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Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ANALYSIS OF AQUEOUS EXTRACTS OF WILD MANGO VARIETIES

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ABSTRACT

Phytochemical screening and antibacterial activity of aqueous extracts of bark and leaf of eighteen mango varieties growing in the village Hanavadi, Malavalli taluk, Mandya district, Karnataka state were conducted. They showed the presence of alkaloids, steroids, flavonoids, tannins, cardiac glycosides, saponnins, resins, carbohydrates and proteins. Antibacterial activity tested against six human pathogens- *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus, Streptococcus mutans, Pseudomonas aeruginosa and Bacillus subtilis*, by disc diffusion method showed significant inhibition zone against pathogens compared to control. These results disclose the inhibiting property of leaf and bark of wild varieties of mangoes against potential pathogens and thus can be subjected to further investigations for their applications in human phytomedicines.

Keywords: Antibacterial assay, *Mangifera indica*, Phytochemicals, Plant metabolites.

1. INTRODUCTION

Anacardiaceae, a dicotyledonous family includes 86 genera and 860 species. The family includes some economically important plants such as cashew, pistachio, mango etc. They are not only valued for their essential nutrients, but also for their medicinal properties [1-3]. Mango, the king of fruits is the plant of choice for the current study. It is loaded with phyto-chemicals such as alkaloids, flavonoids, terpenoids, steroids, etc. Phenolic compounds are exclusively present in mango plant (leaf) which is believed to have medicinal properties [4]. However, the amount and number of phenolic compounds may vary from variety to variety. It is also reported that the plant also contains amino acids such as lysine, alanine, hystidine, arginine, valine, tyrosine, etc. Vitamins such as A, B1, B2, B6, B12, C, K, etc. and minerals such as Calcium, sodium, Potassium, Magnesium, Manganese, iron, zinc [5-8] were reported to be present. Each and every part of the plant is beneficial in some or the other way to the mankind.

India is well known for its traditional medicine which can be traced from the oldest records. '*Charaka Samhita*' is one such work. Many parts of the plant namely stem, leaves, flowers, seed kernel have shown antimicrobial properties in them [9-14]. The use of herbal products is in demand all round the world because of its less or no side-effects. Many researches on mango varieties have been done so far. But less effort has been made to analyze the phytochemical constituents and their medicinal properties in local, wild varieties of mango in India.

With this background, qualitative phytochemical analysis and antimicrobial properties of aqueous extracts of leaf and bark of eighteen wild mango varieties from the village of Hanavadi, Malavalli taluk, Mandya district, Karnataka state were done.

2. MATERIAL AND METHODS

2.1. Collection of samples

Eighteen varieties of mangoes were selected from the village of Hanavadi, Malavalli taluk, Mandya district, Karnataka state. They have been named locally as *Arasu mavina mara* (Ars), *Menasu mavina mara* (Mns), *Nara mavina mara* (Nar), *Sihi mara* (Sih), *Sappa mara* (Spp), *Kenjagada mara* (Knj), *Oobatti unda mara* (Obt), *Onti mavina mara* (Ont), *Gini mavina mara* (Gin), *Emme joddina mara* (Emj), *Uppinkai mara* (Upp), *Raspuri* (Rsp), *Gund mavina mara* (Gnd,variety A), *Chukki mara* (Chk, variety B), *Bhood mavina mara* (Bhd, variety C), *Thogate mara*

(Tkt, variety D), *Holapu mavina mara* (Hlp, variety E) and *Chand mavina mara* (Cnd, variety F).

2.2. Preparation of aqueous extracts of leaves

Fresh mature leaves of all the eighteen varieties of mangoes were collected. Mid rib was removed and leaves were cut into small pieces. Sample of each variety was macerated in a blender with distilled water in a ratio of 1:2. The product obtained was filtered and centrifuged at 4000 rpm for 30 min at 4°C. The supernatant thus obtained was autoclaved at 121°C for 20 min. The extracts were cooled and stored in freezer for further studies.

2.3. Preparation of aqueous extracts of bark

Bark devoid of any fungal infections and above the height of 4 feet, were collected from eighteen varieties of mango. They were cut into small pieces and then macerated in a blender with distilled water in a ratio of 1:3. The products obtained were filtered separately and centrifuged at 6000 rpm for 30 min at 4°C. The supernatant obtained was autoclaved at 121°C for 20 min. The extracts were cooled and stored in freezer for further studies.

2.4. Phytochemical tests

Tests were carried out for detecting alkaloids, flavonoids, carbohydrates, resins [15] steroids, tannins [6] cardiac glycosides, [16] proteins [17] and saponins [18] as per the standard protocols.

2.5. Antimicrobial assay

Aqueous extracts of leaf and bark of eighteen varieties of mangoes were subjected for antimicrobial assay.

2.5.1. Collection of bacterial strains

Six human pathogens such as *E coli* (MCC 3099), *Enterobacter aerogenes* (MCC 3092), *Staphylococcus aureus* (MCC 2043), *Pseudomonas aeruginosa* (MCC 3097), *Bacillus subtilis* (MCC 4239) from NCMR, Pune and *Streptococcus mutans* (MTCC 497) from MTCC, Chandigarh, India were procured.

2.5.2. Preparation of inoculums

The above six pathogens were inoculated into nutrient broth and incubated at 37°C for 24 hrs.

2.5.3. Deduction of antibacterial activity

Twenty-four hrs old cultures of bacteria were subjected for disc diffusion method in triplicates. Distilled water was used as negative control and Gentamicin (30 micro liters) antibiotic, as positive control. Minimum inhibition zone was measured to analyze the activity.

3. RESULTS

The qualitative phytochemical analysis of leaf and bark extracts of eighteen mango varieties revealed the presence of nine compounds (Table 1 and 2) alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides, carbohydrates, proteins and resins.

Table 1: Phytochemical tests of aqueous extracts of leaf of 18 mango varieties of Hanavadi village, Malavalli Taluk, Mandya district, Karnataka state

Samples	Alkaloids	Steroids	Flavonoids	Tannins	Saponins	Cardiac glycosides	Carbo-hydrates	Proteins	Resins
Ars	+	+	+	+	+	+	+++	+	+
Emj	+	+	+	+	+	+	+++	+	+
Gin	+	+	+	+	+	+	++	+	++
Knj	+	+	+	+	+	+	++	+	+++
Mns	+	+	+	+	+	+	+++	+	++
Nar	-	+	+	+	+	+	+++	+	++
Obt	+	+	+	+	+	+	+++	+	+
Ont	+	+	+	+	+	+	+	+	+++
Rsp	-	+	+	+	+	+	+	+	-
Sih	+	+	+	+	+	+	+++	+	+++
Spp	+	+	+	+	+	+	++	+	+++
Upp	-	+	+	+	+	+	+	+	+
Gnd	+	+	+	+	+	+	++	+	+
Chk	+	+	+	+	+	+	+	+	++
Bhd	+	+	+	+	+	+	+	+	+++
Tkt	-	+	+	+	+	+	+	+	++
Hlp	+	+	+	+	+	+	+	+	++
Cnd	-	+	+	+	+	+	++	+	++

'+' present, '-'absent, '++' more, '+++' abundant

The presence of these compounds has indicated their possession for antimicrobial properties. The anti-microbial property was analyzed by calculating the diameter of minimum inhibition zone. Among the eighteen varieties, leaf extracts of Rsp, Nar and Mns varieties and bark extracts of Gnd, Gin, and Ars were good inhibitors of selected microbes (Table 3 and 4).

Table 2: Phytochemical tests of aqueous extracts of bark of 18 mango varieties of Hanavadi village, Malavalli Taluk, Mandya district, Karnataka state

Samples	Alkaloids	Steroids	Flavonoids	Tannins	Saponins	Cardiac glycosides	Carbo-hydrates	Proteins	Resins
Ars	+	-	+	+	+	-	+	+	+
Emj	+	-	+	+	+	-	+	+	++
Gin	+	+	+	+	+	-	+	+	++
Knj	+	+	+	+	-	+	+	+	++
Mns	+	+	+	+	-	+	+	+	+
Nar	+	+	+	+	-	-	+	+	++
Obt	+	+	+	+	+	-	+	+	+
Ont	+	+	+	+	+	-	+	+	++
Rsp	+	+	+	+	+	-	+	+	++
Sih	+	-	+	+	-	-	+	+	++
Spp	+	+	+	+	+	-	+	+	+
Upp	+	-	+	+	+	-	+	+	+
Gnd	+	-	+	+	+	-	+	+	+
Chk	+	-	+	+	+	-	+	+	+
Bhd	+	+	+	+	+	-	+	+	++
Tkt	+	+	+	+	+	-	+	+	++
Hlp	+	-	+	+	+	-	+	+	+
Cnd	+	+	+	+	+	-	+	+	+

'+' present, '-' absent, '++' more, '+++' abundant

Table 3: Average inhibition zone of aqueous of aqueous extracts of leaves of 18 mango varieties of Hanavadi village, Malavalli Taluk, Mandya district, Karnataka state on 6 human pathogens

	Inhibition zone on the growth of bacterial strains					
Varieties	***S. aureus***	***P. aeruginosa***	***B. subtilis***	***E. coli***	***E. aerogenes***	***S. mutans***
Ars	09.66±0.5^{a}	13.16 ± 3.17^{a}	10.00 ± 0.00^{a}	10.66 ± 0.57^{a}	10.66 ± 0.57^{a}	0.00± 0.00
Emj	09.33 ± 0.57^{c}	12.33 ± 0.57^{b}	10.66 ± 0.57^{c}	14.33 ± 0.57^{b}	10.33 ± 0.57^{c}	0.00 ± 0.00
Gin	9.33 ± 0.57^{b}	10.66 ± 0.57^{a}	9.00 ± 0.00^{b}	8.83 ± 0.28^{b}	9.00 ± 0.00^{b}	0.00 ± 0.00
Knj	11.66 ± 0.57^{a}	08.00 ± 0.00^{c}	10.66 ± 0.57^{ab}	10.33 ± 0.57^{b}	9.66 ± 0.57^{b}	0.00 ± 0.00
Mns	19.33 ± 1.15^{a}	08.00 ± 0.00^{d}	10.33 ± 0.57^{c}	14.66 ± 1.15^{b}	10.33 ± 0.57^{c}	0.00 ± 0.00
Nar	11.00 ± 0.00^{c}	15.66 ± 0.57^{a}	12.33 ± 0.57^{b}	16.33 ± 0.57^{a}	11.00 ± 0.00^{c}	0.00 ± 0.00
Obt	09.66 ± 0.57^{c}	12.33 ± 0.57^{ab}	10.00 ± 1.73^{c}	14.00 ± 0.00^{a}	10.66 ± 0.57^{bc}	0.00 ± 0.00
Ont	11.66 ± 2.08^{a}	09.66 ± 0.57^{ab}	8.66 ± 0.57^{b}	8.83 ± 0.28^{b}	9.00 ± 0.00^{b}	0.00 ± 0.00
Rsp	11.66 ± 0.57^{b}	09.66 ± 0.57^{b}	18.66 ± 1.15^{a}	13.66 ± 5.50^{ab}	10.33 ± 0.57^{b}	9.00 ± 0.00^{b}
Spp	9.16 ± 0.28^{b}	17.00 ± 2.64^{a}	10.00 ± 0.00^{b}	16.33 ± 0.57^{a}	9.00 ± 0.00^{b}	0.00 ± 0.00
Sih	10.66 ± 0.57^{b}	10.66 ± 0.57^{b}	11.00 ± 1.00^{b}	13.66 ± 0.57^{a}	10.00 ± 0.00^{b}	0.00 ± 0.00
Upp	0.0 0.00	00.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ±0.00	0.00 ± 0.00
Gnd	10.00 ± 0.00^{a}	00.00 ± 0.00	0.00 ± 0.00	8.83 ± 0.28^{b}	0.00 ± 0.00	0.00 ± 0.00
Chk	9.00 ± 0.00^{b}	09.66 ± 0.57^{a}	10.00 ± 0.00^{a}	9.00 ± 0.00^{b}	8.00 ± 0.00^{c}	0.00 ± 0.00
Bhd	0.0 ± 0.00	09.66 ± 0.57^{a}	8.83 ± 0.28^{b}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Tkt	8.83 ± 0.28^{a}	00.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Hlp	10.33 ± 0.57^{ab}	12.00 ± 1.73^{a}	11.33 ± 1.15^{ab}	9.33 ± 0.57^{b}	10.66 ± 0.57^{ab}	0.00 ± 0.00
Cnd	8.66 ± 1.15^{a}	09.16 ± 0.28^{a}	8.66 ± 0.57^{a}	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Figures having same letters (horizontal direction) are not significantly different according to Tukey's post hoc test, $P \leq 0.05$, all values in mm.

Table 4: Average inhibition zone of aqueous extracts of leaves of 18 mango varieties of Hanavadi village, Malavalli Taluk, Mandya district, Karnataka state on 6 human pathogens

	Inhibition zone on the growth of bacterial strains					
Varieties	***S. aureus***	***P. aeruginosa***	***B. subtilis***	***E. coli***	***E. aerogenes***	***S. mutans***
Ars	9.66 ± 0.57^{b}	8.16 ± 0.28^{c}	0.00 ± 0.00	9.66 ± 0.57^{b}	11.33 ± 0.57^{a}	0.00 ± 0.00
Emj	8.66 ± 0.28^{b}	9.33 ± 0.28^{a}	0.00 ± 0.00	0.00 ± 0.00	7.00 ± 0.00^{c}	0.00 ± 0.00
Gin	9.00 ± 0.00^{a}	8.66 ± 0.28^{a}	9.00 ± 0.50^{a}	8.50 ± 0.00^{a}	9.33 ± 0.57^{a}	0.00 ± 0.00
Knj	8.16 ± 0.28^{b}	8.83 ± 0.28^{a}	8.00 ± 0.00^{b}	0.00 ± 0.00	7.16 ± 0.28^{c}	0.00 ± 0.00
Mns	8.33 ± 0.28^{ab}	8.83 ± 0.28^{a}	8.16 ± 0.28^{b}	0.00 ± 0.00	8.66 ± 0.28^{ab}	0.00 ± 0.00
Nar	8.83 ± 0.57^{a}	9.33 ± 0.57^{a}	0.00 ± 0.00	8.50 ± 0.00^{a}	8.66 ± 0.28^{a}	0.00 ± 0.00
Obt	8.33 ± 0.28^{a}	8.16 ± 0.28^{a}	8.6 ± 0.28^{a}	0.00 ± 0.00^{b}	7.83 ± 0.28^{a}	0.00 ± 0.00
Ont	9.33 ± 0.57^{a}	8.66 ± 0.28^{a}	0.00 ± 0.00	8.66 ± 0.28^{a}	9.33 ± 0.57^{a}	0.00 ± 0.00
Rsp	8.33 ± 0.28^{b}	0.00 ± 0.00	8.00 ± 0.00^{c}	0.00 ± 0.00	9.00 ± 0.00^{a}	0.00 ± 0.00
Sih	8.33 ± 0.57^{a}	8.00 ± 0.86^{a}	8.00 ± 0.00^{a}	8.83 ± 0.28^{a}	0.00 ± 0.00	0.00 ± 0.00
Spp	9.00 ± 0.00^{a}	8.66 ± 0.28^{ab}	8.33 ± 0.28^{bc}	8.33 ± 0.28^{d}	8.00 ± 0.00^{c}	0.00 ± 0.00
Upp	8.33 ± 0.28^{bc}	8.33 ± 0.57^{bc}	8.00 ± 0.00^{c}	9.16 ± 0.28^{b}	10.66 ± 0.57^{a}	0.00 ± 0.00
Gnd	10.00 ± 0.00^{ab}	11.00 ± 1.00^{a}	8.33 ± 0.28^{c}	9.00 ± 0.57^{bc}	9.33 ± 0.57^{bc}	0.00 ± 0.00
Chk	9.00 ± 0.00^{ab}	9.00 ± 0.00^{ab}	0.00 ± 0.00	8.5 ± 0.00^{b}	9.33 ± 0.57^{a}	0.00 ± 0.00
Bhd	0.00 ± 0.00	8.83 ± 0.28^{a}	0.00 ± 0.00	0.00 ± 0.00	8.16 ± 0.28^{b}	0.00 ± 0.00
Tkt	8.83 ± 0.28^{b}	7.83 ± 0.28^{b}	0.00 ± 0.00	8.00 ± 0.00^{b}	10.33 ± 1.15^{a}	0.00 ± 0.00
Hlp	8.18 ± 0.31^{b}	9.00 ± 0.00^{a}	0.00 ± 0.00	8.00 ± 0.00^{b}	8.16 ± 0.28^{b}	0.00 ± 0.00
Cnd	8.66 ± 0.28^{ab}	9.16 ± 0.28^{ab}	0.00 ± 0.00	8.50 ± 0.00^{b}	9.33 ± 0.57^{a}	0.00 ± 0.00

Figures having same letters (horizontal direction) are not significantly different according to Tukey's post $P \leq 0.05$, all values in mm.

4. DISCUSSION

From the dawn of time, mango has been used as traditional medicine and is still practiced in several parts of the world. It is due to the presence of some chemicals such as alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides which have proven their potency in medicine [19-23]. In the present study, qualitative phytochemical analysis of aqueous extracts of eighteen mango varieties has revealed the presence of nine compounds (Table 1 and 2) i. e. alkaloids, steroids, flavonoids, tannins, saponins, cardiac glycosides, carbohydrates, proteins and resins. Leaf extracts have revealed the presence of all the above said phytochemicals but in case of bark, steroids, cardiac glycosides and saponins were present in few but absent in other varieties. Similar results were obtained by other researchers by analyzing various parts of the plant (*Mangifera indica* L.) such as in leaf [16] and in bark [24-27], in leaf and bark [28], in fruits [6], in stem, fruit peel and seed [28]; in stem, leaf, flower and seed kernel [15]. This investigation has also revealed their potentiality in controlling the microbial growth. The antimicrobial property tested with aqueous extracts of leaf and bark on six human pathogens namely *E. coli, E. aerogenes, S. aureus, S. mutans, P. aeruginosa* and *B. subtilis*, has significantly inhibited their growth. In the present study, leaf extracts were more efficient than the bark extracts. However, both extracts have shown inhibition against *S. aureus, P. aeruginosa E. aerogenes* and *E. coli*. Leaf extracts of Rsp, Ars, Emj, Gin, Knj, Nar, Mns, Obt, Ont, Sih, Spp, Chk and Hlp are fine inhibitor of *S. aureus, P. aeruginosa, B. subtilis, E. coli* and *E. aerogenes* while variety Cnd has shown inhibition for three of them namely *S. aureus, P. aeruginosa, B. subtilis*. Bhd has revealed inhibition against two microbes namely *B. subtilis* and *P. aeruginosa* and Gnd has shown inhibition against *S. aureus* and *E. coli*. Upp and Tkt both have inhibition against one pathogen that is *P. aeruginosa*, and *S. aureus* respectively. The aqueous extracts of bark have also proven its potential for hindering microbial growth. Gin, Upp and Gnd varieties have revealed good antimicrobial property for four of the selected pathogens namely *P. aeruginosa, S. aureus, E. coli* and *E. aerogenes*. Knj, Mns, Obt and Sih varieties have exhibited inhibition against four of them namely *P. aeruginosa, S. aureus, E. aerogenes* and *B. subtilis*. Nar, Ars, Chk, Tkt, Hlp and Cnd have revealed their inhibition against *P. aeruginosa, S. aureus, E. aerogenes* and *E. coli*. Spp has shown inhibition for *P. aeruginosa, S. aureus B. subtilis* and *E. coli*. Emj has shown inhibition for three of them namely *S. aureus, E. aerogenes* and *P. aeruginosa* and Rsp on *S. aureus, E. aerogenes* and *B. subtilis*. Bhd variety has comparatively less inhibiting property showing inhibition on only *P. aeruginosa* and *E. aerogenes* and Ont inhibited only one pathogen i.e. *S. aureus*. (Table 3 and 4). The study thus shows that each tree is having

different levels of potential against the pathogens and indicates the importance of this type of investigations.

Similar work was carried out with leaf, flower, seed kernel, bark aqueous extracts with different concentrations by several researchers. Antibacterial work with leaf and bark extracts on different strains of *Staphylococcus* ended with the inhibition zone of 6mm [28]. With bark extracts of 60 microlitres on *S. aureus, E. coli* and *P. aeruginosa*, inhibition zone was between 10 to 15 mm [29]. Another work, again with bark extracts, yielded an inhibition of 6 to 10 mm on *Shigella sp. E coli, Staphylococcus sp*. and *Vibrio sp*.[27]. Leaf extracts to analyze antimicrobial property against *E. aerogenes, E. coli* and *P. aeruginosa* obtained an inhibition of 2-6 mm diameter [30]. Again with the leaf extracts (25 microlitres) done on different strains of *Staphylococcus*, inhibition of 5-7 mm of diameter was obtained [31]. In the present study with a lesser amount of extract namely 30 microlitres, good inhibition was seen. Leaf extracts of Nar, Rsp, Spp and Emj and bark of Gnd, Ars and Gin varieties can be subjected for further studies to unveil their antimicrobial property on more virulent microorganisms.

5. CONCLUSION

The preliminary phytochemical study of eighteen varieties of mango has revealed some of the important phytoconstituents which have manifested their antimicrobial property in them. With the emerging virulence of the pathogens, bioactive compounds may be best suited to heal the human or animal diseases caused by them. So, more research on wild varieties of mango is needed to extract more bioactive compounds that may inhibit the growth of drug resistant organisms. This study explains that mango is a plant with a notable antimicrobial property.

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