



Effect of antibacterial and antioxidant properties of flavonoid rich fraction from the flowers of *Mimusops elengi*

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Abstract

To evaluate the antibacterial and antioxidant activities of flavonoid rich fraction from the flowers of *Mimusops elengi*. In this background, the *in vitro* antibacterial and antioxidant activity was demonstrated by ABTS radicals, lipid peroxidation, nitric oxide scavenging activities and antibacterial activity was evaluated first by studying inhibiting bacterial growth by discs diffusion and minimum inhibitory concentration. Total phenolic and flavonoid content were found respectively (72.36 ± 2.34) mg gallic acid equivalent/g, and (58.34 ± 2.45) mg quercetin equivalent/g. The flavonoid rich fraction displayed significant scavenging activity of some radicals such as ABTS EC₅₀ value 57.23 µl/ml. Flavonoid rich fraction from the flowers of *Mimusops elengi* showed *in vitro* antibacterial activity by disc diffusion maximum inhibiting growth of 16.32mm in *Staphylococcus aureus*. Our results show that flavonoid rich fraction from the flowers of *Mimusops elengi* has good antioxidant activity and interesting anti-inflammatory properties. Flavonoid rich fraction from the flowers of *Mimusops elengi* can be used to prevent bacterial infection and oxidative processes.

Keywords: antioxidant; anti-inflammatory; *Mimusops elengi*

Introduction

Plants have the ability to synthesize a wide variety of phytochemical compounds as secondary metabolites. Many of the phytochemicals have been used to effectively treat the various ailments for mankind. World Health Organization has made an attempt to identify all medicinal plants used globally and listed more than 20,000 species. Most of the medicinal plant parts are used as raw drugs and they possess varied medicinal properties (Mahesh and Sathish, 2008) [6]. Antibacterial resistance is defined as the resistance of bacteria to treatment with antibiotic drugs that was originally found to be effective for the treatment of infection caused by that microorganism. This means that antibiotics become ineffective against resistant bacteria allowing infections to persist in patients, putting them at increased risk of worse clinical outcomes and death. In fact, on average, the mortality rate for patients with infections caused by non-resistant bacteria is less than half of that of people with a resistant form of the same infection. Antibiotic resistance is present worldwide and new resistance mechanisms are continuing to emerge, strongly increasing the risk of spread of resistant strains.

Bacterial infections are one of the leading causes of infectious diseases in Africa. Poverty and poor health infrastructure continue to be an impediment to effective health care service delivery (Simoes *et al.*, 2012) [10]. On a global scale, life-threatening infections caused by these

pathogenic prokaryotic microorganisms have become an important cause of morbidity and mortality in immune-compromised patients in developing countries (Ara *et al.*, 2009) [1]. Despite the availability of a wide range of antibiotics, bacteria are constantly developing resistance to these agents, which makes it difficult for the concerted effort of combating infectious diseases.

Materials and methods

Plant material

Flower of *Mimusops elengi* were obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-312/2021. The flowers were sufficiently air-dried in 5 days at the ambient room temperature, while the flower was cut into smaller pieces and air-dried in 7 days.

Phytochemical screening

The aqueous extract of from the flowers of *Mimusops elengi* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973).

Thin layer chromatography profile

The flavonoid rich fraction of *Mimusops elengi* were loaded on to pre coated TLC (60 F₂ 54) and it was developed using solvent system in the ratio of 1:0.5:0.1 (Hexane, Chloroform and Methanol) visible and the non-visible spot given and it is fluorescent with UV light at 360nm.

Antibacterial activity

The antibacterial activities of the flavonoid rich fraction of *Mimusops elengi* were assayed using the disc diffusion method. Bacteria were grown overnight on Mueller Hinton agar plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to $\sim 3 \times 10^8$ CFU/ml. A sterile cotton swab was dipped into the suspension and the swab rotated several times with firm pressure on the inside wall of the tube to remove the excess fluid. The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90° to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile disc of 9 mm diameter. Each disc was placed firmly on to the agar to provide uniform contact with the bacteria. Alkaloid rich fraction (1 mg) was weighed and dissolved in 1 ml of chloroform. The different concentration of flavonoid rich fraction of *Mimusops elengi* was introduced on to each disc and the control disc received only 7% ethyl acetate. The plates were incubated at 37°C for 24 h and the inhibition zone was measured and calculated. The experiments were carried out in duplicate three times. The results (mean value, $n=3$) were recorded by measuring the zones of growth inhibition surrounding the discs.

Minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of the flavonoid rich fraction of *Mimusops elengi* were determined by dilution method (Brantner and Grein, 1994). The strains were grown in Mueller Hinton broth to exponential phase with an A560 of 0.8, representing 3.2×10^8 CFU/ml. Different dilutions of the flavonoid rich fraction of *Mimusops elengi* were prepared to give solutions of 25, 50, 75, and 100 µg/ml. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 10^6 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% ethyl acetate used as bacterial control, 4.5 ml of uninoculated MH broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 °C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at 560 nm.

ABTS (2, 2'-Azino-bis-3-Ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay

ABTS radical scavenging activity of flavonoid rich fraction of *Mimusops elengi* was followed by Re et al. (1999). ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was diluted with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity. The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid.

Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of each extracts. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734nm against distilled water by using a Deep Vision (1371) UV-Vis Spectrophotometer and compared with the control ABTS solution. Ascorbic acid was used as reference antioxidant compound.

$$\text{ABTSS scavenging Effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance of extract.

Inhibition of lipid peroxidation activity

Lipid peroxidation induced by Fe²⁺ascorbate system in egg yolk was assessed as thiobarbituric acid reacting substances (TBARS) by the method of Ohkawa et al. (1979). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); FeSO₄ (NH₄)₂SO₄·7H₂O (0.06 mM); and different concentrations of flavonoid rich fraction of *Mimusops elengi* in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period, 0.4 ml was collected and treated with 0.2 ml sodium dodecyl sulphate (SDS) (1.1%); 1.5 ml thiobarbituric acid (TBA) (0.8%); and 1.5 ml acetic acid (20%, pH 3.5). The final volume was made up to 4.0 ml with distilled water and then kept in a water bath at 95 to 100 °C for 1 hour. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1 v/v) were added to the reaction mixture, shaken vigorously and centrifuged at 4000 rpm for 10 min. The absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV-Vis Spectrophotometer) to quantify TBARS. Inhibition of lipid peroxidation was determined by comparing the optical density (OD) of test sample with control. Ascorbic acid was used as standard.

Inhibition of lipid peroxidation (%) by the each extracts was calculated according to $1 - (E/C) \times 100$,

Where C is the absorbance value of the fully oxidized control and E is absorbance of the test sample.

Nitric oxide radical scavenging activity

Nitric oxide scavenging ability of flavonoid rich fraction of *Mimusops elengi* was measured according to the method described by Olabinri et al. (2010). 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of extracts and incubated at room temperature for 150 min. After treated period, 0.2 ml of Griess reagent (1% Sulfanilamide, 2% Phosphoric acid and 0.1% N-(1-Naphthyl) ethylene diamine dihydrochloride) was added. The absorbance of the experimental sample was read at 546 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The percentage of inhibition was calculated by following equation:

$$\% \text{ Nitric oxide radical scavenging capacity} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Result and discussion

Phytochemical screening

The phytochemical screening of the flowers of *Mimosops*

elengi was studied presently showed the presence of alkaloids, flavonoids, phenol, terpenoids, glycosides and saponin, and absence of glycosides and Saponin (Table -1).

Table 1: Phytochemical screenings of *Mimosops elengi*

Sl. No.	Phytochemical Constituents	Observation	<i>Mimosops elengi</i> flower extract
1	Alkaloids -Dragendorff's test -Mayers test	Orange / red precipitate Creampie ppt	+ +
2.	Flavonoids -Alkalai Reagent -Lead aceate test	Intense yellow colour Precipitate formed	+ +
3.	Glycosides -Keller-Killiani test	Pink colour (Ammonia layers)	-
4.	Tannin -FeCl ₃ test	Blue-black colour	+
5.	Saponins -Frothing test	Foam	-
6.	Terpenoids -Salkowski test	Reddish brown colour ring formed in interface	+
7.	Polyphenols -Ferrozine test	Reddish blue	+
8.	Anthocyanin -Ammonia test	Pink color in ammonia layer	+

+ Positive activity; - Negative activity

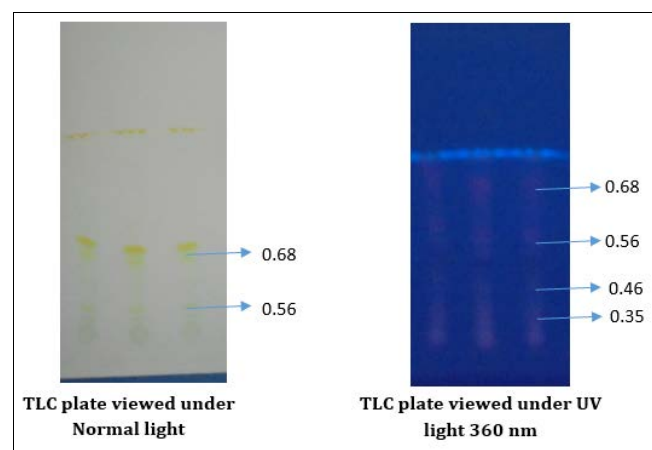


Fig 1: TLC finger print profile of flavonoid rich fraction from the flowers of *Mimosops elengi*

Effect of flavonoid rich fraction from the flowers of *Mimosops elengi* on the growth of Pathogenic bacteria by disc diffusion method

The disc diffusion test showed that flavonoid rich fraction from the flowers of *Mimosops elengi* were active on all tested microorganisms, including Gram-positive, and Gram-negative bacteria. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* in the ranges of concentration of extract 5, 10, 15 and 20µg/ml respectively (Table-2). Flavonoid rich fraction showed the excellent activities against both Gram-positive, and Gram-negative bacteria *Staphylococcus aureus* and *Escherichia coli* with inhibition zones of 16.32 ± 2.3 mm and 15.6 ± 1.8 mm respectively. In contrast, the alkaloid extract less inhibited only a single Gram negative bacterial *Proteus vulgaris* (12.8 ± 2.1).

Table 2: Antibacterial activity of the flavonoid rich fraction on the growth of Pathogenic bacteria by disc diffusion method

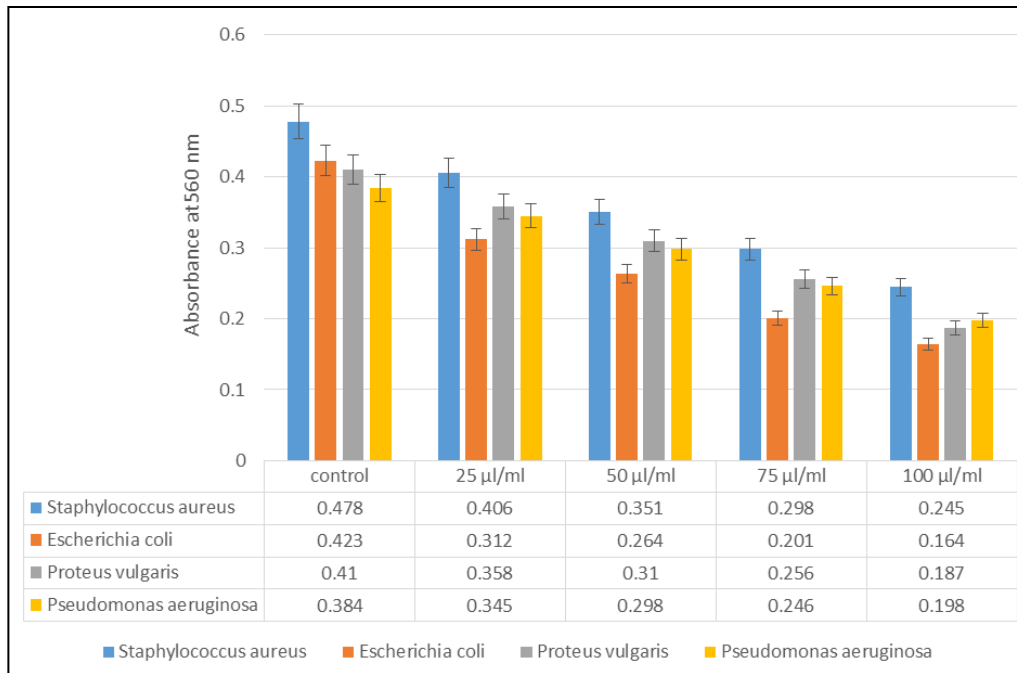
Pathogenic bacteria	Flavonoid rich exhibited the Zone of inhibition (mm) ^a				
	Positive control 10 µl Ampicillin	Different concentrations Crude extract (µl/ml)			
		25 µl	50 µl	75 µl	100 µl
<i>Staphylococcus aureus</i>	13mm	8.3±1.6	10.2±1.4	14.5±1.3	16.32±2.3
<i>Escherichia coli</i>	15mm	7.5±1.5	9.8±1.3	13.2±1.7	15.6 ± 1.8
<i>Pseudomonas aeruginosa</i>	14mm	6.5±1.8	8.1±1.7	10.2±1.6	13.7±1.2
<i>Proteus vulgaris</i>	14mm	6.8±1.3	8.3±1.6	9.7±1.3	12.8±2.1

^aThe inhibitory diameter was measured by means of calipers. All the assays were duplicated, and the mean values were recorded.

Minimum inhibitory concentrations (MICs)

Minimum Inhibitory Concentration (MIC) assays were also performed to determine the antibacterial activities of flavonoid rich fraction from the flowers of *Mimosops elengi* at 18 hours incubation. Flavonoid rich fraction was effective at inhibiting bacterial growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* at low concentrations, with MIC values against the bacterial species that they inhibited generally 25µl/ml potent

antimicrobial activity of alkaloid extract. These MIC values compare favorably with the dosages of the pure standards ampicillin which was tested using 10µl/ml. (Graph-1). Many plants have been known to synthesis active secondary metabolite to protect themselves from microbial attack such as peptide, unsaturated long chain fatty acid, alkaloids, antioxidant and phenolic compound which have potential therapeutic applications (K and has amy and Arunachalam, 2008)



Graph 1: Minimum inhibitory concentrations of flavonoid rich fraction from the flowers of *Mimusops elengi*

Free Radical-Scavenging ability using ABTS assay

The radical scavenging ability was measured by ABTS assay as per given in table 4. The inhibition percentage of the ABTS radical activity was assessed on average and high free radical-scavenging values were found in flavonoid rich fraction from the flowers of *Mimusops elengi*. In ABTS assay, inhibition percentage was high flavonoid rich fraction from the flowers of *Mimusops elengi* 78.32% with EC₅₀ value 57.23µl/ml. The pure ascorbic acid was lower activity (72.34% with EC₅₀ value 63.31) (Table-3). Nevertheless, in present study, it is showed that these activities were mainly due to flavonoids compounds. It is known that vitamin C (ascorbic acid) and carotenoids are chief source of discrepancy of antioxidant/ antiradical activities in plant materials.

Table 3: Free radical-scavenging ability using ABTS assay of flavonoid rich fraction from the flowers of *Mimusops elengi*

Different concentration of extract	Percentage of ABTS radical activity	
	Flavonoid rich fraction from the flowers of <i>Mimusops elengi</i>	Ascorbic acid (+ve control)
25 µl/ml	19.34±0.23	16.34±2.36
50 µl/ml	41.34±2.97	37.32±1.78
75 µl/ml	59.32±2.48	54.32±0.89
100 µl/ml	78.32±1.47	72.34±2.46
EC ₅₀ value	57.23	63.31

^aResults are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

Inhibition of lipid peroxidation activity

The flavonoid rich fraction from the flowers of *Mimusops elengi* also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in flavonoid rich fraction from the flowers of *Mimusops elengi* (80.24%) and lowest inhibition percentage of ascorbic acid was found in 74.32% (Table-4). As it is identified that lipid peroxidation is the net result of any free radical attack on membrane and other lipid components present in the system, the lipid

peroxidation may be enzymatic (Fe/NADPH) or non-enzymatic (Fe/ascorbic acid). In the present study, egg yolk was used as substrate for free radical mediated lipid peroxidation, which is a non-enzymatic method. Normally, the mechanism of phenolic compounds for antioxidant activity includes neutralizing lipid free radicals and preventing decomposition of hydroperoxides into free radicals (Chinenye *et al.*, 2013) [2].

Table 4: Inhibition of lipid peroxidation activity of flavonoid rich fraction from the flowers of *Mimusops elengi*

Different concentration of extract	Percentage of lipid peroxidation	
	Flavonoid rich fraction from the flowers of <i>Mimusops elengi</i>	Standard Vitamin-C
25 µl/ml	22.34±0.84	18.32±2.34
50 µl/ml	43.35±2.89	38.32±1.73
75 µl/ml	59.32±1.78	55.64±1.48
100 µl/ml	80.24±0.23	74.32±2.36
EC ₅₀ value	54.23	60.32

^aResults are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Nitric oxide radical scavenging

Flavonoid rich fraction from the flowers of *Mimusops elengi* indicated a strong nitric oxide scavenging ability which was equivalent to the standards ascorbic acid. The EC₅₀ value (59.32) of flavonoid rich fraction from the flowers of *Mimusops elengi* was less than ascorbic acid (62.24). Percentage of nitric oxide radical scavenging activity flavonoid rich fraction from the flowers of *Mimusops elengi* and standards were presented in Table-4. In the present outcome, nitrite was formed by incubation of sodium nitroprusside in standard phosphate saline buffer at 25°C was reduced by polyphenol rich fraction. Imperative scavenging activity may be due to the antioxidant property of polyphenol, compounds present in *Mimusops elengi* flower, which contest with oxygen to respond with nitric oxide, prominent to less production of nitric oxide. Kim *et al.* (2013) [5] evaluated the antioxidant activity of

polyphenolic extracts from cladodes of *Opuntia ficus-indica*. In particular, *O. ficus-indica* cladodes extracts exhibited *in vitro* strong free radical scavenging activity and increased antioxidant activity in human erythrocytes

Table 5: Nitric oxide radical scavenging assay of flavonoid rich fraction from the flowers of *Mimusops elengi*

Different concentration of extract	Percentage of Nitric oxide radical scavenging activity	
	Flavonoid rich fraction from the flowers of <i>Mimusops elengi</i>	Standard Vitamin-C
25 µl/ml	21.45±0.23	18.34±1.79
50 µl/ml	36.32±1.78	34.56±0.28
75 µl/ml	53.64±2.34	51.23±2.47
100 µl/ml	76.32±1.78	72.34±0.23
EC ₅₀ value	59.32	62.34

^a Results are expressed as percentage of Nitric oxide radical activity with respect to control. Each value represents the mean±SD of three experiments.

Conclusion

In conclusion the results of the present study may strengthen the process of standardization of botanicals containing the flavonoid rich fraction from the flowers of *Mimusops elengi* as one of the ingredients. In many instances, the actual compound/s isolated from the plants may not serve as the drug, but leads to the development of potential novel therapeutic agents. With the rapid identification of new molecules from plant resources having significant antibacterial and antioxidant effects are proving to be important agents in the mainstream of bacterial infection drug discovery marathon.

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