Chapter 1

Bioactivities Of Selected Fruits

Research is to see what everybody else has seen,
And to think what nobody else has thought
– Albert Szent-Gyorgyi

INTRODUCTION

Antioxidants nutrients from natural sources are preferred to use in food or medicine to replace synthetic ones, which are being restricted due to their carcinogenicity [Velioglu, et al., 1998]. Hippocrate's famous dictum "Let food be thy medicine and medicine be thy food" has renewed interest in this context. A number of studies have indicated that oxidative stress is reduced in vitro, by a variety of fruits or fruit extracts that contain significant levels of polyphenols, a class of phytochemicals known to have potent antioxidant properties than antioxidant vitamins. (Wilson et al., 1998; Dianne Hyson et al., 2000). Further, conception of 'French Paradox' has evoked interest to explore the polyphenols from selected fruits and vegetable source.

In this contest, natural, multifunctional, stable, non-toxic and natural bioactive compounds from fruits and vegetables may prefer panacea for disease. India is the second largest producer of fruits and vegetables. It produces a variety of fruits and vegetables due its varied agro-climatic conditions. India is endowed with little known fruits and vegetables, which plays a vital role in nutritional, nutraceutical and/or economical role in rural population. The following selected fruits are one belong to such group.

S.No.	Common Name	Scientific Name	Family
1.	Tiny bitter gourd	*Momordica cymbalaria*	Cucurbitaceae
2.	Wood Apple	*Feronia limonia*	Rutaceae
3.	Indian Hog Plum Variety: Kasturi	*Spondias mangifera* Willd.	Anacardiaceae
4.	Indian Hog Plum Variety: Hal	*Spondias mangifera* Willd.	Anacardiaceae

These selected fruit/ vegetables are very important for their nutraceutical value and they are widely consumed in rural India. Further various pharmaceutical properties were attributed in Ayurvedic medicine for these vegetables. Though they were used from time immemorial, the antioxidant properties and associated bioactive molecules in them have not been reported. Hence, the present work has been initiated to screen the above fruit/ vegetables for their antioxidant properties and elucidation of bioactive compounds from the most promising vegetable. The work presented in this chapter describe in detail regarding antioxidant activity of the following vegetables.

MATERIALS AND METHODS

Plant material

Fresh and healthy fruits of *Momordica cymbalaria,* wood apple, *Spondias mangifera* cv. Kasturi and *Spondias mangifera* cv. Hal were procured from the local market, Mysore, India. Hard shell of wood apple was removed before drying, while in case of other fruit species, whole fruits were washed, sliced and dried in a hot air oven at 50°C for 72 hrs and powdered to 100-120 meshes in an apex grinder [Apex Constructions, London].

Preparation of extracts

Sequential extraction was carried out using solvents of different polarity [from non-polar to polar]. Sequential extraction was employed to resolve the compounds of different polarity effectively and completely. About 100 g of different fruit powder was sequentially extracted using n-hexane, followed by chloroform, ethyl acetate, acetone, methanol at room temperature [25±2°C], at normal atmospheric pressure, by shaking at 100 rpm for 48 hrs**.** Each extract was filtered and concentrated by using rotary evaporator [Buchi Rotavapor R-124, Switzerland]. The concentrated extracts were freeze-dried and stored in refrigerator till used.

ANTIOXIDANT ACTIVITY

DPPH free radical scavenging activity

DPPH [1, 1-diphenyl-2-picrylhydrazyl] radical scavenging activity was determined according to the method described earlier [Blois, 1958; Bondet *et al.,* 1997; Moon and Terao, 1998]. The test samples [10- 100 µl] were mixed with 0.8 ml of Tris-HCl buffer [pH 7.4] to which 1 ml of DPPH [500 µM in ethanol] was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV-Visible Spectrophotometer [UV-160A, Shimadzu co. Japan]. The radical scavenging activity was measured as a decrease in the absorbance of DPPH. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Radical scavenging potential was expressed as EC_{50} value, which represents the sample concentration at which 50 % of the DPPH radicals scavenged.

Superoxide radical scavenging activity

The superoxide scavenging ability was assessed according to the method of Nishikimi, *et al.,* [1972] with slight modifications. The reaction mixture contained NBT [0.1 mM] and NADH [0.1 mM] with or without sample to be assayed in a total volume of 1 ml of Tris-HCl buffer [0.02 M, pH 8.3]. The reaction was started by adding PMS [10 μM] to the mixture, and change in the absorbance was recorded at 560 nm every 30 seconds for 2 min. The percent inhibition was calculated against a control without test sample. Radical scavenging potential was expressed as EC_{50} value, which represents the sample concentration at which 50 % of the radicals scavenged.

Lipid peroxidation inhibitory activity

Lipid peroxidation inhibitory activity was determined according to the method described earlier [Duh and Yen, 1997]. In brief, lecithin [3 mg/ ml phosphate buffer, pH 7.4] was sonicated in dr. Hielscher Gmb_H, UP 50H ultraschallprozessor [DrHielscher GmbH, Teltow, Berlin, Germany]. The test samples [100 μl] were added to 1ml of liposome mixture, control was without test sample. Lipid peroxidation was induced by adding 10 μl $FeCl_3$ [400 mM] and 10 μl L-ascorbic acid [400 mM]. After incubation for 1 hour at 37°C, the reaction was stopped by adding 2 ml of 0.25 N HCl containing 15 % TCA and 0.375 % TBA and the reaction mixture was boiled for 15 min. then cooled, centrifuged and absorbance of the supernatant was measured at 532 nm. Inhibitory activity was expressed as EC_{50} value, which is sample concentration inhibited 50 % of lipid peroxidation.

Metal chelating activity

The chelating of ferrous ions by the test sample was estimated by the method described earlier [Decker and Welch, 1990]. Briefly, the test samples at different concentrations were added to a solution of 2mM $FeCl_2$ [0.05 ml]. The reaction was initiated by the addition of 5mM ferrozine [0.2 ml] and the mixture was vigorously shaken and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the mixture was read at 562nm against a blank. EDTA was used as positive control. Results were expressed as EC_{50} value, which represents the sample concentration at which 50 % of metal chelation occurred.

Platelet-aggregation inhibitory activity

Platelet preparation

Blood samples were taken from healthy volunteers who assured not to have taken any drugs during the 2 weeks prior to the blood sampling. Blood was collected into buffered sodium citrate [3.8 % w/v] pH 6.5 as the anticoagulant at a ratio of 9:1 v/v and used within 3 hr of collection. Platelet-rich plasma [PRP] was obtained by centrifugation of the citrated blood at 1100 rpm for 20 min. 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 10^7 platelets per µl of PRP.

Platelet-aggregation inhibitory assay

Aggregation was measured turbidimetrically at 37°C with constant stirring at 1000 rpm in a Chronolog Dual Channel Aggregometer. About 0.45 ml of PRP was kept stirred at 1200 rpm at 37°C, and aggregation was induced by collagen [10 µM] and. The change in turbidity was recorded with reference to PPP using an omniscribe recorder for at least 5 min. The slope was calculated and it was used as control. Similarly, 100-500 µM of the different fruit extracts and isolated bioactive compounds were added to PRP, incubated for five min after which collagen [10 µM], was added. Platelet aggregation was recorded using an omniscribe recorder for 5 min. The slope was calculated. The difference in the slope between the control and the treated was expressed as percent inhibition of platelet aggregation by different extracts.

Antibacterial activity

Bacterial strains and inoculum preparation

The antibacterial activity was tested against *Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Enterobacter aerogenes, Proteus mirabilis, Yershenia enterocolitica, Micrococcus luteus, Staphylococcus aureus, Enterococcus fecalis, Bacillus subtilis, Bacillus cereus* and *Listeria monocytogenes*. The above bacterial strains isolated from clinical samples were obtained from the Department of Microbiology, Mysore Medical College, Mysore, India. Their cultural characteristics and morphological features were reconfirmed and also subjected to standard biochemical tests [Sneath *et al.,* 1986] before experimentation. The test organisms were maintained on nutrient agar slants.

Agar-well diffusion method

In vitro antibacterial activity was determined by agar-well diffusion method [Mukherjee *et al.,* 1995]. The overnight bacterial culture was centrifuged at 8000 rpm for 10 minutes at 4°C. The supernatant was discarded and bacterial cells were re-suspended in the saline to make suspension 10^5 CFU ml^{-1} and used for the assay. The plating was carried out by transferring bacterial suspension [10^5 CFU ml^{-1}] to sterile Petri plate and mixed with molten Nutrient agar medium [Hi-Media Laboratories Limited, Mumbai, India] and allowed to solidify. About 75 µl of the sample [2 mg ml^{-1}] was placed in the wells and allowed to diffuse for 2 h. Plates were incubated at 37°C for 48 h and the activity was determined by measuring the diameter of inhibition zones. Solvent control and amoxicillin [Galpha Lab. Mumbai, India] were also maintained. The assay was carried out in triplicate.

Minimum inhibitory concentration [MIC]

The minimum inhibitory concentration was determined according to the method described by Jones *et al.,* [1985]. Different concentrations [20 ppm to 300 ppm] of hexane, chloroform, ethyl acetate, acetone and methanol extracts and 100 µl of the bacterial suspension [10^5 CFU ml^{-1}] was placed aseptically in 10 ml of nutrient broth separately and incubated for 24 h at 37°C. The growth was observed both visually and by measuring O.D. at 600 nm at regular intervals followed by pour plating as described above. The lowest concentration of test sample showing no visible growth was recorded as the minimum inhibitory concentration. Triplicate sets of tubes were maintained for each concentration of test sample.

ANTIFUNGAL ACTIVITY

Poisoned Food Technique or radial growth (RG) test

Poisoned Food Technique was determined as described by Golembiewski et al. (1995) method. Different concentration of fruit extracts in ethanol were prepared and mixed with about 20 ml of molten PDA at about 60° C to give a final concentration of 500 ppm (v/v) poured the mixture of medium into petriplates and allowed the medium to set. Agar disc (8 mm) of five day old culture in which pathogen is cultivated was transferred to agar disc of the plates containing media amended with fruit extracts petriplates were

incubated at 27 °C for 5 days. After 5 days, colony diameter was measured at two positions at right angle to each other and calculated the mean diameter of the colony. The relative growth in different treatments was calculated with the following formula.

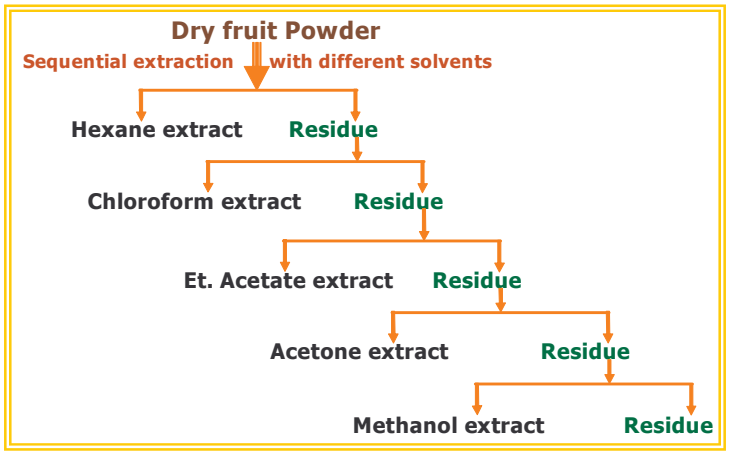
$$\frac{\text{Mean colony diameter in fungicide amended medium}}{\text{Mean colony diameter in un-amended medium (control)}} \times 100$$

RESULTS AND DISCUSSION

Sequential extraction of different fruit powder:

Extraction Method I:

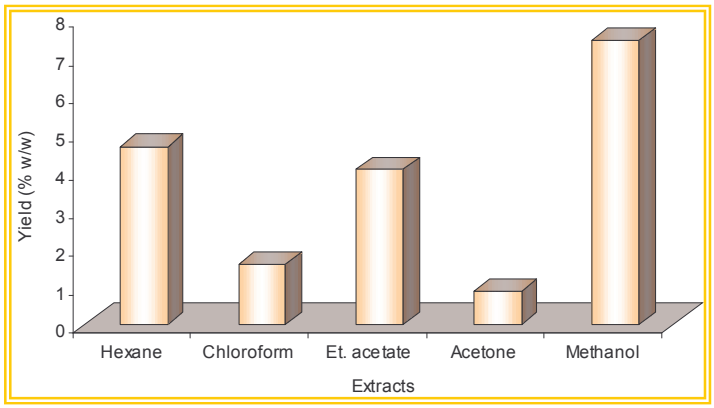
Fig. 1.1: Schematic representation of sequential extraction (method I) of four different fruit powder



Sequential extraction of 100 g of dried fruit powder from *Momordica cymbalaria,* wood apple, *Spondias mangifera* cv. Kasturi fruit and *Spondias mangifera* cv. Hal powder were carried out using hexane, chloroform, ethyl acetate, acetone and methanol **[Fig. 1.1]**.

Yield of the fruit extracts:

Fig. 1.2: Yield of the *Momordica cymbalaria* fruit extracts with different solvents.

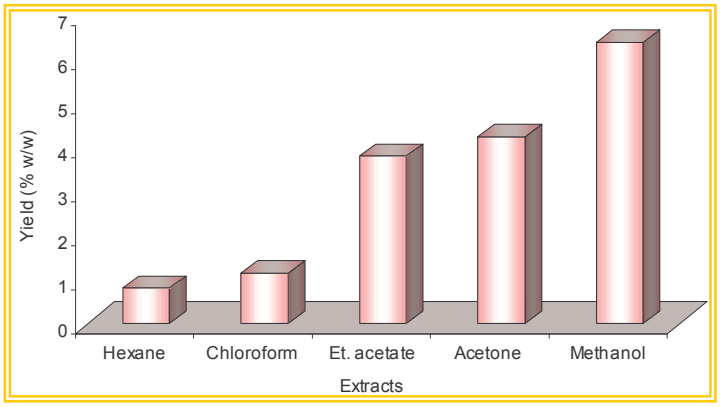


Momordica cymbalaria fruit yielded highest methanol extract (7.40 g) followed by hexane (4.60 g) and chloroform extracts (4.05 g) **[Fig. 1.2]**. Increase in yield of the solvent extracts followed the following order:

Acetone < Chloroform < Ethyl acetate < Hexane < Methanol

Wood apple, methanol extract was observed to have highest yield **[Fig. 1.3]**. Lowest yield was observed in hexane (0.80 g) and it increased gradually up to methanol (6.40 g). Yield increased with increase in the polarity of the solvents. Increase in yield of the solvent extracts followed the following order:

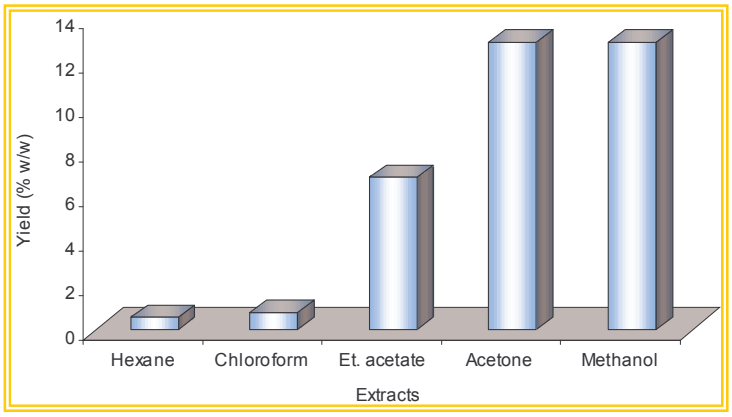
Fig. 1.3: Yield of the wood apple fruit extracts with different solvents.



Hexane < Chloroform < Ethyl acetone < Acetone <Methanol

Fig. 1.4: Yield of the *Spondias mangifera* cv. Kasturi fruit extracts with different solvents.

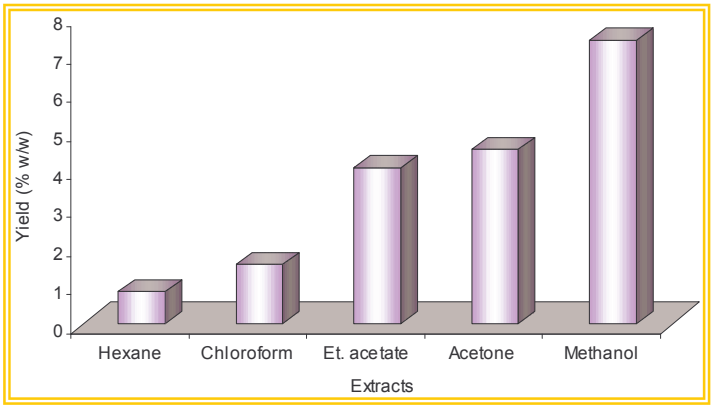
Indian hog plum (cv. Kasturi) fruit powder yielded highest methanol extract (12.95). Lowest yield was observed in hexane (0.56 g) and it increased gradually up to methanol **[Fig. 1.4]**. Yield of the extracts increased as the polarity increased. Increase in yield of the solvent extracts followed the following order:



Hexane < Chloroform < Ethyl acetone < Acetone <Methanol

Fig. 1.5: Yield of *Spondias mangifera* cv. Hal fruit extracts with different solvents.

Indian hog plum (cv. Hal) fruit powder yielded highest methanol extract (7.40 g). Lowest yield was observed in hexane (0.85 g) and it increased gradually up to methanol **[Fig. 1.5]**. Yield of the extracts increased as the polarity increased. Increase in yield of the solvent extracts followed the following order:



Hexane < Chloroform < Ethyl acetone < Acetone <Methanol

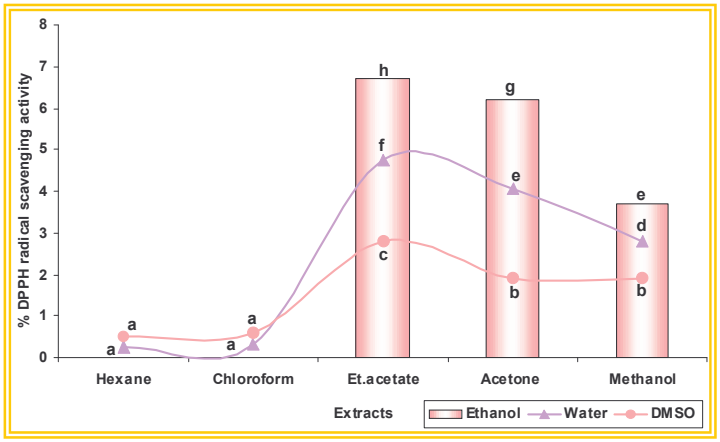
Among different solvents used for extraction from different fruits, methanol extract was found to be having highest yield.

In the present study, sequential extraction of will be helpful to extract wide range of [both non-polar and polar] bioactive molecules in a plant material. Highest yield was observed in methanol extracts in all the fruit extracts. It appears that methanol is the best solvent for extraction of polyphenols, lactones, phenones, quassinoids, flavones, saponons and some terpenoids as observed in earlier studies [Suhaj, 2006]. It has also been indicated that acetone/water mixtures are more useful for extracting polyphenols from proteic matrices, since they appear to degrade the polyphenols-protein complexes [Cowan, 1999].

ANTIOXIDANT ACTIVITY OF THE DIFFERENT FRUIT EXTRACTS

To find out the potential antioxidant activity rich fruit, five different solvent extracts of the *Momordica cymbalaria*, wood apple, *Spondias mangifera* cv. Kasturi and *Spondias mangifera* cv. Hal were subjected to DPPH radical scavenging assay. BHA was used as a standard for antioxidant assay.

Fig. 1.6: DPPH radical scavenging activity of *Momordica cymbalaria* fruit extracts

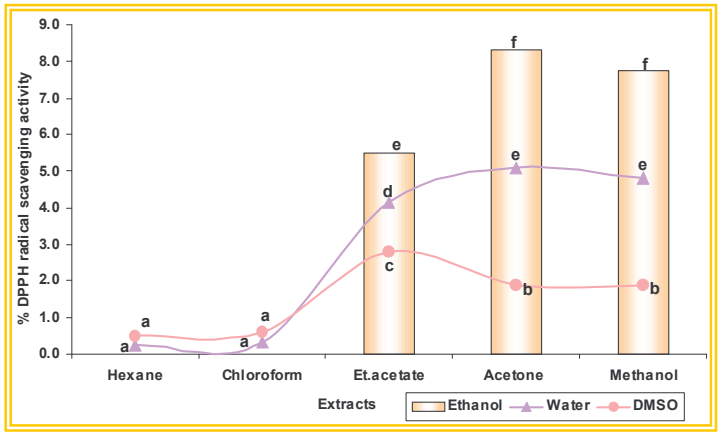


Values with different letters [a, b, c, d, e, f, g and h] differ significantly at P<0.05

Momordica cymbalaria:

Momordica cymbalaria fruit extracts were found to have very less DPPH radical scavenging activity. Highest activity of only 2.8 % of the radicals was scavenged by ethyl acetate extract dissolved in ethanol, while activity shown to be reduced by 50% and 25% when sample was dissolved in DMSO water respectively **[Fig. 1.6]**. This increase may be due to the fact that antioxidant component responsible for the activity is more soluble in ethanol than in DMSO.

Fig. 1.7: DPPH radical scavenging activity of wood apple fruit extracts



Values with different letters [a, b, c, d and f] differ significantly at P<0.05

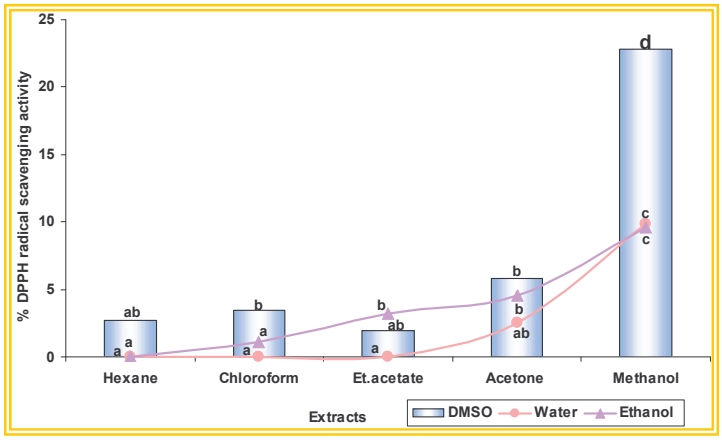
Wood apple:

Wood apple fruit extracts were also found to have less DPPH radical scavenging activity. Acetone and methanol extracts were active. Acetone extract dissolved in ethanol recorded highest antioxidant activity of 8.30%, which is four times higher than DMSO dissolved sample **[Fig. 1.7]**.

***Spondias mangifera* cv. Kasturi:**

Methanol extract of *Spondias mangifera* cv. Kasturi fruit showed highest DPPH radical scavenging activity of 22.8%. Increase in antioxidant activity observed in almost all the extracts when the test sample dissolved in ethanol and it further increased in DMSO solvent **[Fig. 1.8]**. Percentage of antioxidant activity doubled from the ethanol and water dissolved (9.6 and 9.85 %) to DMSO dissolved (22.8 %) methanol extract of *Spondias mangifera* cv. Kasturi. Antioxidant activity of an extract may be depending upon the degree of solubility of antioxidant component of the extracts.

Fig. 1.8: DPPH radical scavenging activity of *Spondias mangifera* cv. Kasturi

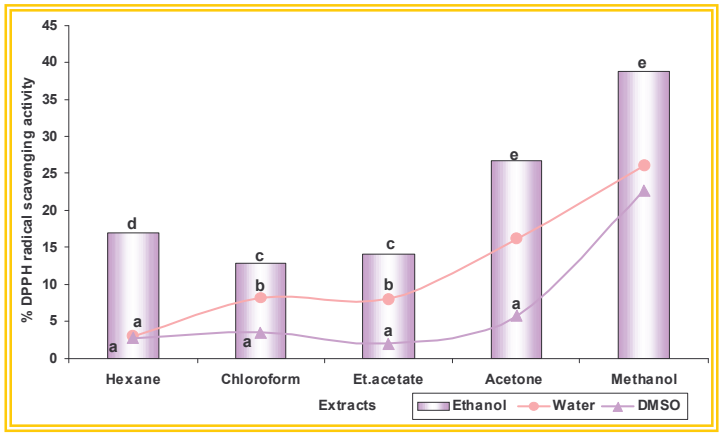


Values with different letters [a, b, c and d] differ significantly at P<0.05

Spondias mangifera cv. Hal:

All *Spondias mangifera* fruit extracts showed considerable DPPH scavenging activity. Methanol extract exhibited highest potential radical scavenging ability of 38.8%. Except, hexane extract, remaining extracts exhibited a steady increase in the antioxidant activity with increase in polarity of the solvent used for extraction **[Fig. 1.9]**.

Fig. 1.9: DPPH radical scavenging activity of *Spondias mangifera* cv. Hal



Values with different letters [a, b, c, d and e] differ significantly at P<0.05

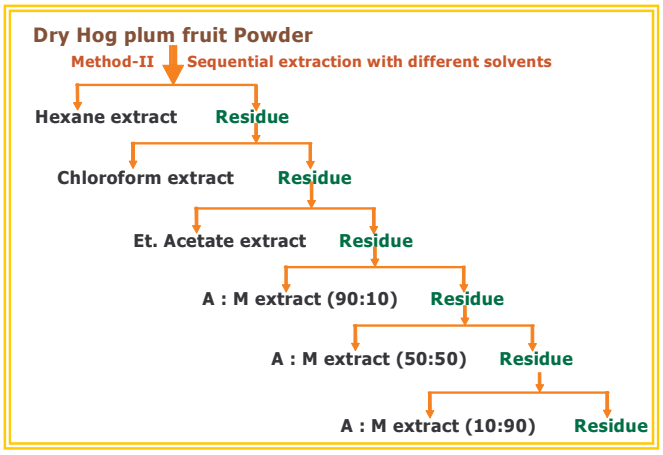
Screening of *Momordica cymbalaria*, wood apple, *Spondias mangifera* cv. Kasturi and *Spondias mangifera* cv. Hal for potential source of antioxidants, lead to the discovery of considerable percentage of antioxidant activity in two different varieties of *Spondias mangifera*. After taking into consideration of the extract yield against antioxidant activity, *Spondias mangifera* cv. Kasturi fruits yielded higher methanol extract (12.95 % w/w) compared to the *Spondias mangifera* cv. Hal variety (7.4 % w/w), but, the antioxidant activity of former was lower (22.8 %), than that of the later (38.8 %).

To exploit the higher yield of *Spondias mangifera* cv. Kasturi and also to find out suitable and efficient sequential solvent extraction system or solvent mixture for effective extraction of available antioxidant components in the fruit, following extraction method carried out apart from the above mentioned extraction system:

Spondias mangifera cv. Kasturi Vs *Spondias mangifera* cv. Hal:

Extraction Method II

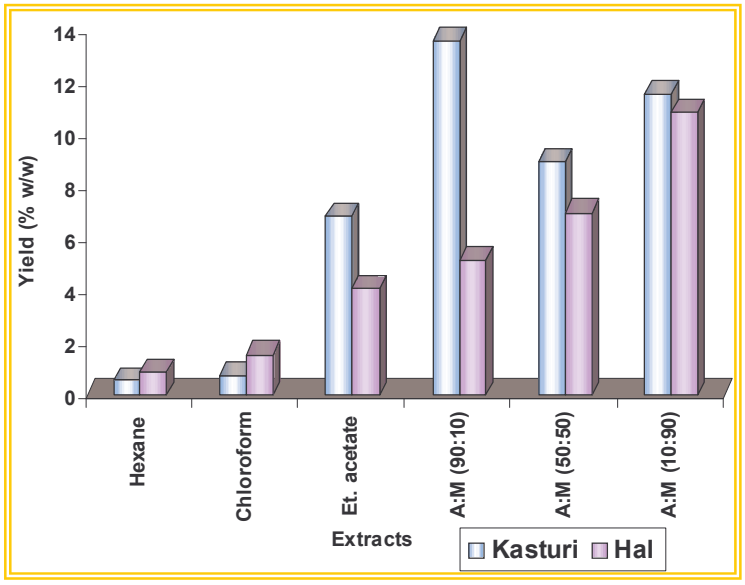
Fig. 1.10: Schematic representation of sequential extraction (method II) of *Spondias mangifera* cv. Kasturi and *Spondias mangifera* cv. Hal. fruit powder



Sequential extraction of 100 g of dried *Spondias mangifera* cv. Kasturi and *Spondias mangifera* cv. Hal fruit powder using hexane, chloroform, ethyl acetate, acetone : methanol (A : M) (90:10), A : M (50:50) and A : M (10:90) was carried out in the manner depicted in **figure 1.10**., and their corresponding yield is shown in **fig. 1.11**.

Yield of the fruit extracts:

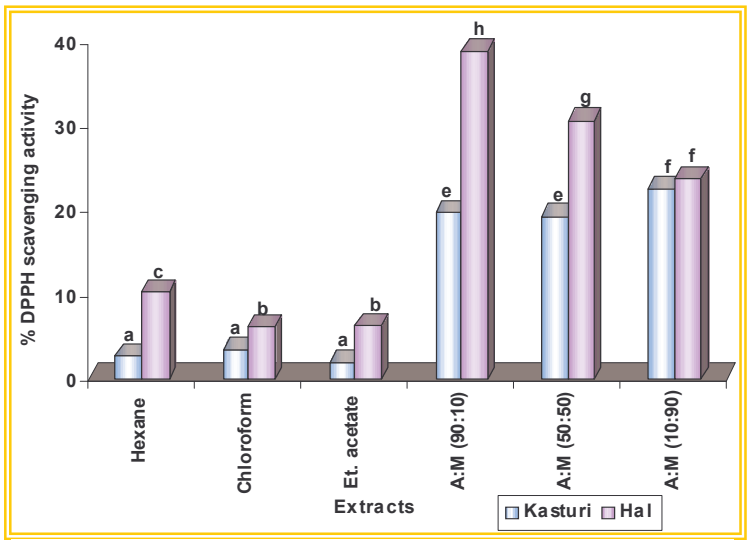
Fig. 1.11: Yield of *Spondias mangifera* cv. Kasturi and *Spondias mangifera* cv. Hal fruit extracts from extraction method II.



Spondias mangifera cv. Kasturi yield was higher than that of the *Spondias mangifera* cv. Hal. There was no significant increase in yield of the any extract in case of the former compared to that of the first method of extraction **[Fig. 1.4 & 1.11]**. But, there was significant increase (30 %) in yield component of the active extract in *Spondias mangifera* cv. Hal **[Fig. 1.5 & 1.11]**.

Antioxidant activity:

Fig. 1.12: DPPH Radical scavenging activity of *Spondias mangifera* var Kasturi and *Spondias mangifera* cv. Hal fruit extracts with different solvents.



Values with different letters [a, b, c, d, e, f, g and h] differ significantly at P<0.05

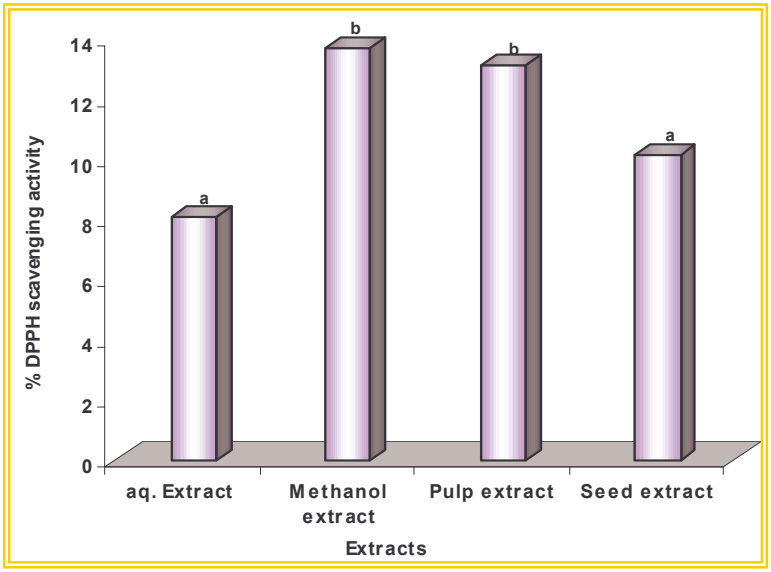
These extracts when tested for their antioxidant activity by DPPH assay, highest antioxidant activity of 38.77 % was observed in fruits of *Spondias mangifera* cv. Hal extracted in A : M (90:10) solvent mixture **[Fig. 1.12]**. This activity was similar to that of the methanol extract in first method **[Fig. 1.9]**. Antioxidant activity decreased with the increase of the methanol concentration in solvent mixture. Similarly, highest antioxidant activity of 22.5 % **[Fig. 1.12]** was observed in fruits of *Spondias mangifera* cv. Kasturi extracted in A : M (10:90) solvent mixture. This activity was similar to that of the methanol extract in first method **[Fig. 1.8]**.

Though there was an increase in the yield of the active extract in second method, but, this increase in extract yield was not expedient in effective extraction of the antioxidant component of the extracts in any of the sequential extraction systems or solvent mixtures. Hence, **the highest antioxidant activity (38.8 %) showed by *Spondias mangifera* cv. Hal extracted from methanol** was found to be a better source of antioxidant screened among the other fruits. This fruit in fresh form was also tested for DPPH radical scavenging activity.

Fresh Extracts:

Fresh fruits of *Spondias mangifera* cv. Hal extracted in methanol showed highest antioxidant activity of 13.7 %, while it was lowest (8.1%) in aqueous extract of the whole fruit **[Fig. 1.13]**. When pulp and seed were separated and extracted in methanol for the radical scavenging activity, pulp extract exhibited higher (13.1 %) activity than seed extract (10.2 %).

Fig. 1.13: DPPH Radical scavenging activity *Spondias mangifera* var. Hal fresh fruit extracts.



The high antioxidant activity of methanol extract may be due to the cumulative effect of its potential antioxidant compounds along with phenolics**.** Phenolics are very important bioactive constituents of *Spondias mangifera* fruit and other fruit crops which are known for their radical scavenging ability due to their hydroxyl groups [Hatano, *et al.,* 1989]. DPPH radical scavenging activity may be attributed to the presence of hydrogen-donating ability of -OH and -CH_3 groups in extracts/ compounds [Chen and Ho, 1995; Nikolaos, *et al.,* 2003].

ANTIOXIDANT ACTIVITIES OF THE METHANOL EXTRACT

Methanol extract of *Spondias mangifera* cv. Hal showed highest antioxidant activity among all the extracts. So, this extract was screened for different antioxidant assays such as DPPH radical scavenging activity, superoxide radical scavenging activity, lipid peroxidation inhibitory [LPI] activity and metal chelating activity.

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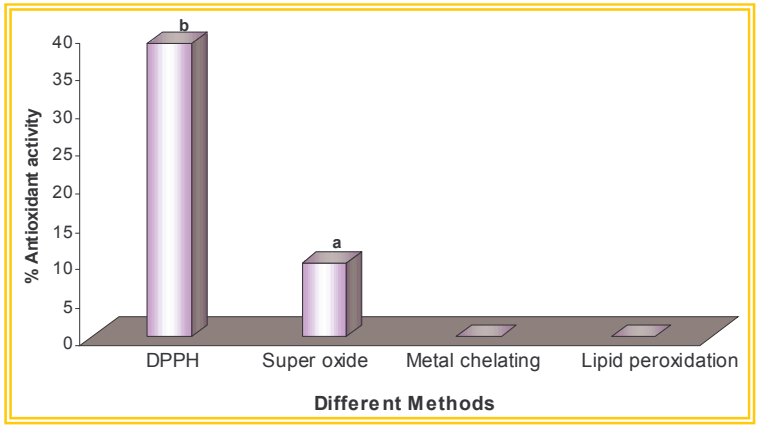
DPPH Radical scavenging activity

Fig. 1.14: Antioxidant activities of *Spondias mangifera* cv. Hal fruit extracts.

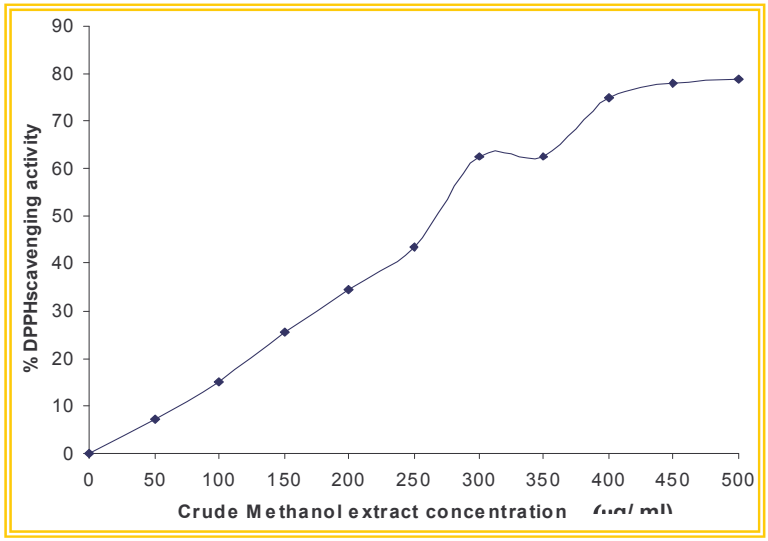


Values with different letters [a and b] differ significantly at P<0.05.

Methanol extract showed highest antioxidant activity compared to that of the other methods of antioxidant assays **[Fig. 1.14]**. So, the concentration required for scavenging of 50% radicals was carried out for DPPH assay. Methanol extract of 290µg of was required for 50% quenching of the DPPH free radicals **[Fig. 1.15].**

Superoxide radical scavenging activity

Fig. 1.15: EC_{50} value for DPPH Radical scavenging activity of methanol extract of *Spondias mangifera* cv. Hal.



Super oxide radicals were successfully quenched by methanol extract **[Fig. 1.14]**. Superoxide anion plays an important role in the formation of reactive oxygen species [ROS] such as hydrogen peroxide, hydroxyl radical, and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA [Dahl and Richardson, 1978; Halliwell and Gutteridge, 1989; Pietta, 2000].

Methanol extract of *Spondias mangifera* cv. Hal did not show lipid peroxidation inhibitory activity and metal chelating activity.

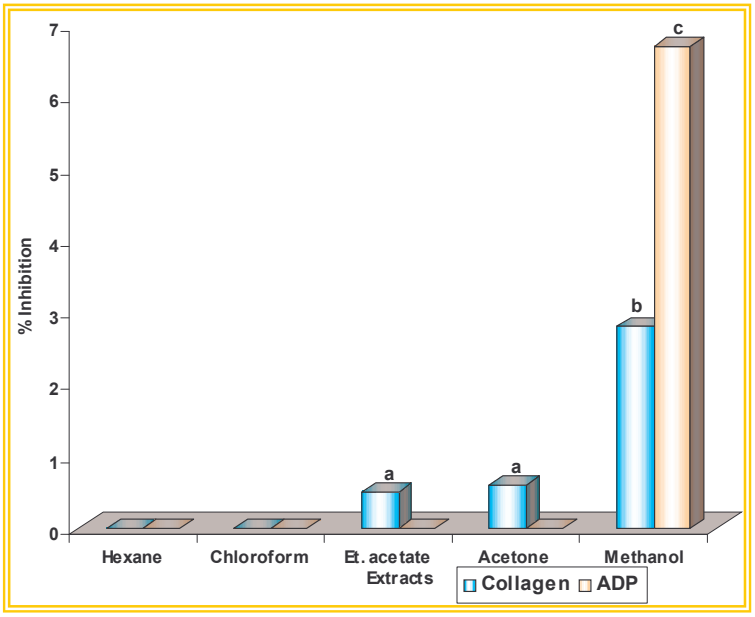
PLATELET-AGGREGATION INHIBITORY ACTIVITY

Among the different fruits viz., *Momordica cymbalaria,* wood apple, *Spondias mangifera* var, Kasturi and *Spondias mangifera* cv. Hal, screened for platelet-aggregation inhibitory activity, except *Momordica cymbalaria,* other fruits showed activity.

Wood apple

Methanol extract (500 µg) of wood apple exhibited platelet aggregation inhibitory activity of 6.7 and 2.8 % against ADP and collagen respectively **[Fig. 1.16]**. Ethyl acetate and acetone extracts showed insignificant activity. Whereas, hexane, chloroform and acetone extracts did not show platelet aggregation inhibitory activity.

Fig. 1.16: Platelet-aggregation inhibitory activity of wood apple fruit extracts.

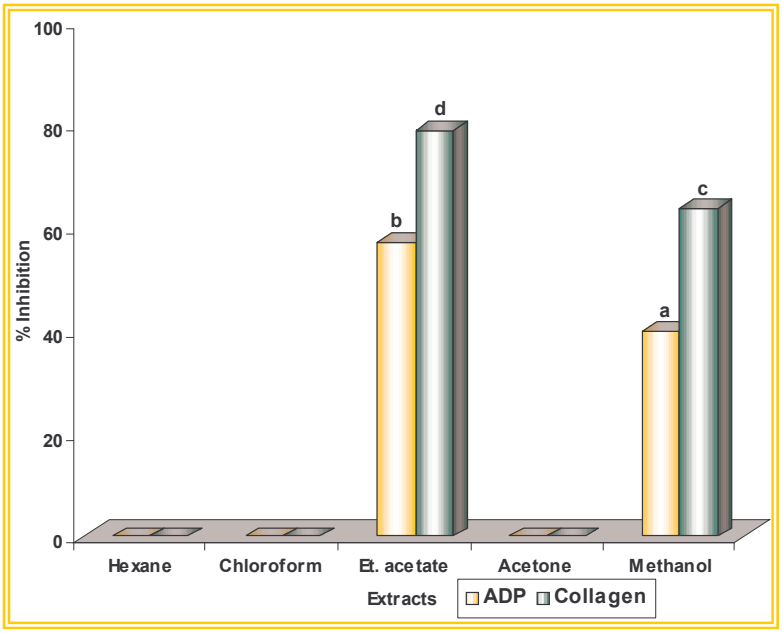


Values with different letters [a, b, and c] differ significantly at P<0.05

Spondias mangifera cv. Kasturi

Ethyl acetate extract (500 µg) of *Spondias mangifera* cv. Kasturi exhibited highest platelet aggregation inhibitory activity of 57.3 and 78.9 % followed by methanol extract inhibiting 40 and 63.9 % against ADP and collagen respectively **[Fig. 1.17]**. Hexane, chloroform and acetone extracts did not show platelet aggregation inhibitory activity.

Fig. 1.17: Platelet-aggregation inhibitory activity of *Spondias mangifera* var. Kasturi fruit extracts.

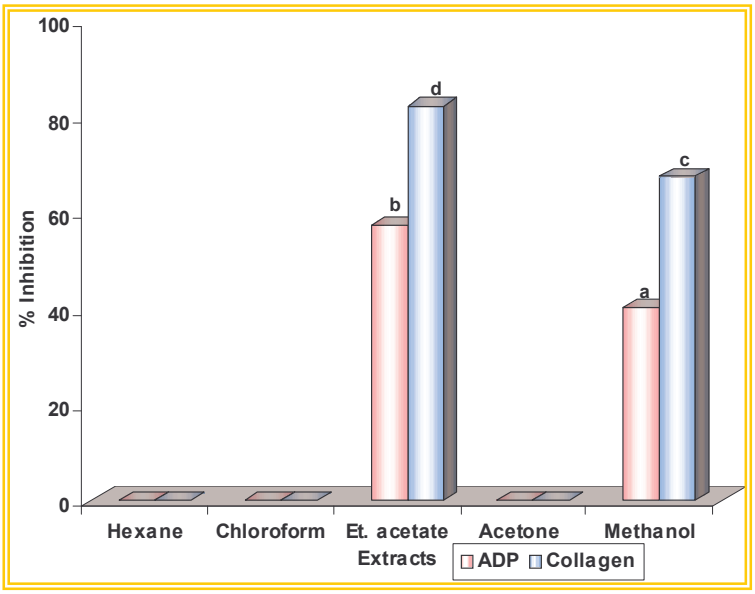


Values with different letters [a, b, c, and d] differ significantly at P<0.05

Spondias mangifera cv. Hal

Ethyl acetate extract (500 µg) of *Spondias mangifera* cv. Hal similar to that of the *Spondias mangifera* cv. Kasturi showed highest platelet aggregation inhibitory activity of 57.3 and 82.3 % followed by methanol extract inhibiting 40 and 67.4 % against ADP and collagen respectively **[Fig. 1.18]**. Hexane, chloroform and

Fig. 1.18: Platelet-aggregation inhibitory activity of *Spondias mangifera* var. Hal fruit extracts.

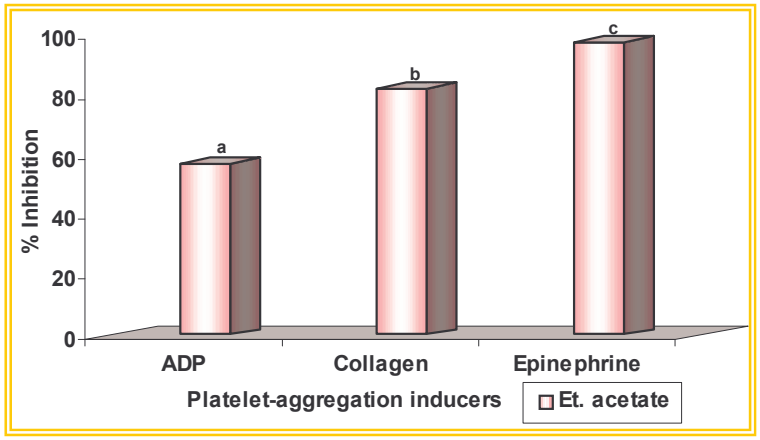


Values with different letters [a, b, c and d] differ significantly at P<0.05

acetone extracts did not show platelet aggregation inhibitory activity.

Ethyl acetate extract of *Spondias mangifera* cv. Hal found to show higher platelet aggregation inhibitory activity than *Spondias mangifera* cv. Kasturi against collagen agonist. To test the potential inhibitory nature of ethyl acetate extract, it was also tested against another agonist 'epinephrine'. It was also promising against epinephrine revealing 98 % activity **[Fig. 1.19]**.

Fig. 1.19: Platelet-aggregation inhibitory activity of *Spondias mangifera* var. Hal fruit extracts against different inducers.



Values with different letters [a, b, and c] differ significantly at P<0.05

Platelets readily aggregate in response to a variety of endogenous substances and they can initiate thrombus formation, leading to ischemic diseases. In addition, the interactions between platelets and blood vessel walls are important in the development of thrombosis and cardiovascular diseases [Ross, 1978; Hirsh, 1987; Dinerman and Mehta, 1990]. Therefore, the inhibition of platelet function represents a promising approach for the prevention of thrombosis.

Platelet aggregation consists of a series of exquisitely co-coordinated responses. The membrane surface of human platelets is responsible for a variety of reactions to various agonists. Collagen is an important platelet agonist, which enhances tissue factor activity rapidly on the platelet surface bound to monocytes and neutrophils, thus playing a crucial role in physiological haemostasis (Zillmann et al., 2001). Not only that it is associated with a burst in hydrogen peroxide, an oxidant species but also contributes to activation of platelets by acting as secondary messenger (Pignatelli et al., 1999a). It also stimulates arachidonic acid metabolism through the production of thromboxane A_2, a potent platelet aggregator as well as liberation of IP_3 and calcium mobilization (Pignatelli et al., 1999b).

Glycoprotein IV (CD 36) present on platelets plays an important role by acting as a specific receptor for collagen in the early stages of aggregation (Tandon, et al., 1989).

Another receptor Glycoprotein VI has also been identified under flow condition specifically again for collagen induced aggregation (Moroi and Jung, 2004).

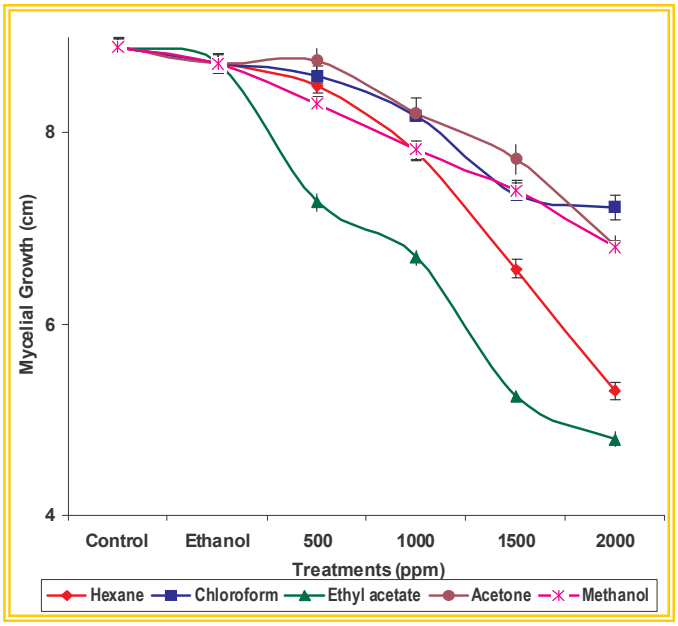
Hence it may be said that wood apple extracts of acetone and methanol and *Spondias mangifera*'s ethyl acetate extracts were acting on the platelet surface resulting in their binding to glycoprotein IV and VI. As a result of this, collagen induced aggregation was being inhibited. Not only that, due to the binding of these components to the glycoproeins, release of hydrogen peroxide, which acts as a secondary messenger was found to be affected.

ANTIFUNGAL ACTIVITY

Among the different fruits viz., *Momordica cymbalaria,* wood apple and *Spondias mangifera* cv. Hal screened for antifungal activity, all fruits showed activity. *Spondias mangifera* cv. Kasturi was not tested for antifungal activity and it was confined to *Spondias mangifera* cv. Hal only.

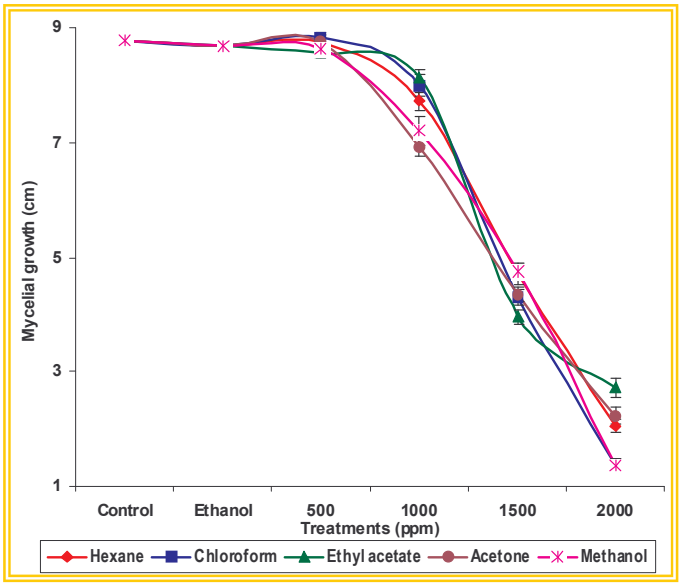
Momordica cymbalaria :

Fig. 1.20: Antifungal activity of *Momordica cymbalaria* fruit extracts .



Ethyl acetate extract of *Momordica cymbalaria* fruit inhibited the mycelia growth better than chloroform, acetone or methanol. Ethyl acetate extract concentration of 1000 ppm restricted the mycelial growth to 7.28 cm while, 1500 ppm of hexane and 2000 ppm of other extracts restricted the same **[Fig. 1.20]**. With the highest concentration of 2000 ppm ethyl acetate extract restricted the mycelial growth to a least of 4.80 cm. So, ethyl acetate extract of *Momordica cymbalaria* may be having component(s) responsible for such antifungal activity.

Fig. 1.21: Antifungal activity of wood apple fruit extracts.

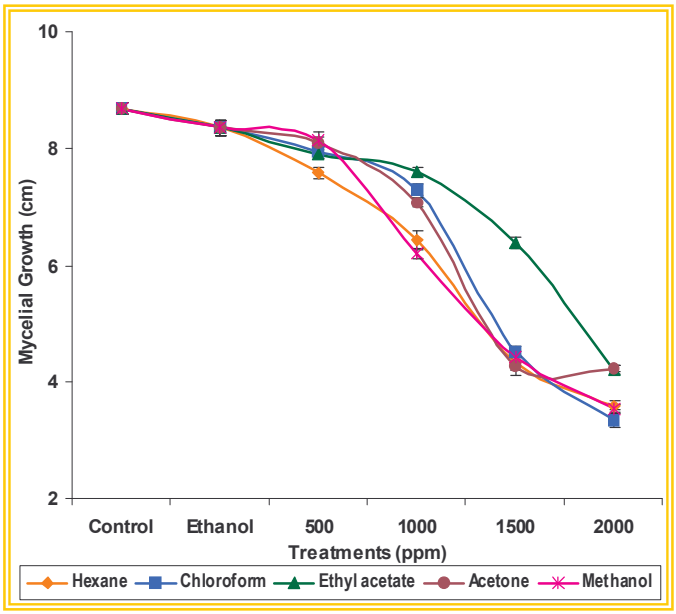


Wood apple:

Methanol and chloroform extracts of wood apple at 2000 ppm concentration inhibited and restricted the fungal growth to 1.35 cm which is highest, compared to that of hexane (2.05 cm), acetone (2.23 cm) and ethyl acetate (2.73 cm) extracts **[Fig. 1.21]**. Observing the zone of inhibition, wood apple extracts (1.35 cm) were found to 4-5 times effective and having higher antifungal properties compared to that of the *Momordica cymbalaria* extracts (4.80 cm).

Spondias mangifera cv. Hal:

Fig. 1.22: Antifungal activity of fruits of *Spondias mangifera* var. Hal extracts.



Chloroform, methanol and hexane extracts from the fruits of *Spondias mangifera* cv. Hal at 2000 ppm concentration restricted the mycelial growth of *Colletotrichum capsici* to 3.35 to 3.55 cm, while, ethyl acetate and acetone extracts inhibited 4.2 cm **[Fig. 1.22]**.

Colletotrichum capsici, a widely distributed fungal pathogen in the tropical world, causing anthracnose disease in commercially important fruits and vegetables. Wide array of fungicide sprays on the standing crop as well as on the harvested produce as pretreatments, reported to cause

biological magnification finally hazard to human health. Environmental harmful and health hazardous practice can be substituted by the use of residue free, harmless, non-toxic, environmental friendly natural products. In the wake of this, three different tropical fruits were selected for their potential antifungal activity, as they were reported in the involvement of preformed antifungal compounds for the resistance of subtropical fruits to fungal decay (Prusky and Keen, 1993).

Among five extracts of three fruit species tested for the antifungal activity against this fungal disease revealed that, concentration of 2000 ppm of chloroform and methanol extracts of wood apple (1.35 cm), followed by hexane, chloroform and methanol extracts of *Spondias mangifera* (3.35 to 3.6 cm) and ethyl acetate extract of *Momordica cymbalaria* (4.8 cm) inhibited the mycelial growth to 1.35 cm.

ANTIBACTERIAL ACTIVITY OF HOG PLUM EXTRACTS

Spondias mangifera cv. Hal showed antioxidant activity, platelet-aggregation inhibitory activity and antifungal activity. So, antibacterial activity was restricted to these fruit extracts only. Chloroform extract of *Spondias mangifera* fruits, exhibited effective antibacterial activity against five out of 12 bacteria screened viz. *Bacillus Subtilus*, *Micrococcus luteus*, *Streptococcus faecalis, Staphylococcus aureus*, *Salmonella typhi*, *Enterobacter aerogenes*, *Klebsiella*

Table 1.1: Minimum inhibitory concentrations [MIC] for different extracts of *Spondias mangifera* cv. Hal

Bacteria	MIC [in ppm] * for *Spondias mangifera* extracts				
	Hexane extract	Chloroform extract	Ethyl acetate extract	Acetone extract	Methanol extract
B. Subtilus	140^{c}	60^{a}	140^{c}	180^{d}	--
M. luteus	180^{d}	60^{a}	--	--	220^{e}
S. faecalis	200^{e}	100^{b}	--	--	--
S. aureus	--	--	--	--	200^{e}
S. typhi	--	--	100^{b}	--	--
E. aerogenes	--	--	--	--	--
K. pneumoniae	--	180^{d}	--	--	--
S. dysenteriae	--	100^{b}	--	180^{d}	--
E. coli	--	--	--	--	--
P. mirabilis	--	--	--	--	--
P. aureginosa	--	--	--	--	--
Y. enterocolitica	--	--	--	--	--

* Each value represents mean of three different observations.
Mean values with different superscripts [a, b, c, d and e] differ significantly at P<0.05

pneumoniae, *Shigella dysenteriae*, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonos aureginosa*, and *Yershenia enterocolitica*. Chloroform extract showed inhibition similar to hexane extract, in addition to that it also showed inhibition against *K. pneumoniae* and *S. dysenteriae*. Ethyl acetate extract showed inhibitory effect as shown by acetone extract, but, in addition to that, ethyl acetate was showing inhibition against *S. dysenteriae* while acetone extract against *S. dysenteriae.* Methanol extract showed inhibition against *M. luteus,* and *S.aureus*. The chloroform extract of *Spondias mangifera* exhibited highest antibacterial activity against wide range of bacteria, when compared to other solvent extracts **[Table 1.1].** However, *E. aerogenes E. coli, P. mirabilis, P. aureginosa and Y. enterocolitica* were not inhibited by any of the solvent extracts of *Spondias mangifera*.

Different solvent extracts of *Spondias mangifera* showed MIC values ranged from 60- 220 ppm **[Table 1.1].** The high antibacterial activity i.e. low MIC for wide range of bacteria was exhibited by chloroform extract. Chloroform extract was very effective against *B. subtilis, M. luteus* with MIC of 60 ppm and also inhibited the growth of *S. faecalis* and *S. dysenteriae* with MIC of 100ppm, while *K. pneumoniae* was completely inhibited at 180 ppm **[Table 1.1].** Lack of outer polysaccharides layer in Gram-positive bacteria may be responsible for more permeable to amphipathic compounds [Cowan 1999]. High resistance of Gram-negative bacteria to *Spondias mangifera* extracts may be due to the presence of their outer layer composed of lipo-polysaccharides.

The results indicated differential activity between polar and non-polar solvent extracts of *Spondias mangifera*. According to Cowan [1999], polar extracts are less effective against microbes than non-polar extracts. It may be due to the presence of polysaccharides, polypeptides and lectins that are more effective as inhibitors of pathogen adsorption and would not be identified in the screening techniques commonly used.

CONCLUSION

In the present investigation, screening of hog plum extracts for different functional properties has revealed that, they are potential inhibitors of platelet aggregation and also scavengers of free radicals and super oxide radicals. Hog plum extracts also showed antibacterial and antifungal properties. The highest platelet aggregation inhibitory activity exhibited by ethyl acetate extract and the highest antioxidant activity shown by methanol extract of hog plum cv. Hal, prompted us to select the *Spondias mangifera* cv. Hal, for the present study. Hence, a detailed studies "Characterization of bioactive and biochemical changes associated during fruit growth maturation and during storage was carried out on *Spondias mangifera* cv. Hal. Accordingly, further, purification and characterization of bioactive compounds from the active extracts of *Spondias mangifera* fruit was carried out. The details are presented in the next chapter.