	Amount (mg)	Anisotropy parameter *
Control	0.0	1.62 ± 0.015
Coriander (raw)	0.360	1.52 ± 0.017
Coriander (boiled)	0.480	1.47 ± 0.013
Coriander (roasted)	0.480	1.51 ± 0.010
Cumin (raw)	0.512	1.37 ± 0.011
Cumin (boiled)	0.608	1.39 ± 0.014
Cumin (roasted)	0.656	1.43 ± 0.009
Coriander + Cumin mix (raw)	0.546	1.45 ± 0.012
Coriander + Cumin mix (boiled)	0.147	1.39 ± 0.016
Coriander + Cumin mix (roasted)	0.210	1.41 ± 0.010
Coriander dhal	0.096	1.40 ± 0.007

* Values are mean ± S.D of triplicates.

Lipid peroxidation, which adversely affects the membrane bilayers due to the production of the free radicals, was inhibited by the spice extracts. In the absence of the spice extracts, due to lipid

peroxidation a decrease in membrane fluidity was seen as shown by the increase in the anisotropy parameter value (1.62) compared with the control (1.18) as shown in table 40. But in the presence of these spice extracts there was increase in the fluidity.

Also this increase in the membrane fluidity may stimulate adenylate cyclase activity, which in turn increases the c-AMP levels. An increase in c-AMP levels causes the inhibition of platelet aggregation. This may also be one of the reasons for spice extracts to inhibit human platelet aggregation.

These spice extracts may stabilize the platelet membranes and resist the rigidity induced by free radical attack. It may therefore be presumed that the extracts of coriander and cumin possess the ability to stabilize cellular membranes by scavenging the free radical activity, which is related to its ability to reduce lipid peroxidation.

3. EFFECT OF CORIANDER AND CUMIN EXTRACTS ON THE KINETICS OF HUMAN PLATELET MEMBRANE BOUND ENZYMES:

Platelets, though anucleated, like other mammalian cells have a distinct plasma membranes with its complement of proteins, enzymes, receptors, transport channels and execute several membrane related functional activities.

Platelets which play an important role in haemostasis were found to be influenced by a variety of cations like Na^{+}, K^{+}, Ca^{+2}, Mg^{+2} or H^{+} to name a few. $Na^{+}K^{+}$ pump was found to be responsible for the active extrusion of

Na^+ and the active intrusion of K^+ in red cells. This is called the 'Na pump', which uses the energy from ATP generated in the EM pathway for pumping action. The rate at which pumping occurs depends on the internal Na^+ and external K^+ and may be considered as substrates along with ATP (Harlan *et al.*, 1982).

Many hormones and drugs act via the membrane receptors to decrease cellular c-AMP levels. Attenuation of c-AMP accumulation in intact cells has been linked, at least in part, to a decreased synthesis of adenylate cyclase. α_2-adrenergic receptors of human platelets mediate inhibition of adenylate cyclase in broken cell preparations. The effects of Na^+ on the catalytic activity of adenylate cyclase are accompanied by modulation of receptor-ligand interaction. In a number of model systems that show receptor-mediated inhibition of adenylate cyclase, Na^+ significantly decreases receptor affinity for agonists and partial agonists while slightly increasing receptor affinity for antagonists.

Also platelets in Na^+ free buffer could not aggregate and secrete to maximal dose of the α-adrenergic agent, epinephrine (Connolly and Limbird, 1983). In the absence of Na^+, apart from epinephrine stimulation, ADP and low concentrations of thrombin also did not cause human platelet aggregation mainly due to the non-mobilisation of arachidonic acid from human platelets (Sweatt, *et al.*, 1985).

Although Na^+ is usually thought of as an extracellular ion, intracellular Na^+ may play a key role in regulating the affinity and function of

cell surface receptors and that settings of altered intracellular Na^+ may be associated with altered receptor functions (Motulsky and Insel, 1983).

Table 42 - Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.45	42.3	37.5	3.8	8.9	1.25
0.6	49.1	44.5	4.6	9.2	
0.9	58.6	52.6	6.0	10.4	
1.2	61.7	54.3	7.4	12.0	
1.5	66.3	58.3	8.0	12.1	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Na^+K^+ATPase assayed in human platelet membranes had a K_m of 1.25 mM. This was observed in the absence of any of the added spice extracts as inhibitors. The same has been reported at three different concentration of the each extract of the spices to determine their respective K_m values.

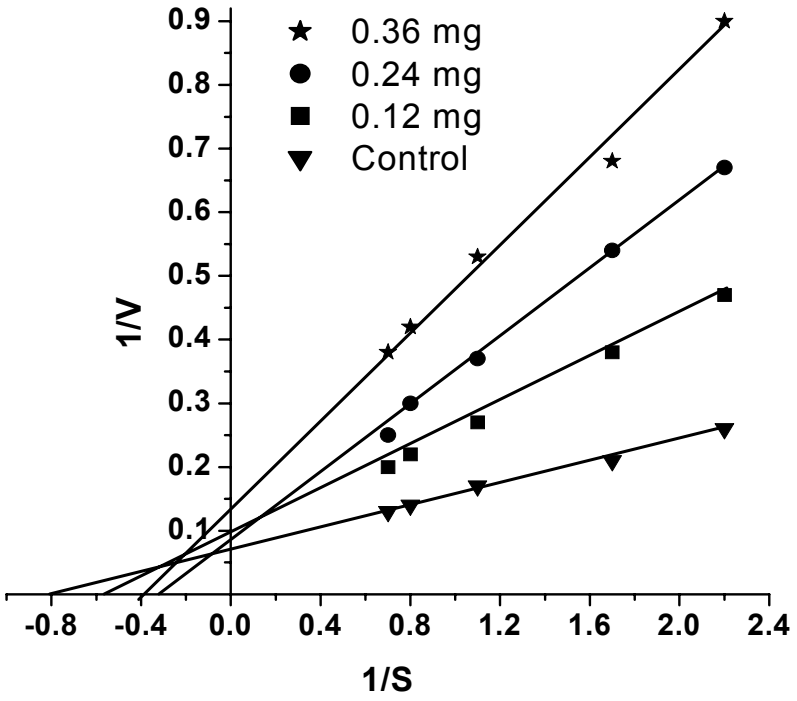


Figure 21 - Effect of coriander (raw) extract on Na^+K^+ATPase Kinetics of human platelet membranes

Concentrations of 0.12, 0.24 and 0.36 mg of raw coriander extract were used and the K_m of 2.5, 3.1 and 5.0 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. The extract could inhibit the activity competitively.

Table 43 - Effect of boiled coriander extract on Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.12	0.45	17.8	15.2	2.6	14.6	2.4
″	0.6	21.2	18.0	3.2	15.1	
″	0.9	22.8	18.4	4.4	19.3	
″	1.2	26.7	21.1	5.6	20.9	
″	1.5	32.6	26.3	6.3	19.4	
0.18	0.45	24.9	23.0	1.9	7.6	4.5
″	0.6	29.3	26.9	2.4	8.2	
″	0.9	31.6	28.9	3.5	11.0	
″	1.2	36.5	31.9	4.6	12.6	
″	1.5	42.0	37.0	5.0	11.9	
0.24	0.45	33.3	31.6	1.7	5.1	5.0
″	0.6	37.3	35.1	2.2	5.9	
″	0.9	39.5	36.5	3.0	7.6	
″	1.2	43.1	39.1	4.0	9.3	
″	1.5	43.9	39.3	4.6	10.5	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Concentrations of 0.12, 0.18 and 0.24 mg of boiled coriander extract were used and the K_m of 2.4, 4.5 and 5.0 mM respectively were obtained.

Table 44 - Effect of roasted coriander extract on Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.06	0.45	48.2	46.0	2.2	4.6	2.5
″	0.6	58.4	55.7	2.7	4.6	
″	0.9	62.1	58.4	3.7	6.0	
″	1.2	70.4	65.6	4.8	6.8	
″	1.5	76.9	71.6	5.3	6.9	
0.12	0.45	72.1	70.5	1.6	2.2	3.6
″	0.6	77.9	75.9	2.0	2.6	
″	0.9	83.9	81.0	2.9	3.5	
″	1.2	96.0	92.3	3.7	3.9	
″	1.5	101.7	97.7	4.0	3.9	
0.18	0.45	77.0	75.8	1.2	1.6	5.0
″	0.6	81.2	79.6	1.6	2.0	
″	0.9	85.4	83.1	2.3	2.7	
″	1.2	93.8	90.9	2.9	3.1	
″	1.5	98.1	94.9	3.2	3.3	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Concentrations of 0.06, 0.12 and 0.18 mg of roasted coriander extract were used and the K_m of 2.5, 3.6 and 5.0 mM respectively were obtained.

Of the three extracts, roasted extract of coriander seems to have a significant affect on Na^+K^+ATPase, as very low concentrations of this were required.

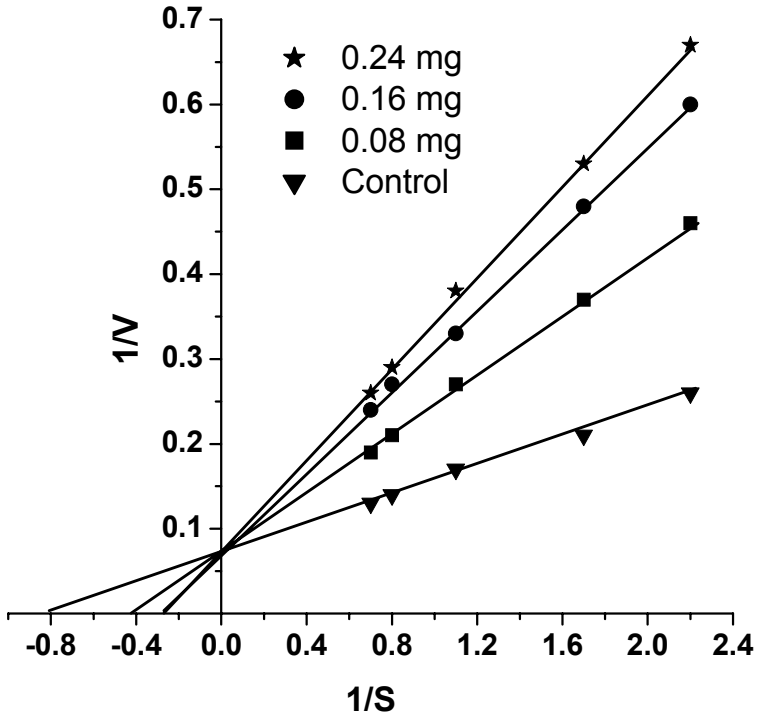


Figure 22 - Effect of cumin (raw) extract on Na^+K^+ATPase Kinetics of human platelet membranes

Concentrations of 0.08, 0.16 and 0.24 mg of raw cumin extract were used and the K_m of 2.5, 3.1 and 3.6 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. The extract inhibited the activity competitively as seen in figure 22.

Table 45 - Effect of boiled cumin extract on Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.24	0.45	49.2	46.4	2.8	5.7	1.9
″	0.6	54.3	51.0	3.3	6.1	
″	0.9	59.2	54.6	4.6	7.8	
″	1.2	70.5	64.8	5.7	8.1	
″	1.5	75.7	69.4	6.3	8.3	
0.32	0.45	56.6	54.2	2.4	4.2	2.1
″	0.6	65.0	62.0	3.0	4.6	
″	0.9	70.7	66.7	4.0	5.7	
″	1.2	73.7	68.7	5.0	6.8	
″	1.5	81.7	76.1	5.6	6.9	
0.40	0.45	54.9	52.8	2.1	3.8	2.4
″	0.6	60.2	57.6	2.6	4.3	
″	0.9	70.1	66.4	3.7	5.3	
″	1.2	75.6	71.0	4.6	6.1	
″	1.5	83.6	78.6	5.0	6.0	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Concentrations of 0.24, 0.32 and 0.40 mg of boiled cumin extract were used and the K_m of 1.9, 2.1 and 2.4 mM respectively were obtained.

Table 46 - Effect of roasted cumin extract on Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.19	0.45	34.6	32.3	2.3	6.6	2.2
″	0.6	41.8	39.0	2.8	6.7	
″	0.9	47.7	43.8	3.9	8.2	
″	1.2	53.2	48.4	4.8	9.0	
″	1.5	64.9	59.6	5.3	8.2	
0.27	0.45	38.3	36.3	2.0	5.2	2.9
″	0.6	44.7	42.3	2.4	5.4	
″	0.9	50.3	46.9	3.4	6.8	
″	1.2	59.5	55.1	4.4	7.5	
″	1.5	66.2	61.4	4.8	7.3	
0.35	0.45	42.9	41.3	1.4	3.3	4.6
″	0.6	46.6	45.0	1.7	3.7	
″	0.9	52.7	50.3	2.4	4.6	
″	1.2	58.7	55.6	3.1	5.3	
″	1.5	68.2	64.8	3.4	5.0	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Concentrations of 0.19, 0.27 and 0.35 mg of roasted cumin extract were used and the K_m of 2.2, 2.9 and 4.6 mM respectively were obtained.

Of the three extracts, raw extract of cumin seems to have a significant effect on Na^+K^+ATPase, as very low concentrations of this were required.

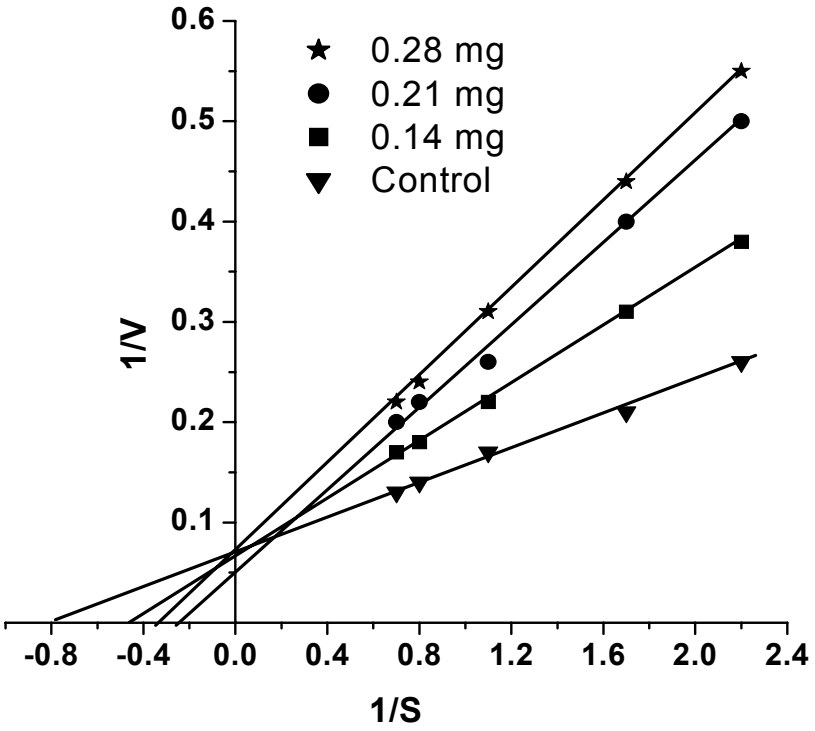


Figure 23 - Effect of coriander and cumin mix (raw) extract on Na^+K^+ATPase Kinetics of human platelet membranes

Concentrations of 0.14, 0.21 and 0.28 mg of raw coriander and cumin mix extract were used and the K_m of 2.2, 2.9 and 3.1 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. The mix extract inhibited the ATPase activity competitively as shown in figure 23.

Table 47 - Effect of boiled coriander and cumin mix extract on Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.14	0.45	40.9	38.2	2.7	6.6	1.6
″	0.6	53.7	50.4	3.3	6.1	
″	0.9	61.7	57.3	4.6	7.5	
″	1.2	63.9	58.3	5.6	8.8	
″	1.5	66.5	59.8	6.7	10.1	
0.21	0.45	50.6	48.4	2.2	4.3	1.7
″	0.6	53.2	50.4	2.8	5.3	
″	0.9	56.4	52.5	3.9	6.9	
″	1.2	61.7	56.7	5.0	8.1	
″	1.5	66.4	59.0	5.6	8.4	
0.28	0.45	55.5	53.6	1.9	3.4	2.6
″	0.6	56.7	54.5	2.2	3.9	
″	0.9	66.1	62.8	3.3	5.0	
″	1.2	72.6	68.4	4.2	5.8	
″	1.5	77.1	72.1	5.0	6.5	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Concentrations of 0.14, 0.21 and 0.28 mg of boiled coriander and cumin mix extract were used and the K_m of 1.6, 1.7 and 2.6 mM respectively were obtained.

Table 48 - Effect of roasted coriander and cumin mix extract on Na^+K^+ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	Ouabain insensitive activity	Ouabain sensitive activity *	Ouabain sensitive component	K_m
0.14	0.45	51.2	48.2	3.0	5.9	1.7
″	0.6	59.6	55.9	3.7	6.2	
″	0.9	65.7	60.7	5.0	7.6	
″	1.2	74.5	68.2	6.3	8.4	
″	1.5	81.0	74.3	6.7	8.2	
0.21	0.45	63.3	60.6	2.7	4.3	2.2
″	0.6	70.1	66.8	3.3	4.8	
″	0.9	79.6	75.1	4.6	5.7	
″	1.2	85.2	79.6	5.6	6.5	
″	1.5	90.4	84.1	6.3	6.9	
0.28	0.45	68.7	66.2	2.5	3.6	2.3
″	0.6	75.2	72.1	3.1	4.2	
″	0.9	83.3	79.1	4.2	5.0	
″	1.2	88.9	83.6	5.3	5.9	
″	1.5	94.4	88.8	5.6	5.9	

Values are expressed as μmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
* Na^+K^+ATPase activity.

Concentrations of 0.19, 0.27 and 0.35 mg of roasted coriander and cumin mix extract were used and the K_m of 1.7, 2.2 and 2.3 mM respectively were obtained.

Of the three extracts, raw extract of coriander and cumin mix seems to have a significant affect on Na^+K^+ATPase, as very low concentrations of this were required.

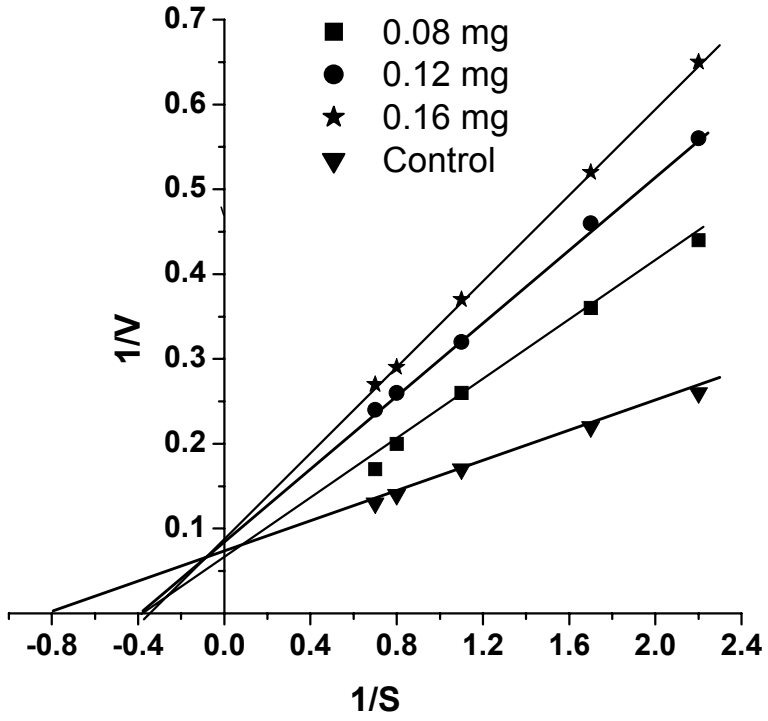


Figure 24 - Effect of processed coriander dhal on Na^+K^+ATPase Kinetics of human platelet membranes

Concentrations of 0.08, 0.16 and 0.24 mg of processed coriander dhal extract were used and the K_m of 2.5, 2.6 and 2.9 mM respectively were obtained.

Impairment in the ability to extrude Na^+ from cells can result in hypertension both in rats and humans mainly because of the increase in the vasotonicity and blood pressure without an increase in extracellular fluid. Also a decrease in Na^+K^+ATPase activity is associated with diabetes (Mimmura *et al.*, 1992; Rabini *et al.*, 1994). So a normal or increased Na^+K^+ATPase may have beneficial effects.

Na^+K^+ATPase is an integral membrane protein, comprising of glycoprotein subunits and hence its activity may be affected when the composition and organisation of the membranes is altered in the presence of agonists (Kaul and Krishnakantha, 1994). Curcumin has been reported to inhibit Na^+K^+ATPase activity in tissue microsomes (Shantha and Krishnakantha, 1987).

Quercetin inhibited Na^+K^+ATPase activity from the electric organ of electric eel or from lamb kidney may be due to altered state in the enzyme that interferes with either the acceptance or release of Pi or may block the site concerned with these reactions (Kuriki and Racker, 1976). The wide spread usage of tea may be linked with increase in the concentrations of an inhibitor of Na^+K^+ATPase which was ultimately leading to hypertension (Sagnella and MacGregor, 1984).

Aqueous extracts of spices like nutmeg, cinnamon, cloves, cumin, coriander, turmeric and caraway inhibited Na^+K^+ATPase activity in dog kidney, rat kidney and intestine microsomes *in vitro*. Also the extracts of cloves and cinnamon inhibited significantly *in vivo* absorption of alanine, which is co-transported with sodium and is dependent on the sodium gradient created by the Na^+K^+ATPase (Sigrist-Nelson *et al.*, 1975). This inhibition may be due to permeation into the enterocytes mucosal membrane and reach the serosal side where the Na^+K^+ATPase is located. Consequently, all the cellular activities and transport processes that are geared by the sodium gradient established by the Na^+ - K^+ pump are expected to be impaired both in the intestine and kidney, like the sodium

coupled transport of glucose and some amino acids, the Na^+ H^+ counter transport involved in the regulation of intracellular pH and osmotic balance (Kreydiyyeh *et al.*, 2000).

On the other hand, along with sodium, calcium ions also play an important role in the cascade of events of platelet aggregation. When haemostatic agents like ADP, thrombin or collagen induce platelet aggregation they require calcium. The dissociation of microtubules, contraction of actomyosin, glycogenolysis, the action of lipases in the release of arachidonic acid for prostaglandin synthesis, phosphorylation of key substrates by protein kinase are few of the intracellular mechanisms in the platelets triggered by the surface membrane signals and all these are regulated by the mobilisation of internal calcium stores (Rink *et al.*, 1982; Stoffel *et al.*, 1958)

Thromboxane A_2 may be involved in transporting calcium out of the intracellular storage site into the cytoplasm. The mobilisation of platelet intracellular calcium accumulating property of these membranes and some internally generated ionophores involved in its release could constitute a dynamic system central to the regulation of platelet shape change, pseudopod formation, secretion processes and other motile events known to be triggered by the haemostatic agents acting at the plasma membranes (Menashi *et al.*, 1982). The influx of calcium during platelet stimulus secretion is mediated through the intracellular calcium ionophores, which disrupt the intracellular ion gradient and thus trigger secretion (Feinman and Detwiler, 1974).

Human blood platelets release major part of their calcium when incubated with inducers which release of adenine nucleotides. The release is dependent on active energy metabolism and is inhibited when synthesis or levels of ATP in the platelet's energetically active pool is reduced by addition of inhibitors like aspirin or actomycin. The released calcium may possibly participate in clotting process by raising the ionic calcium concentration in the platelet surface above the surrounding plasma. Also calcium may be a relic of an old coagulation system where the coagulation factors when stored inside formed elements of blood like amoebocytes of the horseshoe crab. Calcium may be stored in platelets along with adenine nucleotides and serotonin (Murer and Holme, 1970).

A relaxing factor in skeletal muscles is a Ca^{+2}, Mg^{+2}, ion dependent ATPase bound to the microsomes, which pumps Ca^{+2} from the surrounding media into vesicular components. This relaxing factor may be the ecto-ATPase of the intact discoid platelets which helps as calcium extrusion pump and that the membranes of the surface and canalicular system functions as sarcoplasmic reticulum (Statland *et al.*, 1969). The (Ca^{+2} + Mg^{+2}) – ATPase activity and the Ca^{+2} uptake is probably to a large extent affected by membranes from dense tubular system, an intracellular compartment, which resembles the sarcoplasmic reticulum of muscle (De Metz, et al., 1984).

Calcium uptake is stimulated in platelet membranes by cyclic AMP and protein kinase. An increase in the intracellular cyclic AMP by stimulation of adenylate cyclase or by inhibition of phosphodiesterases will

also interfere with platelet activity. Cytoplasmic cyclic AMP level is related to platelet activity through contractile activity, which is required for clot retraction, for the spontaneous contraction of platelet aggregates, and most likely for shape change, is calcium dependent. In the presence of cyclic AMP and protein kinase, a significant increase in calcium uptake by membranes vesicles is observed (Kaser-Glanzmann, *et al.*, 1977).

An increase in the cyclic AMP levels will lead to inhibition of platelet activity as some of the most powerful known inhibitors of platelet activity like prostaglandins PG E_1 and PG I_1 (prostacyclin), are potent inhibitors of adenylate cyclase activity (Kaser-Glanzmann, *et al.*, 1978).

Subcellular location of calcium uptake in platelets is ATP dependent. Also concomitant inorganic phosphate release is associated with ATPase activity and calcium transport (Javors *et al.*, 1982).

Calcium transport is an important event in the process of platelet aggregation. This activity is controlled by the membrane bound enzyme $Ca^{+2}Mg^{+2}$ATPase. Hence, it was of interest to study the kinetics of this enzyme in the presence and absence of the spices extracts on platelet membranes.

Table 49 - $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

ATP Concentration (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
1.25	43.7	29.5	14.2	32.5	3.3
0.83	57.5	34.8	22.7	39.5	
0.63	67.3	39.4	27.9	41.5	
0.50	74.7	42.1	32.6	43.6	
0.42	86.4	50.3	36.1	42.8	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase activity.

$Ca^{+2}Mg^{+2}$ATPase assayed in human platelet membranes had a K_m of 3.3 mM. This was observed in the absence of any of the inhibitors. The same has been reported at three different concentration of the each extract of the spices to determine their respective K_m values.

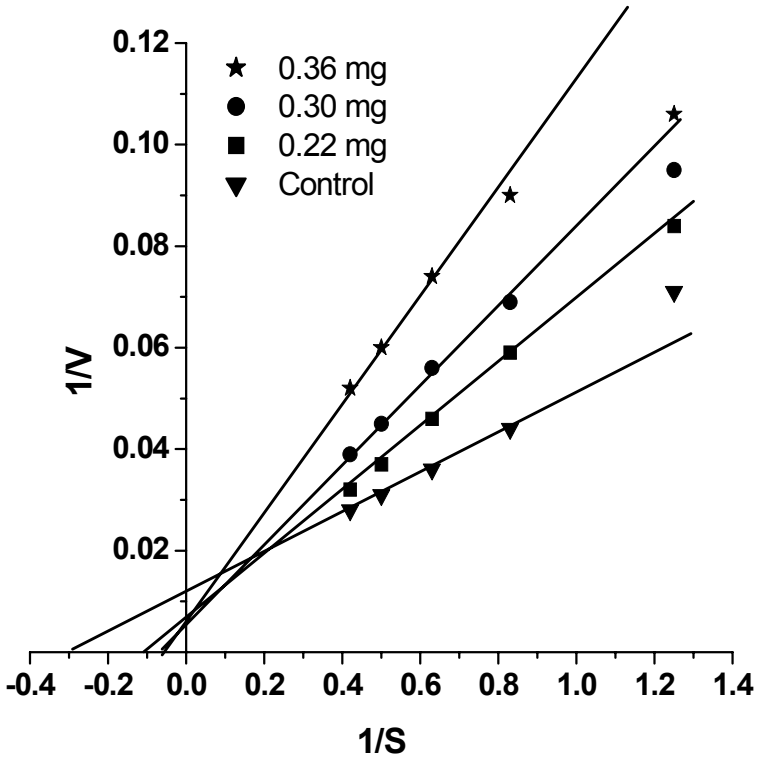


Figure 25 - Effect of coriander (raw) extract on $Ca^{+2}Mg^{+2}$ATPase Kinetics human platelet membranes

Concentrations of 0.22, 0.29 and 0.36 mg of raw coriander extract were used and the K_m of 4.6, 5.9 and 6.7 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. The enzyme activity was inhibited competitively as shown in figure 25.

Table 50 - Effect of boiled coriander extract on $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
0.06	1.25	44.7	30.6	14.1	31.5	4.2
″	0.83	60.3	40.1	19.6	32.5	
″	0.63	71.7	47.9	23.8	33.2	
″	0.50	85.8	57.2	28.6	33.3	
″	0.42	90.1	57.8	32.3	35.9	
0.12	1.25	63.9	50.6	10.9	17.1	5.3
″	0.83	80.4	64.3	16.1	20.0	
″	0.63	88.3	68.7	19.6	22.2	
″	0.50	91.7	67.9	23.8	26.0	
″	0.42	96.6	69.6	27.0	27.9	
0.18	1.25	80.2	70.9	9.3	11.6	6.7
″	0.83	85.4	71.5	13.9	16.3	
″	0.63	89.3	72.1	17.2	19.3	
″	0.50	93.9	73.1	20.8	22.2	
″	0.42	102.8	78.4	24.4	23.7	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase activity.

Concentrations of 0.06, 0.12 and 0.18 mg of boiled coriander extract were used and the K_m of 4.2, 5.3 and 6.7 mM respectively were obtained.

Table 51 - Effect of roasted coriander extract on $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
0.12	1.25	53.0	43.0	10.0	18.9	5.6
″	0.83	58.1	42.5	15.6	26.9	
″	0.63	72.1	52.5	19.6	27.2	
″	0.50	76.9	53.6	23.3	30.3	
″	0.42	80.4	54.7	26.3	32.7	
0.24	1.25	72.0	63.7	8.3	11.5	6.7
″	0.83	73.4	60.2	13.2	18.0	
″	0.63	79.0	62.6	16.4	20.8	
″	0.50	83.8	64.2	19.6	23.4	
″	0.42	89.9	67.6	22.3	24.8	
0.36	1.25	72.8	65.8	7.0	9.6	8.3
″	0.83	78.6	67.5	11.1	14.1	
″	0.63	86.4	74.5	11.9	13.8	
″	0.50	89.5	72.5	17.0	19.0	
″	0.42	96.2	76.6	19.6	20.4	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase activity.

Concentrations of 0.12, 0.24 and 0.36 mg of roasted coriander extract were used and the K_m of 5.6, 6.7 and 8.3 mM respectively were obtained.

Of these three extracts, boiled extract of coriander seems to have a significant effect on $Ca^{+2}Mg^{+2}$ATPase, as very low concentrations of this extract were required.

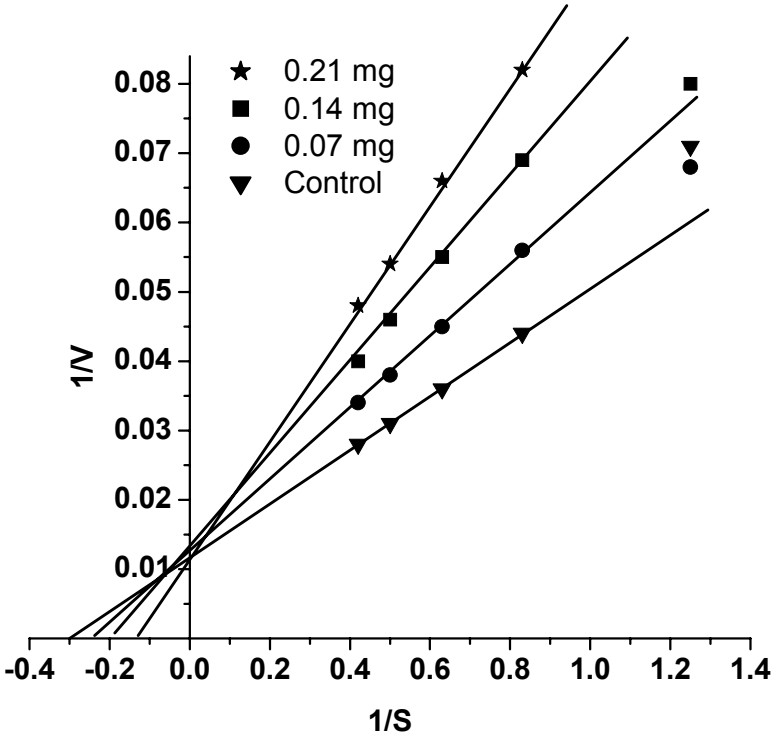


Figure 26 - Effect of cumin (raw) extract on $Ca^{+2}Mg^{+2}$ATPase Kinetics on human platelet membranes

Concentrations of 0.08, 0.16 and 0.24 mg of raw cumin extract were used and the K_m of 4.6, 5.9 and 6.7 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. The extract inhibited the activity competitively as shown in figure 26.

Table 52 - Effect of boiled cumin extract on $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
0.24	1.25	83.5	71.0	12.5	15.0	5.0
″	0.83	91.7	74.8	16.9	18.4	
″	0.63	96.5	75.7	20.8	21.6	
″	0.50	101.7	76.7	25.0	24.6	
″	0.42	104.3	76.1	28.2	27.1	
0.32	1.25	88.9	78	10.9	12.3	5.6
″	0.83	96.4	85.5	15.4	16.0	
″	0.63	98.1	78.9	19.2	19.6	
″	0.50	107.8	85.1	22.7	21.1	
″	0.42	109.9	84.3	25.6	23.3	
0.40	1.25	94.8	93.7	10.1	10.7	5.9
″	0.83	99.6	85.1	14.5	14.6	
″	0.63	105.9	87.9	18.0	17.0	
″	0.50	112.8	91.14	21.7	19.2	
″	0.42	113.3	88.9	24.4	21.5	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase activity.

Concentrations of 0.24, 0.32 and 0.40 mg of boiled cumin extract were used and the K_m of 5.0, 5.6 and 5.9 mM respectively were obtained.

Table 53 - Effect of roasted cumin extract on $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
0.16	1.25	54.6	41.3	13.3	24.4	3.9
″	0.83	58.4	38.4	20.0	34.3	
″	0.63	64.9	39.9	25.0	38.5	
″	0.50	72.6	43.2	29.4	40.5	
″	0.42	75.3	43.1	32.2	42.8	
0.24	1.25	57.3	45.1	12.2	21.3	4.7
″	0.83	63.6	46.3	17.3	27.2	
″	0.63	72.9	51.4	21.5	29.5	
″	0.50	81.5	55.9	25.6	31.4	
″	0.42	87.2	58.6	28.6	32.8	
0.32	1.25	63.6	52.4	11.2	17.6	5.9
″	0.83	72.1	57.2	14.9	20.7	
″	0.63	79.0	60.4	18.5	23.4	
″	0.50	88.0	65.8	22.2	25.2	
″	0.42	92.4	67.4	25.0	27.1	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase activity.

Concentrations of 0.16, 0.24 and 0.32 mg of roasted cumin extract were used and the K_m of 3.9, 4.7 and 5.9 mM respectively were obtained.

Of these three extracts, raw extract of cumin seems to have a significant effect on $Ca^{+2}Mg^{+2}$ATPase, as very low concentrations of this extract were required.

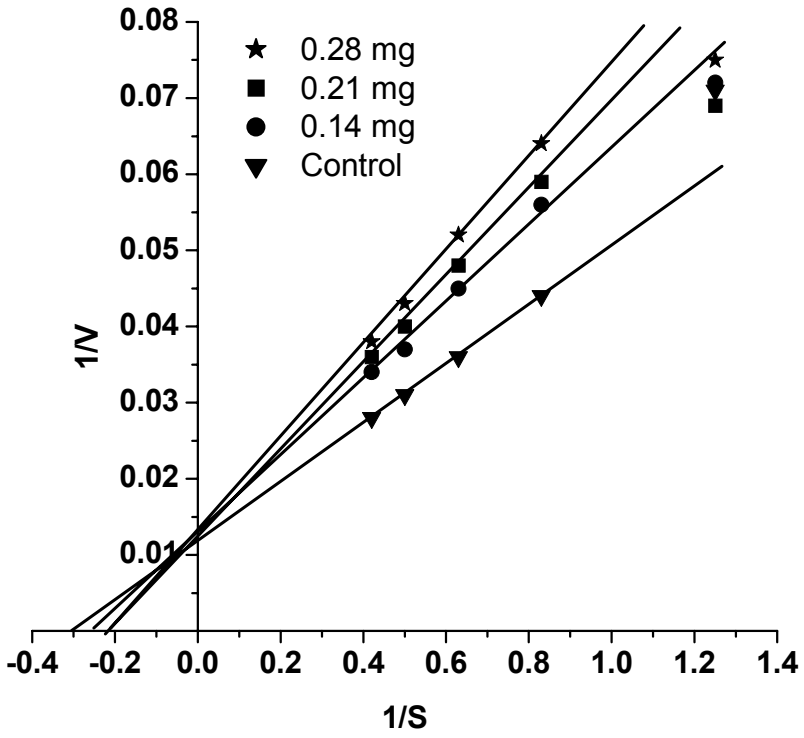


Figure 27 - Effect of coriander and cumin mix (raw) extract on $Ca^{+2}Mg^{+2}$ATPase Kinetics on human platelet membranes

Concentrations of 0.14, 0.21 and 0.28 mg of raw coriander and cumin mix extract were used and the K_m of 4.8, 5.0 and 5.6 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. The kinetics showed a competitive type of inhibition of the activity.

Table 54 - Effect of boiled coriander and cumin mix extract on $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
0.14	1.25	40.6	28.7	11.9	29.3	3.9
″	0.83	51.1	30.7	20.4	39.9	
″	0.63	56.9	31.9	25.0	43.9	
″	0.50	61.5	39.4	29.4	47.8	
″	0.42	71.3	39.9	32.3	45.3	
0.21	1.25	51.8	40.7	11.1	21.4	4.0
″	0.83	59.0	39.4	19.6	33.2	
″	0.63	72.7	48.9	23.8	32.7	
″	0.50	81.7	53.4	27.8	34.0	
″	0.42	99.7	68.4	31.3	31.4	
0.28	1.25	50.4	40.6	9.8	19.4	4.4
″	0.83	62.1	42.2	17.9	28.8	
″	0.63	64.4	42.2	22.2	34.5	
″	0.50	68.4	42.8	25.6	37.4	
″	0.42	72.8	44.2	28.6	39.3	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase activity.

Concentrations of 0.14, 0.21 and 0.28 mg of boiled coriander and cumin mix extract were used and the K_m of 3.9, 4.0 and 4.4 mM respectively were obtained.

Table 55 - Effect of roasted coriander and cumin mix extract on $Ca^{+2}Mg^{+2}$ATPase activity of human platelet membranes at different ATP concentrations

Amount -extract (mg)	ATP Concentra-tion (mM)	Total activity	EGTA insensitive activity	EGTA sensitive activity #	EGTA sensitive component	K_m
0.14	1.25	72.4	59.1	13.3	18.4	4.6
″	0.83	80.5	63.3	17.2	21.4	
″	0.63	86.1	64.8	21.3	24.7	
″	0.50	93.3	68.3	25.0	26.8	
″	0.42	97.4	68.8	28.6	29.3	
0.21	1.25	80.1	67.7	12.4	15.5	5.0
″	0.83	83.6	68.1	15.2	18.2	
″	0.63	91.9	62.7	19.2	20.9	
″	0.50	98.6	76.1	22.7	23.0	
″	0.42	105.0	79.4	25.6	24.4	
0.28	1.25	88.2	76.6	11.6	13.2	5.3
″	0.83	91.7	77.4	14.3	15.6	
″	0.63	97.2	79.1	18.1	18.6	
″	0.50	103.8	82.5	21.3	20.5	
″	0.42	109.5	85.1	24.4	22.3	

Values are expressed as nmoles of pi liberated /mg protein / hr.
Values are mean of triplicates.
$Ca^{+2}Mg^{+2}$ATPase.

Concentrations of 0.14, 0.21 and 0.28 mg of roasted coriander and cumin mix extract were used and the K_m of 4.6, 5.0 and 5.3 mM respectively were obtained.

Of these three extracts, raw extract of coriander and cumin mix seems to have a significant effect on $Ca^{+2}Mg^{+2}$ATPase, as very low concentrations of this extract were required.

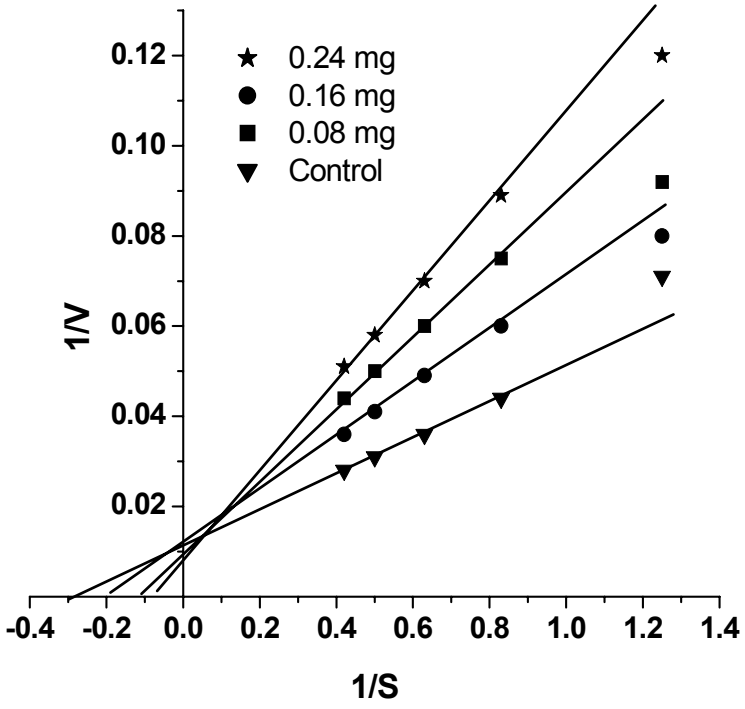


Figure 28 - Effect of processed coriander dhal extract on $Ca^{+2}Mg^{+2}$ATPase Kinetics on human platelet membranes

Concentrations of 0.08, 0.16 and 0.24 mg of processed coriander dhal extract were used and the K_m of 2.5, 3.3 and 3.6 mM respectively were obtained. In the presence of this extract the K_m value increased as the concentration was increased. Results reveal a competitive type of inhibition of activity by this extract.

Table 56 – K_I values for inhibition of Na^+K^+ATPase and $Ca^{+2}Mg^{+2}$ATPase in human platelet membranes treated with raw and processed spice extracts

Extract	Na^+K^+ATPase	$Ca^{+2}Mg^{+2}$ATPase
Coriander (raw)	0.132	0.312
Coriander (boiled)	0.144	0.240
Coriander (roasted)	0.078	0.240
Cumin (raw)	0.104	0.240
Cumin (boiled)	0.472	0.592
Cumin (roasted)	0.272	0.264
Coriander + Cumin mix (raw)	0.245	0.392
Coriander + Cumin mix (boiled)	0.294	0.518
Coriander + Cumin mix (roasted)	0.392	0.434
Processed coriander dhal	0.102	0.240

Values expresses in mg.
Values are mean of triplicates.

K_I values for the spice extracts computed from the results obtained till now for Na^+K^+ATPase and $Ca^{+2}Mg^{+2}$ATPase are given in table 56. It may be seen that of all the extracts inhibiting Na^+K^+ATPase, roasted cumin extract showed the best inhibition of 0.078 mg where as for $Ca^{+2}Mg^{+2}$ATPase four of the extract have the same K_I value of 0.24 mg. Among these spice extracts, the Na^+K^+ATPase activity is more sensitive to coriander than to cumin. $Ca^{+2}Mg^{+2}$ATPase activity did not show much difference in sensitivity for these extracts overall.

In tables 57 and 58, Na^+K^+ATPase and $Ca^{+2}Mg^{+2}$ATPase activity were determined in the platelet membranes which were prepared after incubating the Platelet Rich Plasma (PRP) at IC_{50} of the respective spice extracts followed by aggregation induced by ADP, epinephrine, collagen or A 23187 separately.

After aggregation the platelet membranes isolated were used to determine the percent inhibition of the ATPase activities in them. This study helped to determine the role of Na^+ and Ca^{+2} in platelet aggregation in the presence of the spice extracts and the respective agonists.

Table 57 – Na^+K^+ATPase activity in agonist challenged platelet membranes with spice extracts at IC_{50}

Extract	Total Phosphatase Activity	Ouabain Insensitive Activity	Ouabain Sensitive Activity *	Ouabain Sensitive Component	Inhibition (%)
ADP only	71.67	63.67	8.0	11.2	0.0
Epinephrine only	69.66	64.85	4.81	6.9	0.0
Collagen only	78.40	69.55	8.85	11.3	0.0
A 23187 only	66.56	59.19	7.37	11.1	0.0
Coriander (raw) extract:					
+ ADP	69.12	67.68	1.44	2.1	82.0
+ epinephrine	66.51	65.49	1.02	1.5	78.8
+ collagen	77.24	73.72	3.52	4.6	60.2
+ A 23187	63.52	61.59	1.93	3.0	73.8
Coriander (boiled) extract:					
+ ADP	67.79	65.76	2.03	3.0	74.6
+ epinephrine	64.37	62.5	1.87	2.9	61.2
+ collagen	75.43	72.65	3.68	2.6	68.6
+ A 23187	62.82	60.95	1.87	3.0	59.3
Coriander (roasted) extract:					
+ ADP	68.16	66.13	2.03	2.8	74.6
+ epinephrine	64.74	62.61	2.13	3.3	55.7
+ collagen	75.53	72.86	2.67	3.5	69.8
+ A 23187	62.61	60.47	2.14	3.4	71.0
Cumin (raw) extract:					
+ ADP	70.09	68.96	1.19	1.6	85.1
+ epinephrine	66.39	65.39	1.0	1.5	79.2
+ collagen	74.73	72.28	2.45	3.3	72.4
+ A 23187	64.32	62.23	2.04	3.3	55.2
Cumin (boiled) extract:					
+ ADP	69.23	67.73	1.50	2.2	81.3
+ epinephrine	66.13	64.96	1.17	1.8	75.7
+ collagen	74.04	71.58	2.46	3.3	72.2
+ A 23187	63.68	61.22	2.46	3.9	66.6

Cumin (roasted) extract:					
+ ADP	68.61	66.72	1.89	2.8	76.4
+ epinephrine	65.71	64.64	1.07	1.6	77.8
+ collagen	73.72	71.05	2.67	3.6	69.8
+ A 23187	63.09	60.90	2.19	3.5	70.3
Coriander + cumin (raw) mix extract:					
+ ADP	73.40	70.39	3.01	4.1	62.4
+ epinephrine	65.42	64.0	1.42	2.2	70.5
+ collagen	75.0	73.72	1.28	1.7	85.5
+ A 23187	63.68	62.55	1.13	1.8	84.7
Coriander + cumin (boiled) mix extract:					
+ ADP	70.09	66.72	3.37	4.8	57.8
+ epinephrine	68.70	66.35	2.35	3.4	51.1
+ collagen	77.35	72.86	4.49	5.8	49.3
+ A 23187	64.42	60.95	3.47	5.4	52.9
Coriander + cumin (roasted) mix extract:					
+ ADP	72.89	69.93	2.96	4.1	63.0
+ epinephrine	67.46	65.86	1.60	2.4	66.7
+ collagen	76.73	74.81	1.92	2.50	78.3
+ A 23187	61.75	59.64	2.10	3.40	71.5
Processed coriander dhal extract:					
+ ADP	71.58	69.39	2.19	3.1	72.6
+ epinephrine	66.02	64.11	1.19	2.9	60.3
+ collagen	74.89	72.65	2.24	3.0	74.7
+ A 23187	64.86	63.14	1.72	2.7	80.6
α-Linalool:					
+ ADP	71.69	67.52	4.17	5.8	27.5
+ epinephrine	69.33	65.91	3.42	4.9	28.9
+ collagen	76.82	73.67	3.15	4.1	53.7
+ A 23187	66.56	61.91	4.65	6.99	36.9
α-Terpinene:					
+ ADP	70.9	66.56	4.32	6.1	46.0
+ epinephrine	66.42	63.18	3.26	4.9	32.2
+ collagen	74.39	70.18	4.21	5.7	52.4
+ A 23187	68.12	63.52	4.60	6.7	37.6
Unchallenged platelet membranes	74.03	66.89	7.14	9.7	0.0

Values are expressed as μmoles of pi liberated/mg protein/hr.
* Na^+K^+ATPase released from agonist challenged platelet membranes.

Table 58 – $Ca^{2+}Mg^{2+}$ATPase activity in agonist challenged platelet membranes with spice extracts at IC_{50}

Extract	Total Phosphatase Activity	EDTA Insensitive Activity	EDTA Sensitive Activity #	EDTA Sensitive Component	Inhibition (%)
ADP only	74.40	58.30	16.1	21.6	0.0
Epinephrine only	69.23	50.41	18.82	27.2	0.0
Collagen only	78.02	61.03	16.99	21.8	0.0
A 23187 only	73.18	60.88	12.3	16.8	0.0
Coriander (raw) extract:					
+ ADP	74.85	71.36	3.49	4.7	78.3
+ epinephrine	65.39	63.97	1.42	2.2	92.5
+ collagen	70.60	69.84	0.76	0.01	95.5
+ A 23187	71.26	68.37	2.89	0.04	76.5
Coriander (boiled) extract:					
+ ADP	77.82	73.62	4.20	5.4	76.8
+ epinephrine	68.12	66.44	2.47	3.6	86.9
+ collagen	72.44	70.56	1.88	2.6	88.9
+ A 23187	76.26	72.34	3.92	5.1	68.1
Coriander (roasted) extract:					
+ ADP	76.12	71.5	4.62	6.1	74.5
+ epinephrine	66.28	63.62	2.66	4.0	85.9
+ collagen	71.0	69.06	1.94	2.7	88.6
+ A 23187	74.28	70.24	4.04	5.4	67.2
Cumin (raw) extract:					
+ ADP	74.54	74.39	0.15	0.002	99.1
+ epinephrine	65.74	64.47	1.27	0.02	93.3
+ collagen	68.62	67.26	1.36	0.02	92.0
+ A 23187	71.56	69.13	2.43	3.4	80.2
Cumin (boiled) extract:					
+ ADP	72.18	69.72	2.46	3.4	86.4
+ epinephrine	65.10	63.28	1.82	3.8	90.3
+ collagen	73.29	70.25	3.04	4.2	82.1
+ A 23187	76.14	71.62	4.52	5.9	63.3
Cumin (roasted) extract:					
+ ADP	74.48	72.70	1.78	2.4	90.2
+ epinephrine	67.22	65.68	1.54	2.3	91.8
+ collagen	75.50	72.84	2.66	3.5	84.3
+ A 23187	73.0	69.23	3.77	5.2	69.4
Coriander + cumin (raw) mix extract:					
+ ADP	73.86	71.54	2.32	3.1	87.2
+ epinephrine	66.42	65.22	1.20	1.8	93.6

+ collagen	72.48	71.24	1.24	1.7	92.7
+ A 23187	70.09	67.04	3.05	4.4	75.2
Coriander + cumin (boiled) mix extract:					
+ ADP	75.10	71.33	3.77	5.0	79.2
+ epinephrine	67.92	65.74	2.18	3.2	88.4
+ collagen	74.27	69.63	2.64	3.7	84.5
+ A 23187	75.35	71.27	4.08	5.4	66.8
Coriander + cumin (roasted) mix extract:					
+ ADP	73.22	70.52	2.70	3.7	85.1
+ epinephrine	65.86	63.60	2.26	3.4	88.0
+ collagen	73.43	70.55	2.88	3.9	83.1
+ A 23187	72.07	68.07	4.0	5.6	67.5
Processed coriander dhal extract:					
+ ADP	74.36	69.13	5.23	7.0	71.1
+ epinephrine	65.56	60.63	4.83	7.4	74.3
+ collagen	71.82	66.62	5.20	7.2	69.4
+ A 23187	73.11	67.13	5.98	8.2	51.4
α-Linalool:					
+ ADP	73.56	59.74	13.82	18.8	23.7
+ epinephrine	68.68	54.33	14.05	20.6	25.4
+ collagen	77.84	67.40	10.44	13.4	38.6
+ A 23187	74.07	64.08	9.99	13.5	18.8
α-Terpinene:					
+ ADP	74.0	60.88	13.12	18.1	27.5
+ epinephrine	69.62	55.37	14.25	18.8	24.3
+ collagen	77.22	67.01	10.21	17.0	39.9
+ A 23187	73.82	63.74	10.08	12.3	18.1
Unchallenged platelet membranes	89.92	68.12	21.80	24.2	0.0

Values are expressed as nmoles of pi liberated/mg protein/hr.
$Ca^{+2}Mg^{+2}$ATPase released from agonist challenged platelet membranes.

Discussion:

A variety of cations including Na^{+}, K^{+}, Ca^{+2} and Mg^{+2} play an important role in platelet aggregation. When there was a decrease in the activity of adenylate cyclase, Na^{+} has significantly decreased the receptor affinity to agonists. Also the absence of Na^{+} inhibited the human platelet aggregation mainly due to the non-mobilisation of arachidonic acid from

platelet membranes. It was observed that Na^+ favors the formation of phosphorylated enzymes where K^+ favors its dephosphorylation. The inhibitor Ouabain binds to the phosphorylated intermediate and inhibits the dephosphorylation. As these spice extracts inhibited the Na^+K^+ATPase activity competitively it is possible that unavailability of Na^+ ions may result in the agonists unable to induce the platelet aggregation. Also these spice extracts may tend to prevent either the phosphorylation or dephosphorylation of the enzymes required for platelet aggregation.

On the other hand, calcium ions play an important role in the cascade of events of platelet aggregation. It helps in the regulation of platelet shape change, pseudopod formation, secretion processes and other motile events, which are triggered by the haemostatic agents. Hence it may be said that mobilisation of Ca^{+2} ions across the platelet membranes is being inhibited by the extracts of coriander and cumin (raw and processed). This resulted in decreased platelet aggregation as sequence of events, which require Ca^{+2} ions were being hindered.

In conclusion it may be said that, these extracts possess component(s), which are potent enough to inhibit the availability of Na^+ and Ca^{+2} ions across the platelet membranes. These inhibitors tend to obstruct these two activities in a competitive manner varying the K_I values indicative of their potency to inhibit. Further due to their inhibition of the Na^+ and Ca^{+2} requirement in the platelets resulted in the reduced rate of aggregation.

4. SEPHADEX FRACTIONATION OF CUMIN AND CORIANDER EXTRACTS:

The aqueous extracts of cumin and coriander showed inhibitory activity on human platelet aggregation. These extracts may contain component(s), which are water soluble as well as water insoluble are having the potency to inhibit platelet aggregation. Hence attempts were made to fractionate these spice extracts separately.

Cumin and coriander extracts were lyophilised completely and 100 mg this freeze-dried sample was added to 1 ml water and loaded on the sephadex columns. 1 mg of Sephadex G-25 material swelled to 2.5 ml and G-50 material swelled to 5 ml volume in water. The length of column used was 120 cm with an inner diameter of 1.5 cm. The flow rate was 1 ml/min.

Cumin:

Cumin extract was lyophilised and 100 mg was dissolved in 1 ml water and loaded on to a sephadex G – 50 column. The elutant used was water. The purification profile was shown in figure 29.

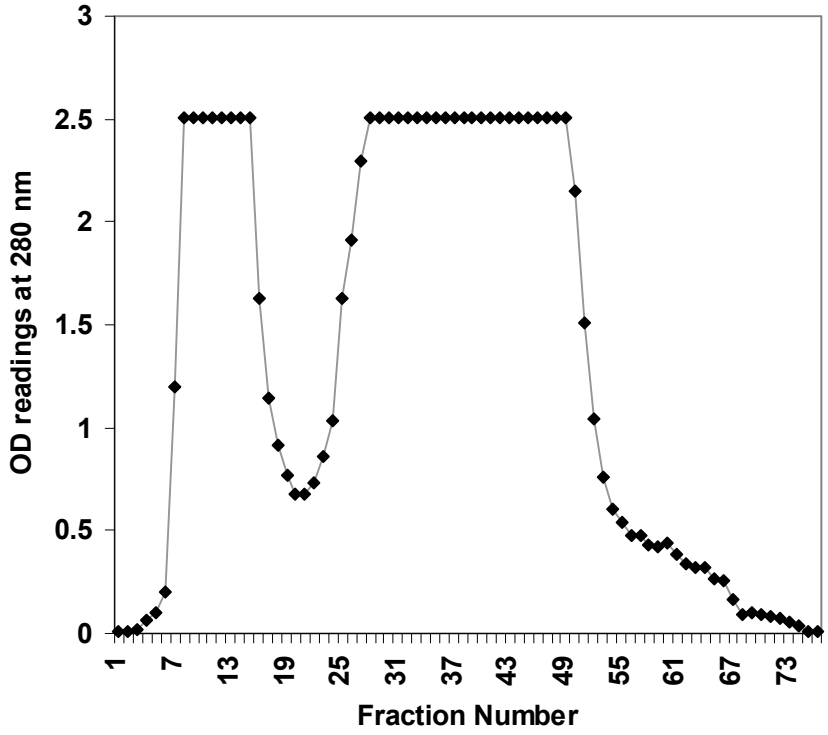


Figure 29 – Fractionation of cumin extract on Sephadex G – 50 column

The two peaks '1' and '2' obtained were again separately lyophilised and from that 16 mg / ml was taken and used on human platelets in the presence of agonists like ADP, epinephrine and collagen and the results are as shown in table 59.

Table 59 - Effect of fractions '1' and '2' of cumin on human platelet aggregation with sephadex G - 50

Extract	Amount (mg)	Slope	Inhibition (%)
ADP	0.0	4.88 ± 0.16	0.0
Epinephrine	0.0	1.40 ± 1.17	0.0
Collagen	0.0	5.60 ± 0.18	0.0
Fraction '1':			
+ ADP	0.48	4.16 ± 0.08	11.8 ± 2.0
″	0.60	3.76 ± 0.09	23.0 ± 2.4
″	0.72	3.51 ± 0.06	28.1 ± 1.8
+ Epinephrine	0.48	1.17 ± 0.03	16.2 ± 2.4
″	0.60	1.09 ± 0.02	22.4 ± 2.0
″	0.72	1.04 ± 0.02	25.6 ± 1.9
+ Collagen	0.48	4.71 ± 0.10	15.9 ± 2.2
″	0.60	4.52 ± 0.04	19.2 ± 0.8
″	0.72	4.40 ± 0.07	21.4 ± 1.5
Fraction '2':			
+ ADP	0.48	4.22 ± 0.05	13.4 ± 1.2
″	0.60	3.92 ± 0.07	19.7 ± 1.9
″	0.72	3.74 ± 0.02	23.4 ± 0.6
+ Epinephrine	0.48	1.24 ± 0.01	11.4 ± 1.4
″	0.60	1.16 ± 0.02	17.1 ± 2.1
″	0.72	1.11 ± 0.02	20.6 ± 1.5
+ Collagen	0.48	5.22 ± 0.07	6.8 ± 1.3
″	0.60	4.97 ± 0.03	11.3 ± 0.7
″	0.72	4.82 ± 0.04	14.0 ± 0.9

Values are mean ± S.D of triplicates.

As fractionation on sephadex G-50 did not yield any active fractions, again lyophilised cumin sample of 100 mg was dissolved in 1 ml water and loaded on to a sephadex G – 25 column. The fractionation profile was shown in figure 30.

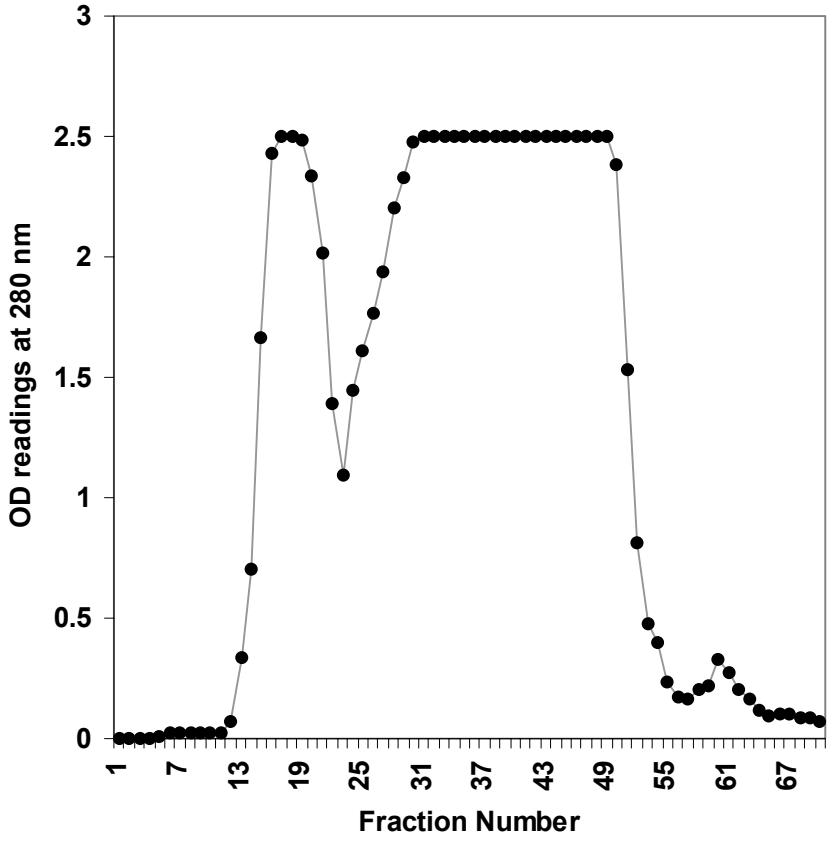


Figure 30 – Fractionation of cumin extract on sephadex G – 25 column

The three peaks ‘a’, ‘b’ and ‘c’ obtained were again separately lyophilised and from that 16 mg / ml water was taken and used on human platelets in the presence of agonists like ADP, epinephrine and collagen as shown in table 60.

Table 60 - Effect of fractions 'a', 'b' and 'c' of cumin on human platelet aggregation with sephadex G - 25

Extract	Amount (mg)	Slope	Inhibition (%)
ADP	0.0	4.88 ± 0.16	0.0
Epinephrine	0.0	1.40 ± 1.17	0.0
Collagen	0.0	5.60 ± 0.18	0.0
Fraction 'a':			
+ ADP	0.48	4.27 ± 0.07	12.5 ± 1.6
″	0.60	4.15 ± 0.08	15.0 ± 2.0
″	0.72	3.90 ± 0.07	20.0 ± 1.8
+ Epinephrine	0.48	1.14 ± 0.01	18.9 ± 1.2
″	0.60	1.10 ± 0.01	21.4 ± 0.8
″	0.72	1.06 ± 0.01	24.2 ± 0.9
+ Collagen	0.48	4.67 ± 0.10	16.7 ± 2.1
″	0.60	4.60 ± 0.07	17.8 ± 1.5
″	0.72	4.32 ± 0.04	22.8 ± 1.0
Fraction 'b':			
+ ADP	0.48	4.58 ± 0.06	6.1 ± 1.4
″	0.60	4.38 ± 0.05	10.2 ± 1.2
″	0.72	4.26 ± 0.07	12.6 ± 1.7
+ Epinephrine	0.48	1.28 ± 0.02	8.2 ± 1.3
″	0.60	1.23 ± 0.03	11.9 ± 1.9
″	0.72	1.20 ± 0.02	14.0 ± 1.5
+ Collagen	0.48	5.21 ± 0.11	7.2 ± 2.2
″	0.60	5.09 ± 0.10	9.1 ± 2.0
″	0.72	4.95 ± 0.11	11.6 ± 2.3
Fraction 'c':			
+ ADP	0.48	3.88 ± 0.04	20.6 ± 1.1
″	0.60	3.71 ± 0.05	23.9 ± 1.4
″	0.72	3.56 ± 0.06	27.1 ± 1.7
+ Epinephrine	0.48	1.19 ± 0.02	15.0 ± 2.0
″	0.60	1.14 ± 0.03	18.3 ± 2.2
″	0.72	1.10 ± 0.01	21.4 ± 1.2
+ Collagen	0.48	4.61 ± 0.07	17.7 ± 1.6
″	0.60	4.23 ± 0.06	24.5 ± 1.5
″	0.72	4.04 ± 0.07	28.0 ± 1.8

Values are mean ± S.D of triplicates.

As seen in tables 59 and 60, it may be observed at concentration of crude extract, the fractions of cumin on sephadex G - 50 and G - 25 columns no significant inhibitions were observed. It may be concluded that

the inhibition of human platelet aggregation observed with crude extract of cumin may be due the synergistic effect of all the components present in it and hence when fractionated no individual fraction has shown any significant inhibition.

This was the reason why the crude extract of cumin itself has been used on human platelets for it mode of action.

Coriander:

Coriander extract was lyophilised and from that 100 mg was taken and dissolved in 1 ml water and loaded on to a sephadex G – 50 column. The fractions were shown in figure 31.

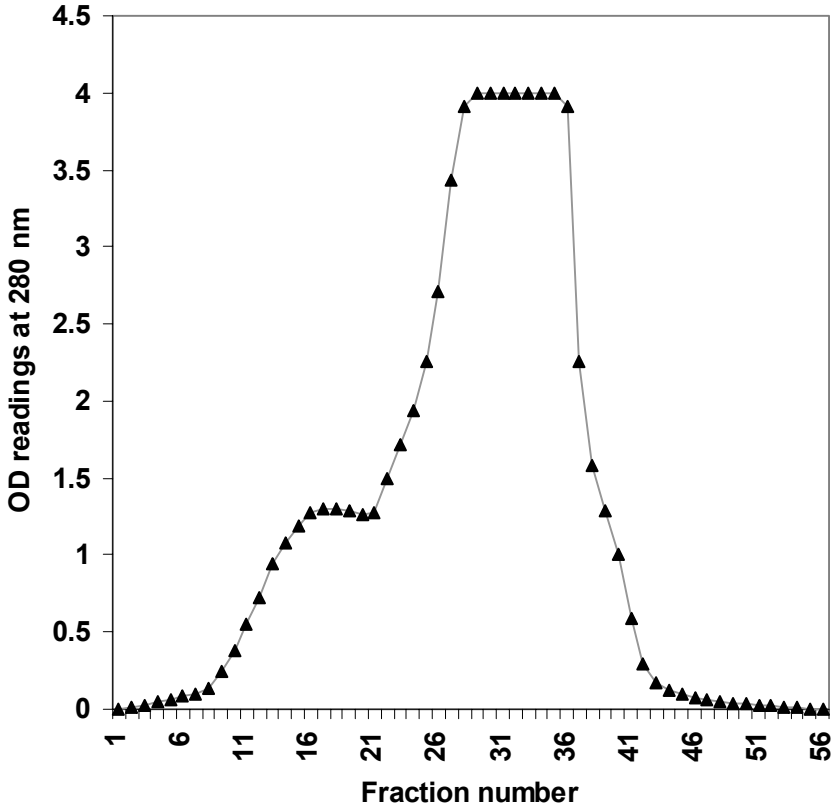


Figure 28 – Fractionation of coriander extract on sephadex G – 50 column

Lyophilised sample of coriander when fractionated on sephadex G – 50 column no separation was seen. Hence this was the reason why the crude extract of coriander itself has been used on human platelets.

5. EFFECT OF OTHER SPICES ON HUMAN PLATELETS:

Spices have been used from ancient times as a flavouring agent in various cuisines. But they possess component(s), which have antioxidant properties. Although coriander and cumin were taken up for extensive study, some of the other spices like cardamom, saffron, swallowroot, coriander leaf, curry leaf, cinnamon, cloves, garlic, ginger and turmeric wee initially screened for their inhibitory effect on human platelets. These studies were done to generate comparative data on the other commonly used spices apart from coriander and cumin.

(a) Cardamom:

Cardamom or *elachi* the 'Queen of spices' Elettaria *cardamomum* Maton (family Zingiberaceae) is valued through out the world as one of the essential ingredient in a variety of cuisine. It consists of dried fruits or pods in which the seeds are reddish brown to black and are highly aromatic (Uhl, 2000). It is commonly used for chewing as a mouth freshener and also as a constituent of various seasonings, sweet meats and bakery items (Pruthi, 1976). Ayurveda, the Indian system of medicine describes it as a carminative, diuretic, stomachic, digestive and cardiac stimulant (Tirtha, 1998).

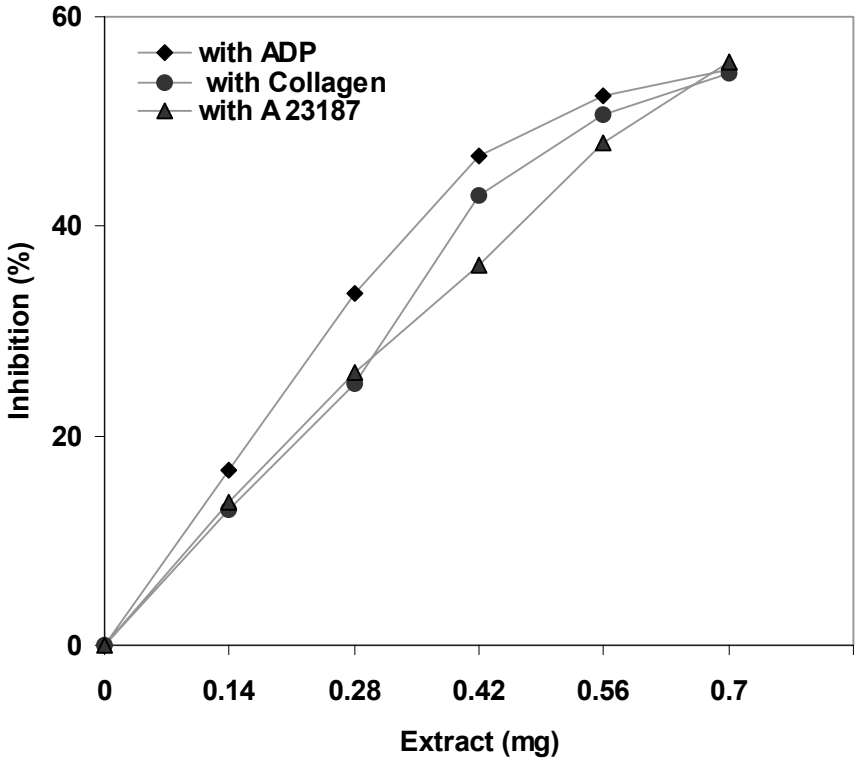


Figure 32 - Effect of cardamom extract on human platelet aggregation

Aqueous extract of cardamom was tested on human platelets using agonists like ADP, epinephrine, collagen and A 23187. IC_{50} were 0.49, 0.21, 0.55 and 0.59 mg with ADP, epinephrine, collagen and A 23187 respectively. A maximum inhibition between 55 to 88% was observed with these agonists. Ristocetin induced aggregation was not significant at 1.2 mg concentration and 30 min incubation.

(b) Saffron:

The exotic spice 'Saffron' or *kesar* Crocus *sativus* Linn. (family Iridaceae), which is one of the oldest and most expensive spice, bears importance to many cultures apart from being a popular colouring and flavouring agent in various cuisine (Uhl, 2000). It usually consists of dried stigma, from a bulbous perennial plant, a native of Southern Europe (Pruthi, 1976). As per the Indian system of medicine, Ayurveda, saffron acts as an antispasmodic, aphrodisiac, carminative and a stimulative agent (Tirtha, 1998). It helps in overcoming problems of reproductive system as well as the gastrointestinal and urinary tracts (Svoboda, 1992).

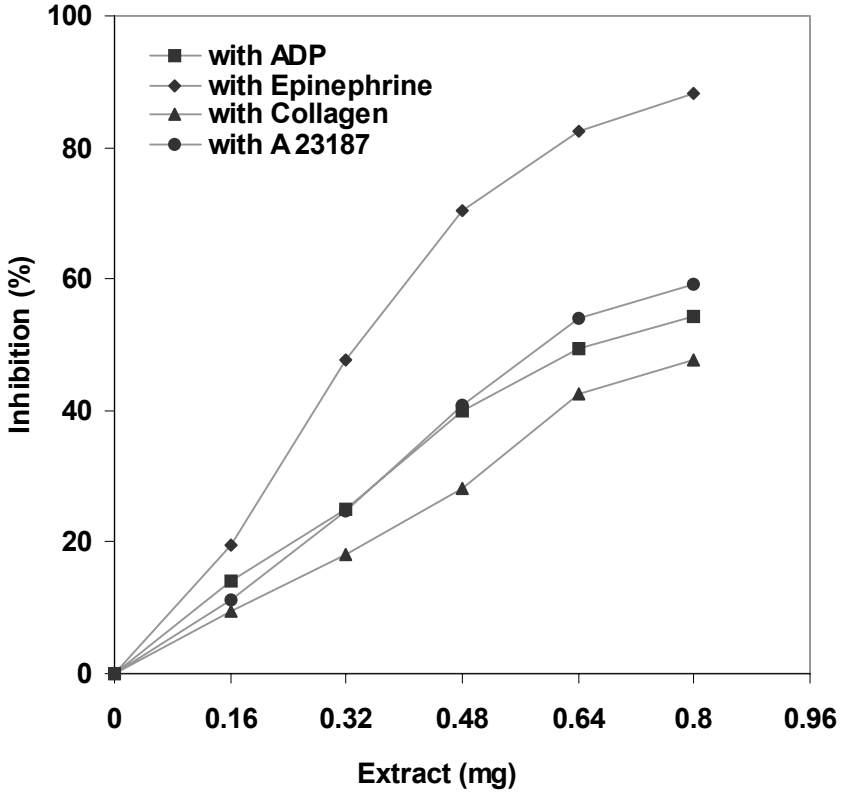


Figure 33 - Effect of saffron extract on human platelet aggregation

Saffron extract was used on human platelets with varying concentrations with agonists like ADP, epinephrine, collagen and A 23187. IC_{50} were 0.66, 0.35, 0.86 and 0.59 mg with ADP, epinephrine, collagen and A 23187 respectively. A maximum inhibition between 55 to 88% was observed with these agonists. Ristocetin induced aggregation was not significant at 1.5 mg concentration and 30 min incubation.

(c) Swallowroot:

The peculiar spice found only in peninsular India, (mostly in the western ghats of India), Swallowroot (Decalepis *hamiltonii*, Wight & Arn family Asclepiadaceae). It is considered to be an appetizer and blood purifier (Jacob, 1937). This root is considered as "Sariva Bheda' in ayurvedic preparations like Amritamalaka taila, Drakshadi churna and Shatavari rasayana to name a few. It also finds popular use in pickles (Nayar, *et al.*, 1978). It is found to possess bacteriostatic and pesticidal properties due to the presence of the volatile principle 4-O-methyl resorcylaldehyde, which is also responsible for its characteristic aroma (Anonymous wealth of India, 1952). Also it possesses component(s), which are inhibitors of osteoporosis (Hayashi, 1985). It is also used in the preparation of beverages and in pickles along with mango ginger in southern parts of India.

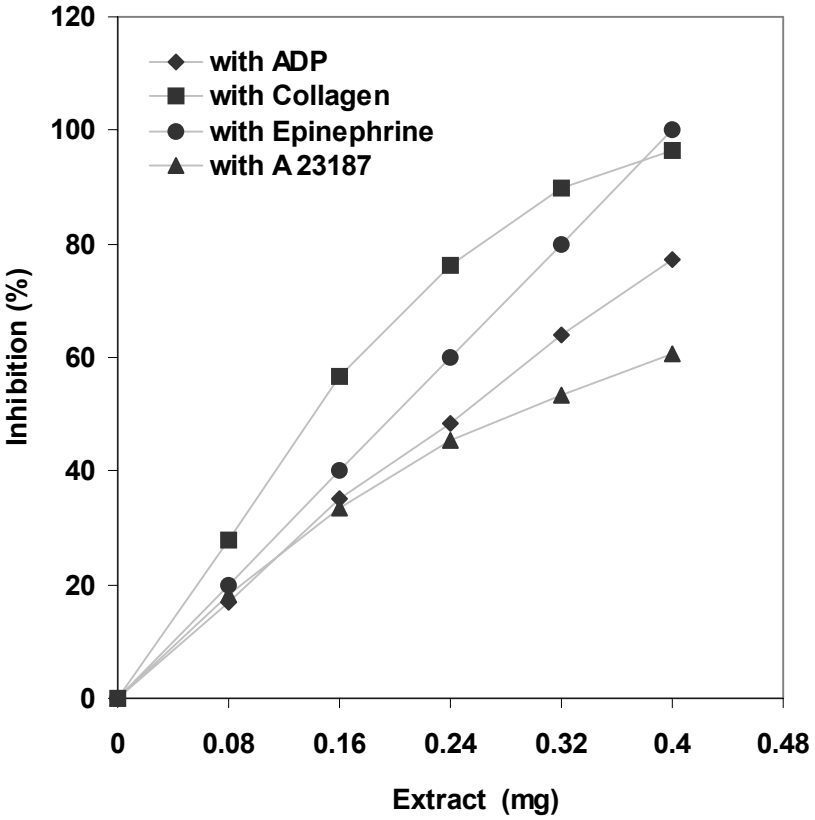


Figure 34 - Effect of swallowroot extract on human platelet aggregation

Swallowroot extract was used on human platelets with varying concentrations with agonists like ADP, epinephrine, collagen and A 23187. IC_{50} were 0.18, 0.20, 0.14 and 0.21 mg with ADP, epinephrine, collagen and A 23187 respectively. A maximum inhibition between 60 to 100% was observed with these agonists. Ristocetin induced aggregation showed no inhibition at concentrations of 0.8 mg and 30 min incubation.

(d) Leaf spices:

The most commonly used greens as spices are coriander and curry leaves through out India. They are added to foods to enhance their

acceptability, as they possess a distinct flavour, which is not available with other spices. Cost of procurement of these leaf spices is very low.

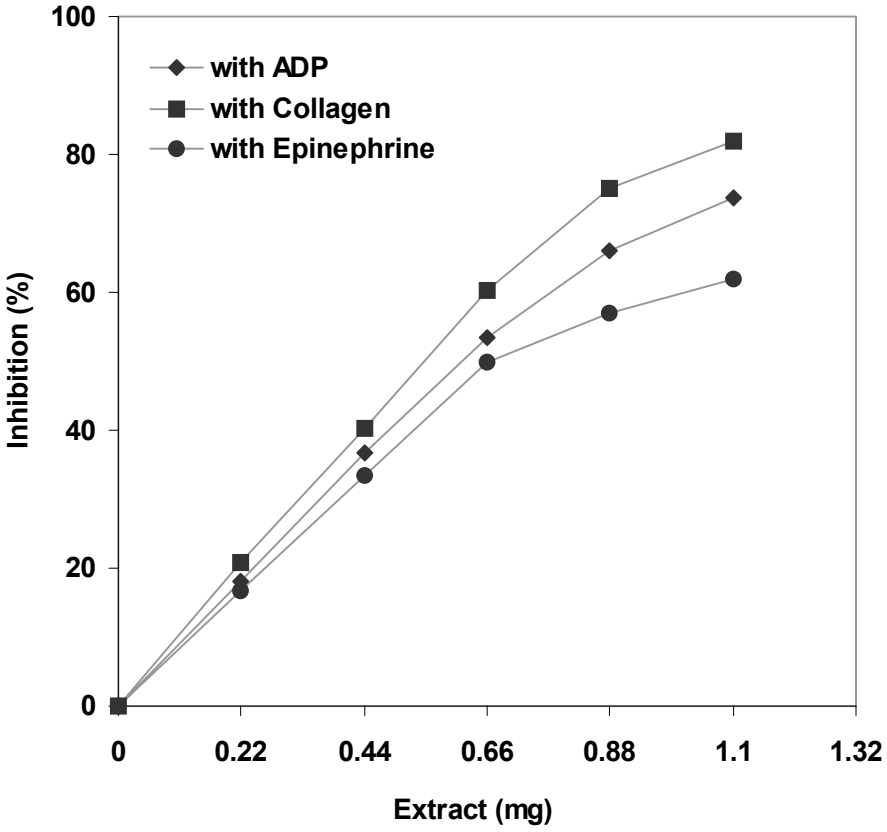


Figure 35 - Effect of coriander leaf extract on human platelet aggregation

Aqueous extract of coriander leaf inhibited human platelet aggregation with IC_{50} of 0.55, 0.66 and 0.57 mg for with ADP, epinephrine and collagen respectively. A maximum inhibition between 62 to 74% was observed with these agonists.

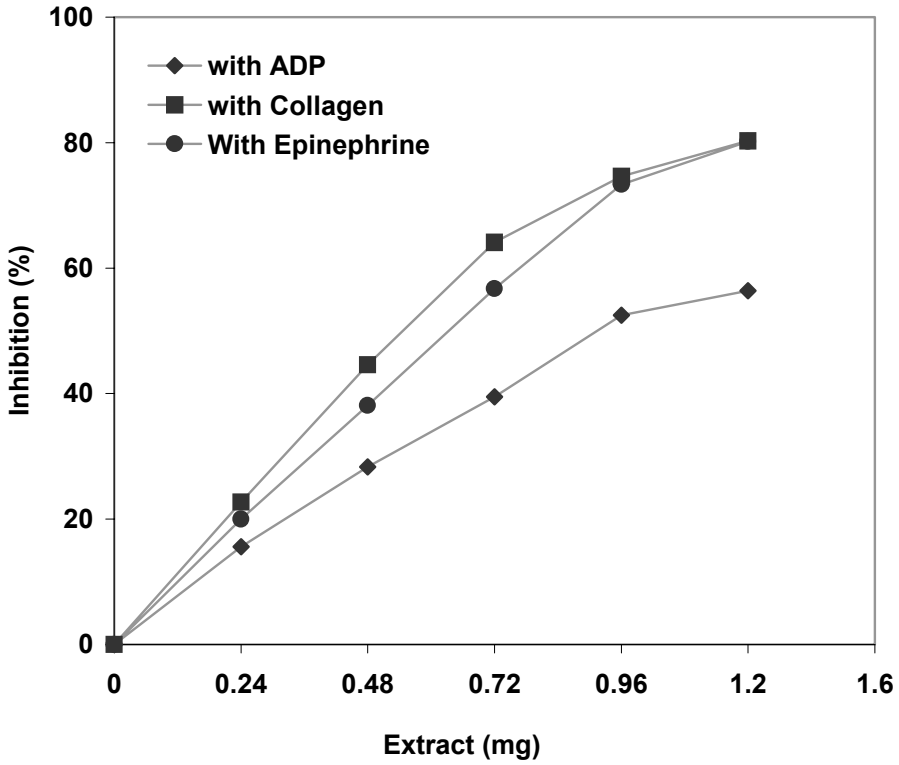


Figure 36 - Effect of curry leaf extract on human platelet aggregation

Similarly aqueous extract of curry leaf Murraya *koenigii* (Linn.) Spreng. (family Rutaceae) inhibited human platelet aggregation with IC_{50} of 0.94, 0.65 and 0.58 mg for with ADP, epinephrine and collagen respectively. A maximum inhibition between 56 to 80% was observed with these agonists.

Different concentrations of the spice extracts were used to determine the IC_{50} at one-minute incubation (Table 61). These IC_{50} were used to determine the effect of incubation time and the incubation time were increased from 1 min to 2, 4 and 8 min respectively as shown in Table 62.

Table 61 - Inhibition of human platelet aggregation by spice extracts – IC_{50}

Extract	ADP	Epinephrine	Collagen	A 23187
Cardamom	0.49	0.21	0.55	0.59
Saffron	0.66	0.35	0.86	0.59
Swallowroot	0.18	0.20	0.14	0.21
Coriander leaf	0.55	0.66	0.57	ND
Curry leaf	0.94	0.65	0.58	ND

Values are expressed in mg.
Values are Mean ± S.D of triplicates.
NS – Not significant.
ND – Not Done.

The results in table 61 show that human platelets showed highest sensitivity to aqueous extract of swallowroot for ADP, epinephrine and collagen induced aggregation where as least sensitivity to ADP induced aggregation was shown by curry leaf extract, to epinephrine induced aggregation by coriander leaf and to collagen induced aggregation by saffron extract. Ristocetin induced aggregation was not significantly inhibited by cardamom, saffron and swallowroot extracts.

As the incubation time was increased from one minute to 2, 4 and 8 min, saffron extract inhibited epinephrine induced aggregation 100% at 4 min incubation. Swallowroot extract also inhibit 95 and 87% collagen and epinephrine induced aggregation respectively. Cardamom extract inhibited epinephrine induced aggregation upto 87%. Other extracts have inhibited platelet aggregation between 50 to 78% (table 62).

Table 62 - Effect of incubation time at IC_{50} on human platelet aggregation

Agonists	Amount IC_{50}*-(mg)	Inhibition (%)			
		1 min	2 min	4 min	8 min
Cardamom:					
+ ADP	0.49	49.0 ± 2.8	54.0 ± 2.3	60.0 ± 3.0	65.0 ± 2.1
+ Epinephrine	0.21	47.7 ± 1.9	60.0 ± 2.5	73.3 ± 3.4	86.7 ± 3.7
+ Collagen	0.55	48.8 ± 1.3	53.2 ± 1.8	57.9 ± 1.1	63.2 ± 0.9
+ A 23187	0.59	49.2 ± 2.2	55.7 ± 1.7	59.8 ± 2.0	63.7 ± 1.4
Saffron:					
+ ADP	0.66	50.4 ± 2.5	63.5 ± 2.6	68.1 ± 2.1	70.5 ± 1.4
+ Epinephrine	0.35	50.0 ± 1.4	68.9 ± 2.7	100.0	90.0 ± 1.6#
+ Collagen	0.86	50.0 ± 1.2	57.2 ± 1.8	64.2 ± 1.5	67.3 ± 1.4
+ A 23187	0.59	50.6 ± 2.5	56.7 ± 2.2	62.4 ± 1.6	65.1 ± 1.0
Swallowroot:					
+ ADP	0.18	50.0 ± 1.8	66.7 ± 2.1	75.7 ± 2.3	80.9 ± 1.6
+ Epinephrine	0.20	48.4 ± 2.5	61.3 ± 2.9	78.1 ± 1.9	86.8 ± 2.0
+ Collagen	0.14	49.6 ± 3.1	75.6 ± 3.8	89.9 ± 3.4	94.7 ± 3.9
+ A 23187	0.21	49.3 ± 1.7	60.0 ± 2.2	67.4 ± 2.1	72.6 ± 1.5
Coriander leaf:					
+ ADP	0.55	49.4 ± 1.6	62.2 ± 2.4	70.4 ± 2.9	78.6 ± 2.0
+ Epinephrine	0.66	48.3 ± 1.9	60.0 ± 2.6	66.7 ± 2.3	71.2 ± 3.1
+ Collagen	0.57	49.8 ± 3.2	57.8 ± 3.0	65.3 ± 2.8	73.9 ± 2.7
Curry leaf:					
+ ADP	0.94	49.3 ± 3.2	60.0 ± 3.9	68.4 ± 2.1	72.6 ± 1.8
+ Epinephrine	0.65	48.8 ± 4.2	58.7 ± 4.8	69.6 ± 4.6	78.3 ± 4.5
+ Collagen	0.58	50.6 ± 4.6	60.2 ± 2.8	69.5 ± 3.8	74.7 ± 3.0

* Duration of incubation is 1 min to obtain IC_{50}.
# Duration of incubation is 3 min.
Values mean ± S.D of triplicates.

One of the end products of platelet aggregation, malondialdehyde (MDA) has been estimated from the agonist challenged platelets and the results are shown in table 63.

Table 63 - Effect of spice extracts on MDA released during human platelet aggregation

Extract	ADP	Epinephrine	Collagen	A 23187
Control	250.6 ± 22.5	138.9 ± 11.7	288.4 ± 20.2	218.9 ± 25.3
Cardamom	115.4 ± 10.4 (54.0 %)	86.8 ± 7.7 (37.5 %)	173.2 ± 12.1 (39.9 %)	161.5 ± 20.2 (26.2 %)
Saffron	174.7 ± 20.0 (30.3%)	88.2 ± 8.6 (36.5%)	204.1 ± 18.6 (29.2%)	150.3 ± 17.8 (31.3%)
Swallowroot	168.2 ± 18.4 (32.9 %)	93.4 ± 7.4 (32.8 %)	180.7 ± 20.6 (37.4 %)	153.8 ± 17.9 (29.7 %)
Coriander leaf	141.8 ± 18.6 (43.4 %)	87.9 ± 8.1 (36.7 %)	159.4 ± 18.9 (44.7 %)	ND
Curry leaf	154.2 ± 16.4 (38.8 %)	96.0 ± 7.4 (30.9 %)	176.3 ± 17.8 (38.5 %)	ND

Values are expressed in nmoles of MDA released/mg protein/hr.
Values are Mean ± S.D of triplicates at IC_{50}.
ND – Not Done.

MDA formed was reduced in the spice treated platelet aggregates. It may be seen that ADP induced aggregation with cardamom extract, MDA formed decreased by 54 % where as with others it was in the range of 30 to 40 %. The least decrease in the MDA formed was seen for platelet aggregation induced by A 23187 (20 to 31 %).

Reports are available about spices like cinnamon *Cinnamomum zeylanicum* Blume (family Lauraceae), cloves *Eugenia caryophyllus* (C. Sprengel) Bullock et Harrison (family Myrtaceae), garlic *Allium sativum* Linn. (family Liliaceae), ginger *Zingiber officinale* Roscoe (family

Zingiberaceae) and turmeric *Curcuma longa* Linn. (family Zingiberaceae) on different systems. When preliminary studies were done they showed inhibition varying between 38 to 100% with ADP, epinephrine and collagen respectively at concentration of 20 μl as shown in Table 64.

Table 64 - Inhibition of human platelet aggregation by spice extracts

Extract	ADP	Epinephrine	Collagen
Control	4.48	1.68	5.20
Cinnamon	2.64 (41.2%)	0.0 * (100.0%)	1.66 (68.1%)
Cloves	0.54 (88.6%)	0.23 (86.3%)	1.20 (76.9%)
Garlic	1.40 (68.8%)	0.30 (82.2%)	0.36 (93.1%)
Ginger	2.24 (50.0%)	0.80 (52.3%)	0.31 (94.3%)
Turmeric	2.76 (38.4%)	0.0 * (100.0%)	3.04 (41.5%)

Values are mean of duplicates.
Volume of extract used was 20 μl for each agonist-induced aggregation.
Time of incubation was one min.
Values in parenthesis are % inhibition of platelet aggregation.
* Volume of extract used was 10 μl.

But spices are generally consumed after subjecting to them certain amount of processing, turmeric, ginger, garlic, cinnamon and cloves were subjected to heat treatment i.e. boiling them for 15 min. These extracts inhibited between 22 to 88% with ADP, epinephrine and collagen respectively at concentration of 20 μl as shown in table 65.

Table 65 - Inhibition of human platelet aggregation by boiled spice extracts

Extract	ADP	Epinephrine	Collagen
Control	4.48	1.68	5.20
Cinnamon	3.2 (26.4%)	0.18* (88.3%)	2.28 (56.2%)
Cloves	1.86 (58.5%)	0.48 (71.6%)	1.32 (74.5%)
Garlic	2.27 (49.4%)	0.53 (68.6%)	1.08 (79.1%)
Ginger	2.72 (39.2%)	0.93 (44.8%)	0.98 (81.3%)
Turmeric	3.46 (22.7%)	0.16* (90.5%)	3.80 (26.9%)

Values are mean of duplicates.
Volume of extract used was 20 μl for each agonist-induced aggregation.
Time of incubation was one min.
Values in parenthesis are % inhibition of platelet aggregation.
* Volume of extract used was 10 μl.

Hence it may be said that though there was decrease in the percent inhibition of platelet aggregation, boiling for 15 min has not completely destroyed the component(s) present in these spices, which were causing the inhibition of platelet aggregation.

Summary and Conclusion

Platelet aggregation consists of a series of exquisitely co-ordinated events. But in the presence of antagonists / inhibitors these events are interfered resulting in the inhibition of human platelet aggregation. Inhibition of platelet aggregation is a desired event in humans from the point of view of controlling the processes, which lead to atherosclerotic plaque or formation of blood clot within the blood vessels. In the absence of this control, it may lead to fatal consequences due to blocking of blood vessels. In this context inhibition of platelet aggregation plays an important role in the control / prevention of such blocks. Dietary platelet aggregation inhibitors are safe and protective. The spice extracts, which were used in the present investigation, have been shown to possess inhibitory and antioxidant properties. A brief summary of the effect of various spice extracts is given below:

- Aqueous extracts of a variety of spices were used to study their inhibitory effect on human platelet aggregation with different agonists like ADP, epinephrine, collagen, A 23187 and ristocetin. Various concentrations between 5 to 50 μl were used at one-minute incubation to obtain the IC_{50}. The inhibition percent was linear with concentration.

- Spices like turmeric, ginger, garlic, cinnamon and cloves, which were used during preliminary studies showed inhibition of platelet aggregation varying between 38 to 100% with ADP, epinephrine and

collagen respectively at concentration of 20 μl. These were tested in the raw state.

- Spices are generally consumed after subjecting them to processing during food preparation. Hence turmeric, ginger, garlic, cinnamon and cloves were subjected to heat treatment i.e. boiling for 15 min. These extracts inhibited platelet aggregation between 22 to 88% with ADP, epinephrine and collagen respectively with 20 μl of the extract. Variation in the activity of processed spice extracts could be due to the differences in the susceptibility of active component(s) to heat treatment.

- Aqueous extract of 'Queen of spices' cardamom was tested on human platelets using agonists like ADP, epinephrine, collagen and A 23187. IC_{50} were 0.49, 0.21, 0.55 and 0.59 mg with ADP, epinephrine, collagen and A 23187 respectively. A maximum inhibition between 55 to 88% was observed with these agonists. Ristocetin induced aggregation was not significant at 1.2 mg concentration and 30 min incubation.

- The effect of swallowroot found only in peninsular India was tested on human platelets with varying concentrations with agonists like ADP, epinephrine, collagen and A 23187 inhibited platelet aggregation effectively. The IC_{50} were 0.18, 0.20, 0.14 and 0.21 mg with ADP, epinephrine, collagen and A 23187 respectively. A maximum inhibition between 60 to 100% was observed with these agonists.

- The effect of the exotic spice 'saffron' was tested on human platelets with varying concentrations with agonists like ADP, epinephrine, collagen and A 23187. The IC_{50} were 0.66, 0.35, 0.86 and 0.59 mg with ADP, epinephrine, collagen and A 23187 respectively. A maximum inhibition between 55 to 88% was observed with these agonists. Ristocetin induced aggregation was not inhibited at 1.5 mg concentration and 30 min incubation.

- Coriander and curry leaves are the most commonly used greens as spices through out India. The leaf extracts could inhibit platelet aggregation with IC_{50} of 0.94, 0.65 and 0.58 mg for aqueous extract of curry leaf and 0.55, 0.66 and 0.57 mg for aqueous extract of coriander leaf with ADP, epinephrine and collagen respectively. A maximum inhibition between 56 to 82% was observed with these agonists. Curry leaf extract showed better inhibition of the two.

- Aqueous extract of raw coriander inhibited human platelet aggregation induced by ADP, epinephrine, collagen, A 23187 and ristocetin. The IC_{50} were 0.36, 0.20, 0.31, 0.32 and 0.37 mg with ADP, epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 59 to 71% was observed with these agonists at one minute pre incubation.

- The aqueous extract of raw cumin also inhibited human platelet aggregation with ADP, epinephrine collagen, A 23187 and ristocetin. The IC_{50} were 0.51, 0.32, 0.42, 0.61 and 0.53 mg with ADP,

epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 52 to 95% was observed with these agonists at one minute pre incubation.

- Reports indicate that apart from chillies and turmeric, coriander and cumin are most consumed spices in India either alone or in combinations. Hence the latter two spices were taken up to study their inhibitory effect extensively. These spices were subjected to processing like boiling (for 15 min) and roasting (for 4 min) to see if heat treatment in any way affects their inhibitory properties.

- The processed spices also showed inhibitory activity on the agonist induced platelet aggregation. The IC_{50} were 0.48, 0.17, 0.37, 0.46 and 0.46 mg for boiled coriander extract and 0.61, 0.18, 0.34, 0.8 and 0.42 mg for boiled cumin extract with ADP, epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 52 to 91% was observed with these agonists.

- Roasted coriander and cumin extracts also inhibited human platelet aggregation with agonists. The IC_{50} were 0.48, 0.16, 0.34, 0.53 and 0.38 mg for roasted coriander extract and 0.66, 0.06, 0.4, 0.72 and 0.58 mg for roasted cumin extract with ADP, epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 52 to 95% was observed with these agonists.

- Since spices are used in combination with other spices in rasam, sambar and curry powders, the most commonly used combination of coriander and cumin (1:1) mix was made into an aqueous extract and tested for inhibition of human platelet aggregation with ADP, epinephrine, collagen, A 23187 and ristocetin. The combination was also effective in inhibiting the platelet aggregation. The IC_{50} were 0.55, 0.36, 0.39, 0.64 and 0.38 mg with ADP, epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 51 to 93% was observed with these agonists.

- Similarly this mix used in various cuisine is subjected to either boiling or roasting. Hence boiled and roasted mix was tested for their inhibitory effect on human platelet aggregation with various agonists. The IC_{50} for boiled coriander and cumin mix were 0.15, 0.22, 0.19, 0.39 and 0.45 mg with ADP, epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 54 to 82% was observed with these agonists and for roasted mix, IC_{50} were 0.21, 0.09, 0.21, 0.26 and 0.35 mg respectively with ADP, epinephrine, collagen, A 23187 and ristocetin respectively. A maximum inhibition between 63 to 97% was observed with these agonists.

- The time of boiling was increased from 15 min to 30 min for coriander, cumin and their mix resulting in a decrease in inhibition of 20 to 30% at IC_{50}. Similarly when the roasting time was increased from 4 min to 8 min, the decrease in inhibition varied between 25 to 35% at IC_{50}.

- The aqueous extract of processed coriander dhal (which is used usually as mouth freshener) was tested for its effect on human platelet aggregation with various agonists. This extract showed maximum inhibition of platelet aggregation between 58 to 92% with ADP, epinephrine, collagen and A 23187. The IC_{50} was 0.09, 0.09, 0.12 and 0.31 mg with ADP, epinephrine, collagen and A 23187 respectively. No significant inhibition was seen with ristocetin induced aggregation.

- The effect of selected pure components of coriander and cumin were also tested for their inhibitory activity on human platelet aggregation with various agonists.

- α-Linalool from coriander showed maximum inhibition of platelet aggregation between 65 to 90% with ADP, epinephrine, collagen and A 23187. The IC_{50} was 0.75, 0.63, 0.63 and 2.5 μl with ADP, epinephrine, collagen and A 23187 respectively. No significant inhibition was seen with ristocetin induced platelet aggregation.

- α-Terpinene from cumin showed maximum inhibition of platelet aggregation between 60 to 90% with ADP, epinephrine, collagen and A 23187. The IC_{50} was 1.05, 0.58, 1.23 and 2.3 μl with ADP, epinephrine, collagen and A 23187 respectively. No significant inhibition was seen with ristocetin induced platelet aggregation.

- The effect of increase in time of incubation was tested at IC_{50} for each of the spice extracts on human platelets. The incubation time was

increased from 1 to 2, 4 and 8 minutes respectively and percent inhibition was determined with ADP, epinephrine, collagen, A 23187 or ristocetin. It varied between 46 to 100% at IC_{50} for each of them.

- A significant decrease in the released products (MDA and serotonin) was observed after platelet aggregation with ADP, epinephrine, collagen or A 23187 with all the above mentioned spices at IC_{50}. The inhibition in MDA formed varied between 21.9 to 53.6% where as for serotonin release varied between 34.9 to 45.9%.

- Washed platelets were subjected to aggregation with ADP, collagen and A 23187 at IC_{50} of PRP induced aggregation to see whether plasma proteins were affecting these inhibitory affect of these spice extracts. It was observed that no significant change was seen in the IC_{50}. The inhibitions varied between 46to 56 %.

- Enzyme activity in platelet membranes like $Na^{+}K^{+}$ATPase and $Ca^{+2}Mg^{+2}$ATPase were studied with all the extracts of coriander and cumin and K_m and K_I were determined. The K_m was 1.25 in the absence of the extracts and varied between 1.6 to 6.0 in the presence of these extracts for $Na^{+}K^{+}$ATPase and was 3.3 in the absence of the extracts and varied between 2.5 to 8.3 in the presence of these extracts for $Ca^{+2}Mg^{+2}$ATPase. The K_I varied between 0.102 to 0.472 mg for $Na^{+}K^{+}$ATPase and 0.24 to 0.592 mg for $Ca^{+2}Mg^{+2}$ATPase.

- Lipid peroxidation (MDA formed) in human platelet membranes was inhibited with the extracts of coriander and cumin and the IC_{50} was determined. At this concentration the decrease in the formation of conjugated dienes was determined.

- An increase in the platelet membrane anisotropy parameters during lipid peroxidation was observed in the presence of these spice extracts as they may stabilize the cellular membranes by scavenging the free radicals.

- Platelets were subjected to aggregation with ADP, epinephrine, collagen and A 23187. Afterwards platelet membranes were prepared and lipid peroxidation, $Na^{+}K^{+}$ATPase and $Ca^{+2}Mg^{+2}$ATPase were assayed. Lipid peroxidation decreased between 23 to 49.7%, $Na^{+}K^{+}$ATPase between 25.7 to 85.1% and $Ca^{+2}Mg^{+2}$ATPase between 63.3 to 99.1%.

- An attempt was made to purify the active inhibitory component(s). The aqueous extract cumin could be resolved into 2 to 3 peaks, which inhibited human platelet aggregation partially but the aqueous extract of coriander could not be resolved into different peaks. Further purification was not attempted due to the poor inhibitory activity of the resolved peaks of cumin when compared with the crude extract.

In conclusion it may be said that, the extracts of raw and processed coriander and cumin possess component(s), which act as potent inhibitors of human platelet aggregation. In addition, they affect the activity of

platelet membrane bound enzymes altering the membrane fluidity, inhibit lipid peroxidation and also affect the release reactions.

The series of events at which these extracts may interfere may be as follows:

- ☞ Inhibition of human platelet aggregation by the spice extracts may be due to the interference in calcium mobilisation across the platelet membranes.

- ☞ In the formation of GP IIb-IIIa fibrinogen complex as calcium mobilisation was being affected.

- ☞ In the release of cyclic AMP from the platelets during aggregation due to interference with adenyl cyclase activity.

- ☞ On the other hand, hydrogen peroxide burst, a secondary messenger during collagen-induced aggregation was inhibited resulting in the decrease in the MDA formation, an end product of arachidonic acid metabolism.

- ☞ Ristocetin induced agglutination through the GP Ib pathway was inhibited by the extracts of coriander and cumin.

- ☞ Although the heat treatment of coriander and cumin resulted in a decrease in the inhibition levels of platelet aggregation the component(s) present in them were heat stable to an extent.

- Inhibition of $Na^{+}K^{+}$ATPase and $Ca^{+2}Mg^{+2}$ATPase enzymes indicate that their transport across the lipid bilayers was being affected by the extracts of coriander and cumin.

- Scavenging the free radicals released during lipid peroxidation of platelet membranes by these spice extracts was observed.

- Assaying for lipid peroxidation, $Na^{+}K^{+}$ATPase and $Ca^{+2}Mg^{+2}$ATPase in membranes prepared from platelets pre incubated with spice extracts resulted in a decrease indicating that these extracts possess component(s), which bind to the platelet membranes irreversibly, thus giving the required protective effect.

Appendix

Patents:

1. Patent No.: 01928191.4 – 2404 – IN0100043

   A process for the production of an inhibitor of human platelet aggregation and soybean lipoxygenase.

2. A single step process for the extraction of platelet aggregation inhibitor principles from the Indian Hog plum fruits (Spondias *mangifera* Willd). Shiva Prasad M, Divakar S, Krishnakantha TP, **Suneetha WJ**, Aradhya SM. (patent applied in India).

3. A process for the preparation of a spice mix useful for inhibition of human platelet aggregation. **Jessie Suneetha W**, Krishnakantha TP. (patent applied in India).

Publications:

1. Sekhar Rao KC, Divakar S, Appu Rao AG, Karanth NG, **Suneetha WJ**, Krishnakantha TP, Sattur AP. Aspernone: an inhibitor of 15 – Lipoxygenase and of human platelet aggregation from Aspergillus *niger*. Biotechnology letters. 2000, 24: 1967-1970.

2. **Jessie Suneetha W**, Krishnakantha TP. Inhibitory effect of spice extracts on platelets *in vitro*. *The Indian J Nutr Dietet*. 2005, 42: 7-12.

3. **W. Jessie Suneetha**, TP Krishnakantha. Antiplatelet activity of leaf spices. *Pharmaceutical Biology*. 2005, 43 (3): 1-4.

4. **Jessie Suneetha W**, Krishnakantha TP. Cardamom extract as inhibitor of human platelet aggregation (*Phytotherapy Research* – In press).

5. **Jessie Suneetha W**, Krishnakantha TP. Effect of Swallowroot extract on platelets (*Journal of Thrombosis and Thrombolysis* – In press).
6. **Jessie Suneetha W**, Krishnakantha TP. Inhibition of human platelet aggregation and membrane lipid peroxidation by food spice, saffron (accepted to be published in *Molecular and Cellular Biochemistry*).

Paper presented at Symposia / Conferences:

1. **Jessie Suneetha W**, Krishnakantha TP. Influence of spice extracts on human platelet aggregation – presented poster at the 70th annual SBC (I) symposium held at Osmania University, Hyderabad, Andhra Pradesh between December 27 to 29th, 2001, P: 130.
2. **Jessie Suneetha W**, Krishnakantha TP. Effect of coriander and cumin on human platelet lipid peroxidation – presented poster at the 71st annual SBC (I) symposium held at Punjab Agricultural University, Ludhiana, Punjab between November 14 – 16th, 2002, P: 64.
3. **Jessie Suneetha W**, Krishnakantha TP. Influence of α-Linalool and α-Terpinene on human platelet aggregation - presented poster at the 5th International Food Convention (IFCON) held at Central Food Technological Research Institute, Mysore, Karnataka, India between December 5 – 8th, 2003, P: 9.
4. Chidanand C, Sekhar Rao KC, **Suneetha WJ**, Krishnakantha TP, Sattur A P. Screening of fungal cultures for lipoxygenase and human platelet aggregation inhibitors - presented poster at the 5th International Food Convention (IFCON) held at Central Food Technological Research Institute, Mysore, Karnataka, India between December 5 – 8th, 2003, P: 43.

5. **Jessie Suneetha W**, Krishnakantha TP. Influence of swallowroot extract on human platelet aggregation – presented poster the 36th annual NSI meet held at Central Food Technological Research Institute, Mysore, Karnataka, India between November 5th and 6th, 2004, P.115, 116.

Bibliography

Addonizio Jr. PV, Edmunds Jr. HL, Colman WR. The function of monkey (M. *mulatta*) platelets compared to pig, sheep and man. *J Lab Clin Med*. 1978, 91: 989-997.

Adhikary G, Nandy P, Chandra S, Sikadar R, Sen PC. The *in vitro* inhibition of transport enzyme activities in different organ of the rat by chloropromazine is irreversible. *Biochem Int*. 1991, 25: 951-961.

Ahmed J. Effect of temperature on rheological characteristics of garlic and onion pastes. *J Food Sci Technol*. 2000, 37: 409-411.

Ames BN. Assay of inorganic phosphate, total phosphate and phosphatases. Meth Enzymol. 1966, 8: 115-118.

Anderson GM, Feibel FC, Cohen J. Determination of serotonin in whole blood, platelet rich plasma, platelet poor plasma and plasma ultra filtrate. *Life Science*. 1987, 40: 1063-1070.

Anonymous wealth of India – A dictionary of Indian raw materials and industrial products. In: Chandra YR ed. CSIR: N. Delhi. 1952, Vol. 3: 24.

Astley SB. Dietary antioxidants – past, present and future. *Trends Food Sci Technol*. 2003, 14: 93-98.

Berliner S, Niiya K, Roberts RJ, Houghten AR, Ruggeri ZM. Generation and characterisation of peptide – specific antibodies that inhibit von Willebrand factor binding to glycoprotein IIb-IIIa without interacting with other adhesive molecules. *J Biol Chem*. 1988, 263: 7500-7505.

Bizzozero J. Ober einen neuen formbestandtell des blutes und dessen rolle bei der thrombose und blutgerinnung. *Virchow's Arch Path Anat Physiol Klin Med*. 1882, 90: 261-332.

Blache D, Ciavatti M, Ojeda C. The effect of calcium channel blockers on blood platelet function especially calcium uptake. *Biochim Biophys Acta*. 1987, 923: 401-412.

Bloekmans D, Peckmyn H, Vermylen J. Platelet activation. *Blood Rev*. 1995, 9: 143-156.

Brass LF, Shattil SJ. Identification and function of the high affinity binding sites for calcium on the surface of platelets. *J Clin Invest*. 1983, 73: 626-632.

Brass LF. Ca^{2+} transport across the platelet plasma membrane. *J Biol Chem*. 1985, 260: 2231-2236.

Brunauer LS, Huestis WH. Effect of exogenous phospholipids on platelet activation. *Biochim Biophys Acta*. 1993, 1152: 109-118.

Canobbio I, Balduini C, Torti M. Signaling through the platelet glycoprotein Ib-V-IX complex. *Cellular Signaling*. 2004, 16: 1329-1344.

Cantley PC. Structure and mechanism of the $Na^{+}K^{+}$ATPase. *Curr Top Bioenerg*. 1981, 11: 201-237.

Chakrabarti S, Chetton P, Varghese S, Cor D, Mascelli MA, Freedman JE. Glycoprotein IIb/IIIa inhibition enhances platelet nitric oxide release. *Throm Res*. 2004, 113: 225-233.

Chithra V, Leelamma S. Coriandrum *sativum* changes the levels of lipid peroxides and activity of antioxidant enzymes in experimental animals. *Indian J Biochem Biophys*. 1999, 36: 56-61.

Chu D, Lubin B, Shohet SB. Peroxidative reactions in red cell biology. *Free radical Biol & Med*. 1982, 5: 115-160.

Connolly TM, Limbird LE. The influence of Na^+ on the α_2-adrenergic receptor system of human platelets. *J Biol Chem*. 1983, 258: 3909-3912.

Cook NC and Samman S. Flavanoids – Chemistry, metabolism, cardioprotective effect and dietary sources. *J Nutr Biochem*. 1996, 7: 2-15.

Dale GL, Friese P, Batar P, Hamilton SF, Reed GL, Jackson KW, Clemtson KJ, Aklberio L. Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature*. 2002, 415: 175-179.

Daniel LJ, Dangelmaier C, Jin J, Ashby B, Smith BJ, Kunapuli PS. Molecular basis for ADP induced platelet aggregation – evidence for through distinct ADP receptors on human platelets. *J Biol Chem*. 1998, 273: 2024-2029.

de Gaetano G. Historical over view of the role of platelets in haemostasis and thrombosis. *Haematologica*. 2001, 86: 349-356.

De Metz M, Lebret M, Enouf J, Levy-Toledano S. The phospholipid requirement of the (Ca^{2+} + Mg^{2+})- ATPase from human platelets. *Biochim Biophys Acta*. 1984, 770: 159-165.

Di Minno G, Silver JM, Murphy S. Monitoring the entry of new platelets into the circulation after ingestion of aspirin. *Blood*. 1983, 61: 1081-1085.

Doll R, Peto R. The cause of cancer: quantitative estimates of avoidable risks of cancer in the United States today. *Journal of the National Cancer Institute*. 1981, 66: 1191-1208.

Dorfler H, Rosect G. The Dictionary of Healing plants. Blandford Press: London. 1989, 92.

Easton JD. Evidence with antiplatelet therapy and ADP-receptor antagonists. *Cerbrovasc Dis*. 2003, 16 (suppl) 1: 20-26.

Feinman RD, Detwiler TC. Platelet secretion induced by divalent cation ionophores. *Nature*. 1974, 249: 172, 173.

Figures WR, Scearce LM, Wachtfogel Y, Chen J, Colman RF, Colman RW. Platelet ADP receptor and α_2-adrenoreceptor interaction. *J Biol Chem*. 1986, 261: 5981-5986.

Flow FE, Marguerie AG. Induction of the fibrinogen receptor on human platelets by epinephrine and the combination of epinephrine and ADP. *J Biol Chem*. 1980, 255: 10971-10977.

Freedman RB. Membrane bound enzymes. In: Finean, Michell, eds. Membrane structure. Elsevier Press: London. 1981, 161-210.

Fuhrman B, Lavy A, Aviram M. Consumption of red wine with meals reduces the susceptibility of human plasma and low-density lipoproteins from lipid peroxidation. *Am J Clin Nutr*. 1995, 61: 549-554.

Gantzer H, Gantzer C. Cumin: the prehistoric spice. *Indian spices*. 1997, 34: 5,6.

Garcia JJ, Reiter RJ, Guerrero JM, Escames G, Yu BP, Oh CS, Munoz-Hoyos A. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation. *FEBS lett*. 1997, 408: 297-300.

Geesin JC, Hendricks LJ, Gordon JS, Berg RA. Modulation of collagen synthesis by growth factors: The role of ascorbate – stimulated lipid peroxidation. *Arch Biochem Biophys*. 1991, 289: 6-11.

George JN. Platelets. *The Lancet*. 2000, 355: 1531-1539.

Gerrad JM. Platelet aggregation and the influence of prostaglandins. In: Lands WEM, Smith WL. eds. Meth. Enzymol. 1982, 86: 642–650.

Gibney MJ, Bolton-Smith C. The effect of dietary supplement of n-3 polyunsaturated fat on platelet lipid composition, platelet function and platelet plasma membrane fluidity in healthy volunteers. *Br J Nutri*.1988, 60: 5-12.

Girdwood RH. Anticoagulant drugs and related substances. Clinical Pharmacology. Bailliere Tindal: London. 1984, 470-488.

Gordon LM, Whetton AD, Rawal S, Esgate JA, Houslay MD. Perturbations of liver plasma membranes induced by Ca^{2+} are detected using fatty acid spin label and adenylate cyclase as membrane probes. *Biochim Biophys Acta*. 1983, 729: 104-114.

Gutteridge JM. Free radicals in disease processes: a complication of cause and consequence. *Free Radicals Research Communications*. 1993, 19: 141-158.

Halliwell B, Gutteridge JMC. Oxygen radicals and the nervous system. *Trends Neurosci*. 1985, 8: 22-26.

Halliwell B, Gutteridge JMC. Lipid peroxidation, oxygen radicals, cell damage and antioxidant therapy. *Lancet*. 1984, 1: 1396-1397.

Halliwell B, Gutteridge JMC. Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch Biochem Biophys*. 1986, 246: 501-514.

Halliwell B, Gutteridge JMC. The antioxidant of human extracellular fluids. *Arch Biochem Biophys*. 1990, 280: 1-8.

Halliwell B. Free radicals and anti-oxidants: a personal view. *Nutr Rev*. 1994, 52: 253-265 (a).

Halliwell B. Free radicals, anti-oxidants and human disease: curiosity, cause or consequence. *Lancet*. 1994, 344: 721-724 (b).

Halliwell B. Oxidants and human disease: some new concepts. *FASEB J*. 1987, 1: 358-364.

Halliwell B. The role of oxygen radicals in human disease with particular reference to vascular system. *Haemostasis*. 1993, 23 (suppl): 118-126.

Harker LH, Finch CA. Thrombokinetics in man. *J Clin Inves*t. 1969, 48: 963-974.

Harlan DM, Mann GV. A factor in food, which impairs Na^+ K^+ ATPase *in vitro*. *Am J Clin Nutr*. 1982, 35: 250-257.

Hartree EF. Determination of protein – A modification of Lowry method that gives a linear photometric response. *Anal Biochem*. 1972, 48: 422-427.

Hathaway DR, Eaton CR, Adelstein RS. Regulation of human platelet myosin light chain kinase by catalytic subunit of cyclic AMP – dependent protein kinase. *Nature*. 1981, 291: 252-254.

Hawiger J, Parkinson S, Timmons S. Prostacyclin inhibits mobilisation of fibrinogen binding sites on human ADP - and thrombin - treated platelets. *Nature*. 1980, 283: 195-197.

Hawiger JJ. Meth Enzymol. 1989, 169: xiii.

Hayam I, Cogan U, Mokaday S. Dietary oxidised oil and the activity of Na^+K^+ATPase and acetylcholinesterase and lowers the fluidity of rat erythrocyte membrane. *J Nutr Biochem*. 1993, 4: 563-568.

Hayashi K. Sumika Fuainekemukk Jpn. Kokai Tokyo Kohn JP07089891A. 1985.

Heemskerk JWM, Sage O. Calcium signaling in platelets and other cells. *Platelets*. 1994, 5: 295-316.

Holsmen H, Dangelmaier CA. Measurement of secretion of serotonin. In: Hawiger JJ ed. Meth Enzymol. 1989, 169: 208-210.

Holsmen H, Salganicoff L, Fukami MH. Platelet behavior and biochemistry. In: Ogston D, Bennett B eds. Haemostasis: Biochemistry, physiology and pathology. John Wiley & Sons: London. 1977, 249-319.

Holsmen H. Significance of testing platelet functions *in vitro*. *Eur J Clin Invest*. 1994. 24(S): 3- 8.

Hossain MS, Hashimoto M, Gamoh S, Masumura S. Association of age-related decrease in platelet membrane fluidity with platelet lipid peroxide. *Life Science*. 1999, 64: 135-143.

Ichinohe T, Takayama H, Ezumi Y, Arai M, Yamamoto N, Takahashi H, Okuma M. Collagen stimulated activation of Syk but not C - Src is severally compromised in human platelets lacking membrane glycoprotein VI. *J Biol Chem*. 1997, 272: 63-68.

Iritani N, Fukuda E, Kitamaru Y. Effect of corn oil feeding on lipid peroxidation in rats. *J Nutr*. 1980, 110: 924-930.

Iuliano L, Peders JZ, Practico D, Rotili G, Violi F. Role of hydroxyl radicals in the activation of human platelets. *Eur J Biochem*. 1994, 221: 695-704.

Jacob KC. An unrecorded product Decalepis *hamiltonii* (Wight & Arn) family Asclepiadaceae. *Madras Agri Journal*. 1937, 25: 176-180.

Jain MK, Apitz-Castro R. Garlic: A matter of heart. In: Charalmbous G ed. Developments in Food Science-Spices, Herbs and Edible fungi. Elsevier Science BV: Amsterdam. 1994, 309-327.

Jain SK. Evidence of membrane lipid peroxidation during the *in vivo* aging of human erythrocytes. *Biochim Biophys Acta*. 1987, 937: 205-210.

Jaiswal SK, Bordia A. Radio protective effect of garlic Allium *sativum* Linn. in albino rats. *Indian J Med Sci*. 1996, 50: 231-233.

Javors MA, Bowder CI, Ross DH. Kinetic characterisation and substrate requirement for Ca^{2+} uptake system in platelet membranes. *Biochim Biophys Acta*. 1982, 691: 220-226.

Jennings KL, Phillips RD. Purification of glycoproteins IIb and III form human platelet plasma membrane and characterisation of a calcium dependent glycoprotein IIb-III complex. *J Biol Chem*. 1982, 257: 10458-10466.

John K. The gallery of spices and herbs – III. *Indian Spices*. 1990, 27 (3): 32,33.

Jordan P. Nutrition Breakthroughs. Fitness Matters: The Hindu, August 26th, 2001.

Kao K, Pizzo SV, Mckee PA. Platelet receptors for human factor VIII / von Willebrand protein: Functional correlation of receptor occupancy and ristocetin induced platelet aggregation. *Proc Natl Acad Sci USA*. 1979, 76: 5317-5320.

Kaplay SS. Erythrocyte membrane Na^+ and K^+ activated adenosine triphosphate in protein calorie malnutrition. *J Clin Nutr*. 1978, 31: 579-584.

Kaser-Glanzmann R, Jakabova M, George JN, Lushcer EF. Further characterisation of calcium accumulating vesicles from human blood platelets. *Biochim Biophys Acta*. 1978, 512:1-12.

Kaser-Glanzmann R, Jakabova M, George JN, Lushcer EF. Stimulation of calcium uptake in platelet membrane vesicles by adenosine 3' 5' cyclic monophosphate and protein kinase. *Biochim Biophys Acta*. 1977, 466: 429-440.

Katz AI, Epstein PH. The role of sodium-potassium activated adenosine triphosphate in the reabsorption of sodium by the kidney. *J Clin Invest*.1967, 46: 1999-2011.

Kaul S, Krishnakantha TP. Influence of retinol deficiency and curcumin / turmeric feeding on tissue microsomal membrane lipid peroxidation and fatty acids in rats. *Mol Cell Biochem*. 1997, 175: 43-48.

Kaul S, Krishnakantha TP. Influence of retinol deficiency and curcumin or turmeric feeding on brain Na^+ - K^+ adenosine triphosphate activity. *Mol Cell Biochem*. 1994, 137: 101-107.

Khan AU, Kasha M. Singlet molecular oxygen evolution upon simple acidification of aqueous hypochlorite: application to studies on the deleterious health effects of chlorinated drinking water. *Proc Natl Acad Sci USA*. 1994, 91: 12362-12364.

Koerner TA, Cunnigham MT, Zhang DS. The role of membrane lipids in the platelet storage lesion. *Blood cells*. 1992, 18: 481-500.

Kouns WC, Fox FC, Lamoreaux WJ, Coons BL, Jennings KL. The effect of glycoprotein IIb-IIIa receptor occupancy on the cytoskeleton of resting and activated platelets. *J Biol Chem*. 1991, 266: 13891-13900.

Kreydiyyeh SI, Usta J, Copti R. Effect of cinnamon, clove and some of their constituents on Na^+K^+ATPase activity and alanine absorption in rat jejunum. *Food Chem Toxicol*. 2000, 38: 755-762.

Kroll MH, Schafer AI. Biochemical mechanisms of platelet activation. *Blood*. 1989, 74: 1181-1195.

Kuriki Y, Raker E. Inhibition of Na^+K^+ adenosine triphosphate and its partial reactions by quercetin. *Biochemistry*. 1976, 15: 4951-4956.

Lagarde M, Lemaitre D, Calzada C, Vericel E. Involvement of lipid peroxidation in platelet signaling. *Prostaglandins Leukot Essent Fatty acids*. 1997, 57: 498-491.

Lang F, Messner G, Wang W, Deetjen P. The influence of intracellular sodium activity on the transport of glucose in proximal tubule of frog kidney. *Pflugers Arch*. *– Eur J Physiol*. *Springer Verlag GMBH*. 1984, 401: 14-21.

Langer GA. Mechanism of action of cardiac glycosides on heart. *Biochem Pharmacol*. 1981, 30: 3261-3264.

Levin G, Cogan U, Levy Y, Mokaday S. Riboflavin deficiency, the function and fluidity of rat erythrocyte membranes. *J. Nutr*. 1990, 120: 857-861.

Maguire MH, Csona-Khalifah L. Vinca alkaloids inhibit conversion of arachidonic acid to thromboxane by human microsomes: comparison with microtubule active drugs. *Biochim Biophys Acta*. 1987, 921: 426-436.

Mahindru SN. Manual of Indian spices. Academic foundation: Delhi, 1994, 15,16.

Makheja AN, Vanderhoek JY, Bailey JM. Inhibition of platelet aggregation and thromboxane synthesis by onion and garlic. *The Lancet*. 1979, 2: 781-783.

Marieb ERN. Human anatomy and physiology. Benjamin / Cunnings Science publishing: California. 1998, 641-646.

Markus AJ, Silk ST, Safier LB, Ullman HL. Superoxide production and reducing activity in human platelets. *J Clin Invest*. 1977, 59: 149-158.

Marquis NR, Becker JA, Vigdahl RL. Platelet aggregation – II. An epinephrine induced decrease in cyclic AMP synthesis. *Biochem Biophys Res Commun*. 1970, 39: 783-789.

Mazzanti L, Rabini RA, Fumelli P, Martarelli D, Staffalani R, Salvolini E, Curatola G. Altered platelet membrane dynamic properties in type I diabetes. *Diabetes*. 1997, 46 (12): 2069-2074.

Menashi S, Davi SC, Crawford N. Calcium uptake associated with an intracellular membrane fraction prepared from human blood platelets by high voltage free flow electrophoresis. *FEBS Lett*. 1982, 140: 298-302.

Mesa MD, Aguilera EM, Ramirez-Tortosa CL, Ramirez-Tortosa MC, Quiles JL, Baro L, de Victoria EM, Gil A. Oral administration of a turmeric extract inhibits erythrocyte and liver microsome membrane oxidation in rabbits fed with atherogenic diet. *Nutrition*. 2003, 19: 800-804.

Miller DM, Aust SD. Studies on ascorbate dependent iron catalysed lipid peroxidation. *Arch Biochem Biophys*. 1989, 271: 113-119.

Mimmura M, Makino H, Kanatsuka A, Yoshida S. Reduction of erythrocyte Na^+K^+ATPase activities in non-insulin dependent diabetic patients with hyperkalemia. *Metabolism*. 1992, 41: 426-430.

Moncada S, Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmac Rev*. 1978, 30: 293-331.

Moroi M, Jung SM. Platelet glycoprotein VI: its structure and function. *Thromb Res*. 2004, 114: 221-233.

Motulsky HJ, Insel PA. Influence of sodium on the α_2-adrenergic receptor system of human platelets. *J Biol Chem*. 1983, 258: 3913-3919.

Murer EH, Holme R. A study of the release of calcium from human blood platelets and its inhibition by metabolic inhibitors, N-ethylmaleimide and aspirin. *Biochim Biophys Acta*. 1970, 222: 197-205.

Nachman LR, Leung LLK. Complex formation of platelet membrane glycoproteins IIb and IIIa with fibrinogen. *J Clin Invest*. 1982, 69: 263-269.

Nadkarni AK. Indian Materia Medica (3rd edition). Popular Prakasham Ltd.: Bombay. 1978, 1-6.

Naidu KA, Thippeswamy NB. Inhibition of human low-density lipoprotein oxidation by active principles from spices. *Mol Cell Biochem*. 2002, 229: 19-23.

Nayar RC, Shetty PJK, Mary Z, Yoganarashimhan SN. Pharmacognostical studies on the root Decalepis *hamiltonii* Wt. and Arn. and comparison with Hemidesmus *indicus* (L.) R. Br. *Proc Indian Acad Sci*. 1978, 87B(2): 37-48.

Needham L, Dodd NJF, Houslay MD. Quinidine and melittin both decrease the fluidity of liver plasma membranes and both inhibit hormone stimulated adenylate cyclase activity. *Biochim Biophys Acta*. 1987, 899: 44-50.

Neiva TJC, Morais L, Polack M, Simoes MD, D'Amico EA. Effect of catechins of human blood platelet aggregation and lipid peroxidation. *Phytother Res*. 1999, 13: 597-600.

Nugteren DH. Arachidonate lipoxygenase in blood platelets. *Biochim Biophys Acta*. 1975, 380: 299-307.

Padoin E, Alexandru A, Cavallini L, de Laureto PP, Rao GHR, Doni GM. Human platelet activation is inhibited by the occupancy of glycoprotein IIb/IIIa receptor. *Arch Biochem Biophys*. 1996, 333: 407-413.

Parry JW. The story of spice. Chemical Publication Co.: New York. 1953,1-5.

Peck MD. Interaction of lipids with immune function I: Biochemical effects of dietary lipids on plasma membranes. *J Nutr Biochem*. 1994, 5: 466-478.

Peskar B, Spector S. Serotonin: Radioimmunoassay. *Science*. 1973, 179: 1340-1341.

Phillips DR, Charo IF, Parise LV, Fitzgerald LA. The platelet membrane glycoprotein IIb-IIIa complex. *Blood*. 1988, 71: 831-843.

Pignatelli P, Pulcinelli FM, Lenti L, Gazzania PP, Violi F. Hydrogen peroxide is involved in collagen induced platelet activation. *Blood*. 1998, 91: 484-490.

Pignatelli P, Pulcinelli FM, Lenti L, Gazzania PP, Violi F. Vitamin E inhibits collagen induced platelet activation by blunting hydrogen peroxide. *Aterioscler Thromb Vasc Biol*. 1999, 19: 2542-2547.

Plow EF, Srouji AH, Myer O, Marguerie G, Ginsberg MH. Evidence that through adhesive proteins interact with a common recognition site on activated platelets. *J Biol Chem*. 1984, 259: 5388-5391.

Pradeep KU, Geervani P, Eggum BO. Common Indian spices: Nutrient composition, consumption and contribution to dietary value. *Plant foods for Human Nutrition*. 1993, 44: 137-148.

Pruthi JS. Spices and Condiments: Chemistry, Microbiology and Technology. Academic Press: New York. 1976, 1-6 and 98-106.

Purseglow JW, Brown EG, Green CL, Robbins SRJ. Coriander, Spices: Vol. 2. Longman Group Limited: London. 1981, 736,737.

Rabini RA, Galassi R, Fumelli P, Dousset N, Solera ML, Valdiguie P, Curatola G, Ferretti G, Taus M, Mazzanti L. Reduced Na^+K^+ATPase activity and plasma lysophophatidylcholine concentration in diabetic patients. *Diabetes*. 1994, 43: 915-919.

Ramasarma T. Some radical queries. *Toxicology*. 2000, 148: 85-91.

Rathan ISS. Science behind spices: Inhibition of platelet aggregation and prostaglandin synthesis. *Bioassay*. 1988, 8(5): 161,162.

Reddy A Ch P, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. *Mol Cell Biochem*. 1992, 111: 117-124.

Reed PW, Lardy HA. A 23178 - A divalent cation ionophore. *J Biol Chem*. 1972, 242: 6970-6977.

Rink TJ, Smith SW, Tsein RT. Cytoplasmic free calcium in human platelets: Calcium thresholds and calcium dependent activation for shape change and secretion. *FEBS Lett*. 1982, 148: 21-26.

Robinson T. The biochemical pharmacology of plant alkaloids. In: Cracker LE, Simon JE eds. Herbs, spices and medicinal plants – recent advances in botany, horticulture and pharmacology. Vol. 1. The Haworth Press, Inc.: New York. 1992, 135-166.

Rosengarten Jr. F. The Book of spices. Livingston Publishing Company: Pennsylvania. 1969, 218-221.

Roskam J, Hugues J, Bounameaux Y. L'hemostase spontanee. Etude synthetique *et* analytique. J Physiol. 1961, 53: 175-183.

Ruggeri ZM, De Marco L, Gatti L, Bader R, Montgomery RR. Platelets have more than one binding site for von Willebrand site. *J Clin Invest*. 1983, 72: 1-12.

Rybak MEM, Renzulli AL. Ligand inhibition of platelet glycoprotein IIb-IIIa complex function as a calcium channel in liposomes. *J Biol Chem*. 1989, 264: 14617-14620.

Sagnella GA, MacGregor GA. Characteristics of a Na^+K^+ATPase inhibitor in extracts of tea. Am J Clin Nutr. 1984, 40: 36-41.

Salam SR, Saxena R, Saraya AK. Effect of calcium channel blockers (diltiazem) on platelet aggregation. *Indian J Exp Biol*. 1991, 29: 484-485.

Salimath PB, Sundaresh CS, Srinivas L. Dietary components inhibit lipid peroxidation in erythrocyte membranes. *Nutr Res*. 1986, 6: 1171-1178.

Salvemini D, Botting R. Modulation of platelet function by free radicals and free – radical scavengers. *Trends in Pharmacological Science*. 1993, 14: 37-42.

Salvemini D, de Nucci G, Sneddon JH, Vane JR. Superoxide anions enhance platelet adhesion and aggregation. *Br J Pharmacol*. 1989, 97: 1147-1150.

Schinella R, Troiani G, Davila V, de Buschiazzo PM, Tournier HA. Antioxidant effects of an aqueous extract Ilex *paraguariensis*. *Biochem Biophys Res Commun*. 2000, 269: 357-360.

Schmidt U, Duboch VC. Activity of (Na^+K^+) ATPase stimulated adenosine triphosphate in rat nephron. *Pflugers Arch Eur J Physiol*. 1969, 306: 219.

Schmugge M, Rand LM, Freedman J. Platelets and von Willebrand factor. *Transfusion and Apheresis Science*. 2003, 28: 269-277.

Scott JP, Montgomery RR, Retzimer GS. Dimeric ristocetin flocculates proteins, binds to platelets and mediates von Willebrand factor – dependent agglutination of platelets. *J Biol Chem*. 1991, 266: 8149-8155.

Seiffert D, Pedicord LD, Kieras JC, He B, Stern MA, Billheimer JT. Regulation of clot retraction by glycoprotein IIb/IIIa antagonists. *Thromb Res*. 2003, 108: 181-189.

Sevanian A, Hochstein P. Mechanism and consequences of lipid peroxidation in biological system. *Ann Rev Nutr*. 1985, 5: 365-370.

Shantha K, Krishnakantha TP. Microsomal Na^+K^+ATPase activity in retinol deficient albino rat. *Nutr Rep Int*.1987, 36: 573-580.

Shattil JS, Hoxie AJ, Cunningham M, Brass FL. Changes in the platelet membrane glycoprotein IIb, IIIa complex during platelet activation. *J Biol Chem*. 1985, 260: 11107-11114.

Shinitzky M, Barenholz Y. Fluidity parameters of lipid regions determined by fluorescence polarisation. *Biochim Biophys Acta*. 1978, 515: 367-394.

Sigrist-Nelson K, Murer H, Hoper U. Active alanine transport in isolated brush border membranes. *J Biol Chem*. 1975, 250: 5674-5680.

Sinigaglia F, Bisio F, Torti M, Balduini CL, Bertolino G, Balduini C. Effect of glycoprotein IIb-IIIa complex ligands on calcium ion movement and cytoskeleton organisation in activated platelets. *Biochem Biophys Res Commun*. 1988, 154: 258-264.

Smith C, Mitchinson MJ, Aruoma OI, Halliwell B. Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. *Biochem J*. 1992, 286: 901-905.

Sreenivasamurthy V, Krishnamurthy K. Place of spices and aromatics in Indian diets. *Food Science*. 1959, 8: 284-288.

Srinivas L, Shalini VK, Shylaja M. Turmerin: A water-soluble antioxidant peptide from turmeric (Curcuma *longa*). *Arch Biochem Biophys*. 1992, 292: 617-623.

Srinivasan K, Sambaiah K, Chandrashekhara N. Loss of active principles of common spices during domestic cooking. *Food Chemistry*. 1992, 43: 271-274.

Srivastava KC, Bordia A, Verma SK. Curcumin, a major component of food spice
turmeric (Curcuma *longa*) inhibits aggregation and alters eicosanoid metabolism
in human blood platelets. *Prostaglandins Leukot Essent Fatty acids*. 1995, 52: 223-227.

Srivastava KC, Justesen U. Inhibition of platelet aggregation and reduced formation of thromboxane and lipoxygenase products in platelets by oil of cloves. *Prostaglandins Leukot Med*. 1987, 29 (2-3): 11-18.

Srivastava KC. Antiplatelet principles from a food spice clove (Syzgium *aromaticum* L.). *Prostaglandins Leukot Essent Fatty acids*. 1993, 48: 363-372.

Srivastava KC. Extracts from two frequently consumed spices – cumin (Cuminum *cyminum*) and turmeric (Curcuma *longa*) – inhibit platelet aggregation and alter eicosanoid biosynthesis in human blood platelets. *Prostaglandins Leukot Essent Fatty acids*. 1989, 37: 57-64.

Srivastava KC. Isolation and effect of some ginger components on platelet aggregation and eicosanoid biosynthesis. *Prostaglandins Leukot Med*. 1986, 52: 187-198.

Statland BE, Heagan BM, White JG. Uptake of calcium by platelet relaxing factor. *Nature*. 1969, 223: 521, 522.

Stoffel W, Ahrens EH. Isolation and structure of the c16 unsaturated fatty acids in Menhaden body oil. *J Am Chem Soc*. 1958, 80: 6604-6608.

Stubbs CD, Smith AP. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim Biophys Acta*. 1984, 779: 89-137.

Subramanyam BR. The lore and lure of spices. *Indian spices*. 1969, 6: 6-11.

Svoboda ER. Ayurveda – Life, health and longevity. Penguin Books India (P) Ltd.: N. Delhi. 1992, 145.

Swaminathan MS. Essentials of Foods and Nutrition Vol. 1. The Bangalore Printing and Publishing Co. Ltd.: Bangalore. 1998, 67.

Sweadner KJ, Goldin SM. Active transport of sodium and potassium ions: mechanism, functions and regulation. *N Engl J Med*. 1980, 302: 777-783.

Sweadner KJ. Two molecular forms of (Na^+K^+) stimulated ATPase in brain. J Biol Chem. 1979, 254: 6060-6067.

Sweatt JD, Johnson SL, Cragoe EJ, Limbird LE. Inhibitors of Na^+ / H^+ exchange block stimulus – provoked arachidonic acid release in human platelets. *J Biol Chem*. 1985, 260: 12910-12919.

Tandon NN, Kralisz U, Jamieson GA. Identification of glycoprotein IV (CD 36) as a primary receptor for platelet collagen adhesion. *J Biol Chem*. 1989, 264: 7576-7583.

The useful plants of India. Publication and Information Directorate, CSIR: N. Delhi. 1986, 141, 150.

Thimmayamma BVS, Rau P, Radhaiah G. Use of spices and condiments in dietaries of urban and rural families. *Indian J Nutr Dietet*. 1983, 20: 153-161.

Tirtha SSS. The Ayurveda encyclopedia – Natural Secrets to healing, prevention and longevity. Sri Satguru Publications: N. Delhi. 1998.

Touyz RM, Milene FJ, Reinach SG. Platelet and erythrocyte Mg^{+2}, Ca^{+2}, Na^{+}, K^{+} and cell membrane ATP activity in essential hypertension in Blacks. *J Hypertens*. 1992, 10 (6): 571-578.

Tyler DD. Role of superoxide radicals in the lipid peroxidation of intracellular membranes. *FEBS lett*. 1975, 51: 180-183.

Uhl RS. A to Z spices – Handbook of spices, seasonings and flavourings. Technomic Publishing Co. Inc.: Pennsylvania. 2000, 94 – 100.

Ummer C. Indian spices from the leaves of history. *Indian spices*. 1991, 28 (3): 9-19.

Wang J, Hsu M, Teng C. Antiplatelet effect of Capsaicin, *Thromb Res*. 1984, 36: 497-507.

Wiseman H. Dietary influence on membrane function: Importance in protection against oxidative damage and disease. *J Nutr Biochem*. 1996, 7: 2-15.

Wolfe SM, Shulman NR. Adenyl cyclase activity in human platelets. *Biochem Biophys Res Commun*. 1969, 35: 265-272.

Wyngaarden JB, Smith LH. Cecil - Textbook of medicine. WB Saunders company IGaker – Shoin / Saunders: Philadelphia. 1982, Vol. 1: 979-992 & 1160-1164.

Yamaguchi A, Yamamoto Y, Kitagawa H, Tanoue K, Yamazaki H. Ca^{2+} influx mediated through the GP IIb / IIIa complex during platelet activation. *FEBS lett*. 1987, 225: 228-232.

Zieve DP, Greenough III BW. Adenyl cyclase in human platelet activity. *Biochem Biophys Res Commun*. 1969, 35: 462-466.

Zillmann A, Luther T, Muller I, Kotzsch M, Spannagl, Kauke T, Oelschlage U, Zahler S, Engelmann B. Platelet associated tissue factor contributes to the collagen triggered activation of blood coagulation. *Biochem Biophys Res Commun*. 2001, 281: 603-609.