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Evaluation of antimicrobial efficacy of flavonoids of *Mimusops elengi* L.

Ruchi Mathur *

Department of Botany; S.S Jain Subodh Girls P.G. College, Jaipur, Rajasthan, India.

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Abstract

Objective: The antimicrobial activity was evaluated using flavonoid extract of leaf; stem; bark and fruit of *Mimusops elengi* L.

Methodology: The flavonoids were extracted using cold extraction method. Antimicrobial activity of free and bound flavonoid extract was evaluated by disc diffusion assay against bacterial strains *viz*. *Escherichia coli*; *Bacillus subtilis*; *Pseudomonas putida*. Minimum inhibitory concentration (MIC) of the extract was evaluated through micro broth dilution method.

Results: Free flavonoid plant part extract of *M. elengi* L. exhibited significant activity against *Escherichia coli*; *Bacillus subtilis* and show no activity against *Pseudomonas putida*. Free flavonoid extract of bark of plant showed maximum inhibitory activity (DIZ = 14.0 ± 1.00 mm) at 50mg/ml against *Escherichia coli* with MIC of 12.5 mg/ml. Bound flavonoid plant part extract of *M. elengi* L. exhibited significant activity against *Escherichia coli*; *Pseudomonas putida* with no activity against *Bacillus subtilis.* Bound flavonoid extracts of leaf significantly showed maximum antibacterial activity (DIZ =21.0 ± 1.00 mm) at 50mg/ml against *Escherichia coli* with MIC of 6.25 mg/ml. Bound flavonoid extracts of bark also showed maximum antibacterial activity (DIZ =19.33 ± 1.15 mm) at 50mg/ml against *Pseudomonas putida* with MIC of 6.25 mg/ml.

Conclusion: Results obtained advocates that use of plant parts especially leaf and bark of the selected plant could be used as natural antimicrobial agent against tested microorganism and upcoming resistant pathogens.

Keywords: *Mimusops elengi* L.; Flavonoids; *Escherichia coli; Bacillus subtilis; Pseudomonas putida*

1. Introduction

Discovery of antibiotics lead to reduction in mortality, saved millions of lives but their irrational use resulted in emergence of resistant microbial populations. Synthetic antibiotic drug had various adverse effects on health like it could cause nausea, vomiting, and diarrhea; even more complex problems of gastrointestinal, neurological, cardiovascular and dermatological can occur [1]. Resistance against antibiotics, and side effects associated with synthetic drugs, shifted the attention towards use of biologically active components isolated from plant species. It is considered that extracts of medicinal plants especially secondary metabolites (alkaloids, flavonoids, polyphenols, phytosterols etc.) could act as alternative substance for resistance modifiers and a safe antibacterial agent. They have the ability to bind to protein domains leading to modification or inhibition protein–protein interactions [2].

In new modern medicinal science plant derived medicine are used as pharmaceutical prescriptions in the world. In India about 80% population is using plant-based medicines nowadays to treat illness [3]. *Mimusops elengi* Linn. (Sapotaceae) has a widespread world distribution and is native to the Western Ghat region of the peninsular India.

* Corresponding author: Ruchi Mathur

It is medium size evergreen tree with very heavy rough brown colored trunk. The various extract of plant (bark, fruit, leaves, seeds and flowers) has been reported to be cardiotonic, alexipharmic, stomachic, hypotensive, antibacterial, antifungal; antihelmintic, antihyperglycemic, anti-gastric ulcer agent and also used in disease of gum and teeth [4,5]. All the parts of *M. elengi* have medicinal properties, but little information cited in the literature on the potentiality of flavonoid extract against microorganism. The present study is, therefore, designed to assess the potency of flavonoid extracts of *Mimusops elengi* L. on some selected bacterial strains.

2. Material and methods

2.1. Plant Material

Plant parts (Leaf, Bark, Stem and Fruits) of *Mimusops elengi* Linn. were collected from district Jaipur, Rajasthan. It was authenticated as (RUBL 211587) by Herbarium, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.

2.2. Extraction of Flavonoids

Leaf, Stem, Bark and Fruit of plant were subjected to the flavonoid extraction by method of Subramanian and Nagarjan. 5 grams of dry material of plant material was cold extracted in 50ml of 80% methanol. The extract was dried and dissolve in distill water. The extraction was done using petroleum ether to remove lipophilic, acidic and other material. Further extract it with diethyl ether and separate the upper layer and label it as F2 (free flavonoids). Extract the lower layer with ethyl acetate and again separate the upper layer and discard the lower layer. Dry the upper layer in petri plate and reflux it with 7% sulphuric acid for two hours. Now extract it with ethyl acetate using separating funnel. Use the upper layer and label it as F3 (Bound flavonoids) and wash it with distilled water to neutralize the pH at 7 to obtain crude flavonoids [6, 7].

2.3. Culture and Maintenance of Bacterial and Fungal Strains

Pure culture of bacterial strains *viz*. *Escherichia coli* (MTCC 40), *Bacillus subtilis* (MTCC 121), *and Pseudomonas putida* (MTCC 102) were collected from the Department of Botany, University of Rajasthan. The bacterial strains were subcultured on Nutrient Agar medium and incubated at 37º C for 48 hours.

2.4. Antibacterial Activity

Agar disc diffusion method was used to investigate antibacterial activity of selected plant parts of *Mimusops elengi* L. [8]. Bacterial culture was maintained in Nutrient Broth. 100µl of inoculum was spread on the Nutrient Agar plates using sterile cotton swab. The plates were dried for 30 minutes. Whatman filter paper disc of 6mm diameter was sterilized by autoclaving at 15 Psi and 121º C. The plant parts extracts of 25mg/ml and 50mg/ml concentrations (prepared using 100 % DMSO) were loaded on disc and later placed it on Nutrient Agar plates. The plates are kept in an incubator at 37º C for 48 hours. Amoxicillin was used as control. The diameter of inhibition zone was measured in mm. Each experiment was repeated 3 times and their means were statistically calculated.

2.4.1. Determination of activity index

Activity Index (A.I.) = Value of zone of inhibition of the sample/ Zone of inhibition of the standard drug.

2.5. Determination of Minimum Inhibitory Concentration (MIC) for Bacteria

The MIC of the extracts against the test microbes was determined using the broth dilution method [9, 10]. During study of MIC for bacteria, different concentrations (3.13mg/ml, 6.25mg/ml, 12.5mg/ml, 25mg/ml and 50mg/ml) of the extract were prepared. 5ml of Nutrient Broth was taken in the test tube. In each test tube, different concentrations of 100µl of crude extract of secondary metabolites of plant parts were added. 100 µl of inoculum of each bacterial culture was added in aseptic conditions and incubated at 37º C $\pm$ 2º C for 48 hours. 100% DMSO was used as negative control. The least concentration of plant extract was observed by taking the optical density (OD) at 600 nm by using spectrophotometer to calculate MIC value.

3. Results and discussion

In present study three bacterial strains were used to investige the antimicrobial efficacy of flavonoid extract of *Mimusops elengi* L. Free flavonoids extract of bark of *M. elengi* L. showed maximum inhibitory activity (DIZ = 14.0 $\pm$ 1.00 mm) at 50mg/ml against *Escherichia coli* (Table 1). Less significant activity was noticed in free flavonoid extract of plant parts of plant against *Bacillus subtilis* while no activity was observed against *Pseudomonas putida*.

Table 1 Antibacterial activity of free flavonoid extracts of plant parts of *Mimusops elengi* L.

Plant parts	Conc. (mg/ml)	*Escherichia coli*		*Bacillus subtilis*		*Pseudomonas putida*	
		IZ	AI	IZ	AI	IZ	AI
Leaf	25	9.33 ± 0.57	0.84	9 ± 1.0	1.08	NA	-
	50	11.33 ± 0.57	0.59	11.00 ± 1.0	0.89	NA	-
Stem	25	NA	-	8.66 ± 0.57	1.03	NA	-
	50	NA	-	10.33 ± 1.52	0.83	NA	-
Bark	25	10.66 ± 0.57	0.96	NA	-	NA	-
	50	14.0 ± 1.00	0.73	NA	-	NA	-
Fruit	25	8.33 ± 0.57	0.75	7.66 ± 0.57	0.91	NA	-
	50	10.33 ± 0.57	0.54	11.33 ± 1.15	0.91	NA	-
Standard Amoxicillin	25	11± 1.00		8.33± 0.57	-	10.66 ± 1.00	-
	50	19.0 ± 1.00		12.33 ± 1.52	-	19.0± 1.00	-

Abbreviations: NA= No activity; IZ= Diameter of Inibition zone in mm; AI= Activity Index

Bound flavonoid extracts of leaf, stem and bark significantly showed maximum antibacterial activity (DIZ = 21.0 ± 1.00 mm; 19.33 ± 1.15 mm; DIZ = 18.66 ± 1.52 mm respectively) at 50mg/ml against *Escherichia coli*. Bound flavonoid extracts of bark also showed maximum antibacterial activity (DIZ =19.33 ± 1.15 mm) at 50mg/ml against *Pseudomonas putida* (Table 2). No activity potential was observed in bound flavonoid extracts of plant parts of *Mimusops elengi* L. against *Bacillus subtilis.*

Amoxicillin was used as standard control. It shows (19 ± 1.00 mm) at 50mg/ml against *Escherichia coli*; whereas (12.33 ± 1.52 mm) and (19 ± 1.00 mm) at 50mg/ml against *Bacillus subtilis* and *Pseudomonas putida* respectively.

Table 2 Antibacterial activity of bound flavonoid extracts of plant parts of *Mimusops elengi* L.

Plant parts	Conc. (mg/ml)	*Escherichia coli*		*Bacillus subtilis*		*Pseudomonas putida*	
		IZ	AI	IZ	AI	IZ	AI
Leaf	25	17.0 ± 1.00	1.54	NA	-	10.00 ± 1.00	0.58
	50	21.0 ± 1.00	1.10	NA	-	14.00 ± 1.00	0.38
Stem	25	17.33 ± 1.15	1.57	NA	-	12.66 ± 1.15	0.74
	50	19.33 ± 1.15	1.01	NA	-	15.0 ± 1.00	0.41
Bark	25	16.0 ± 1.00	1.45	NA	-	15.33 ± 1.15	0.90
	50	18.66 ± 1.52	0.98	NA	-	19.33 ± 1.15	0.53
Fruit	25	16.0 ± 2.00	1.45	NA	-	8.33 ± 0.57	0.49
	50	17.66 ± 0.57	0.92	NA	-	11.0 ± 1.00	0.30
Standard Amoxicillin	25	11± 1.00	-	8.33± 0.57	-	10.66 ± 1.00	-
	50	19.0 ± 1.00	-	12.33 ± 1.52	-	19.0± 1.00	-

Abbreviations: NA= No activity; IZ= Diameter of Inibition zone in mm; AI= Activity Index

Further Minimum Inhibitory Concentration was calculated in range between 3.13mg/ml to 50mg/ml as shown in Table 3. Bound flavonoid extract of leaf, stem, bark and fruit of *M.elengi* L. show MIC of 6.25 mg/ml against *Escherichia coli.* MIC value for *Bacillus subtilis* in free flavonoid extracts of leaf, stem and fruit of *Mimusops elengi* L. was found to be

25mg/ml. In case of *Pseudomonas putida* bound flavonoid extract of stem show MIC of 12.5 mg/ml while bark show MIC of 6.25 mg/ml

Table 3 Minimum Inhibitory Concentration (MIC) of plant part extracts of *Mimusops elengi* L. against bacterial strains

Plant parts		Minimum Inhibitory Concentration (MIC) in mg/ml		
		Escherichia coli	***Bacillus subtilis***	***Pseudomonas putida***
Leaf	Free Flavonoids	25	25	NA
	Bound Flavonoids	6.25	NA	25
Stem	Free Flavonoids	NA	25	NA
	Bound Flavonoids	6.25	NA	12.5
Bark	Free Flavonoids	12.5	NA	NA
	Bound Flavonoids	6.25	NA	6.25
Fruit	Free Flavonoids	25	25	NA
	Bound Flavonoids	6.25	NA	25
Negative Control		NA	NA	NA

Abbreviations: NA= No activity

The vital role of flavonoids and its antimicrobial activity against human disease have been studied earlier also [11, 12]. Some flavonoids those are extracted from plants have ability to reverse the antibiotic resistance and also act as substitutes for antibiotics. Flavonoids can act both synergistically or individually to produce antibacterial effects [13]. Flavonoids can induce structural changes or modulate the function of membrane proteins of bacterial cell wall [14].

4. Conclusion

Plant extracts containing flavonoids show significant activity against *Escherichia coli* and *Pseudomonas putida* with little activity against *Bacillus subtilis.* This might be due to cell wall damage and disruption in the functional property of bacterial cell. The flavonoid extract of plant parts (especially leaf and bark) of the selected plant could be utilized in the treatment of bacterial diseases.

Compliance with ethical standards

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