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Preliminary phytochemical screening and Bioactivity of selected Indian Medicinal plants

Shyma TB[1], Deepa Shree CL[2], Devi Prasad AG[1], Shubha Gopal[2], Komal Kumar J[1]

***Corresponding author:**

Shyma TB

[1]Department of Studies in Environmental Science University of Mysore, Manasagangothri, Mysore - 570006, Karnataka, India.
[2]Department of Studies in Microbiology, University of Mysore, Manasagangothri, Mysore - 570006, Karnataka, India.

Abstract

The methanolic crude extracts of *Chonemorpha fragrans* (Moon) Alston, *Chilocarpus malabaricus* Bedd., *Madhuka longifolia* (Koenig) J.F.Macbr., Pittosporum neelgherrense Wightt., Raphidophora pertusa (Roxb.) Schott., Fagraea ceilanica Thunb., and Rauvolfia tetraphylla L., were screened for the presence of phyto-constituents and their ability to possess antimicrobial and free radical scavenging ability using chloramphenicol, cephoperazone and ascorbic acid as respective standards. Antimicrobial activity was evaluated by Kirby-Bauer disc diffusion method and free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical and reducing power assay. *Pittosporum neelgherrense* showed better overall antimicrobial activity and *Madhuka longifolia* proved better antioxidant ability possessing low IC50 valueof30 µg/ml compared to the other selected medicinal plants. The highest total phenol content was found to be in *Chonemorpha fragrans* with the value 88±0.121mg/g. The present study reveals that the selected plants serve as a source of antimicrobial and antioxidant drugs in future and thus, can find applications in food and pharmaceutical industries.

Keywords: Medicinal plants, methanol extracts, skin diseases, antibacterial agents, antioxidant.

Introduction

The skin is involved in performing a variety of functions such as providing protection to body, thermo-regulation, percutaneaous absorption, secretory and sensory [1].This organ is exposed to a broad variety of attacks by biological, chemical and physical agents such as microorganisms, reactive oxygen species (ROS) etc. These ROS induces oxidative stress in adjacent normal cells and leads to enhancement of pathologic processes [2]. Thus results in skin damage includes erythema, photo ageing cancer, psoriasis, acne and cutaneous vacuities [3,4]. Antimicrobial and antioxidant defense systems have evolved with ability to counteract the destructive effects of microorganisms and ROS to minimize their potential to cause tissue damage by different mechanism [5]. But, resistance towards diseases has been exhibited by many pathogenic microorganisms. This is because of wrong and overuse of antibiotics and has become major problem in recent decades.

Thus there is a need for the substances which can overcome these problems without causing adverse effect on health. Plants acts as a source for such compounds. Many researches have been revealed that plants contain secondary metabolites which possess many biological activity including antimicrobial and antioxidant activity [6]. These metabolites includes flavonoids, tannins, terpenoids, alkaloids etc which acts as antimicrobial by inhibiting protein synthesis and antioxidant by neutralizing free radicals and thus terminating chain reaction. Thus making antibacterial and antioxidant therapy effective has become area of research interest during recent years. In present study efforts has been made to determine the ability of selected medicinal plants for their antibacterial and antioxidant activity along with determination of preliminary phyto-chemicals involved in these activities. Seven medicinal plants were used in present study based upon their traditional use majorly treating variety of skin disorders and are tabulated in Table 1.

Table 1: Selected medicinal plants for their bioactivity

Botanical Name	Local Name	Family	Parts used	Medicinal uses
Chonemorpha fragrans	Perumkurumba	Apocynaceae	Leaf	The juice intake purifies blood. The leaf paste is applied on the affected part to cure skin diseases.
Chilocarpus malabaricus	Vallippala	Apocynaceae	Leaf	The resin of the leaf is applied on the affected part to cure skin diseases.
Madhuka longifolia	Elippa	Sapotaceae	Leaf	Drinking the leaf juice cures dysentery an diarrhea
Pittosporum neelgherrense	Analivenga	Pittosporaceae	Leaf	. The leaf paste is applied on the affected part to cure skin problems and snake bite
Raphidophora pertusa	Anachakkara	Raphidophoraceae	Leaf	The young leaf juice along with salt drinking cures dysentery and diarrhea. Smearing of the leaf paste cures tonsillitis and mumps
Fagraea ceilanica	Modakam	Loganiaceae	Leaf	The leaf juice drinking cures dysentery an diarrhea
Rauvolfia tetraphylla	Pambinkaya	Apocynaceae	Leaf	The leaf paste is applied on the affected part to cure skin diseases.

Materials and Methods

Chemicals

Butylated hydroxyl anisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), trichloroacetic acid (TCA), potassium ferricyanide, ferric chloride, gallic acid and ascorbic acid were obtained from (Sisco Research Laboratories, Mumbai, India), Chloramphenicol and Cephoperazone.

Plant Materials and Test Microorganisms

Seven medicinal plants viz., Chonemorpha fragrans (Moon) Alston., Chilocarpus malabaricus Bedd., Madhuka longifolia (Koenig) J.F.Macbr, Pittosporum neelgherrense Wightt., Raphidophora pertusa (Roxb.) Schott., Fagraea ceilanica Thunb., and Rauvolfia tetraphylla L., were screened against gram-positive bacteria; Staphylococcus aureus and gram-negative bacteria; Escherichia coli.

Extract Preparation

Plant materials were air-dried, coarsely powdered and extracted for four hours with Methanol in Soxhlet apparatus. Then the methanol extracts of all the plants were filtered and allowed to evaporate in open air. The dried extracts were stored in refrigerator until use.

Phytochemical Analysis

Methanol extracts of selected medicinal plants were subjected to standard phytochemical analyses to determine the presence phenols, flavonoids, alkaloids, coumarins, glycosides, tannins, saponins and steroids according to the method of Oguyemi [7].

Antimicrobial Assay

Disc diffusion method

Disc diffusion assay was done according to Kirby-Bauer method [8]. Nutrient agar plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 min and 0.1 ml of inoculum suspension was swabbed uniformly and allowed to dry for 5 min. The different concentrations of extracts (1, 2 and 4 mg/disc) were loaded on 5 mm sterile individual discs. The loaded discs were placed on the surface of medium and the plates were kept for incubation at 37 C for 24 h. Negative control was prepared using respective solvent. Chloramphenicol and Cephoperazone (100 μg/disc) were used as positive control. At the end of incubation, inhibition zones formed around the disc were measured in millimeter. These studies were performed in triplicate.

Antioxidant Assay

Total Phenolic assay

Methanolic extracts were determined for their total phenolic content with Folin– Ciocalteu reagent according to the method of Singleton and Rossi[9] with slight modification using gallic acid as a standard. Briefly, 1.0 ml of extract solution (5 mg/ml) was added in a 100 ml volumetric flask that contained about 60 ml distilled water. Then, 5.0 ml of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 1 - 8 min, 15.0 ml Na_2CO_3 (20 %) was added and the volume was made up to 100 ml using distilled water. The mixture was allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis spectrophotometer. The total phenolic content was determined as mg of gallicacid equivalent (GAE).

DPPH Radical scavenging assay

The DPPH radical scavenging activity of extracts was done according to method described by Sunil K *et al.,*[10]. 0.3 mM DPPH solution was prepared in methanol and 1.0 ml of this solution was added to 3.0 ml of different concentration of methanolic extracts and incubated for 30 min in dark. Later, the absorbance was measured at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations was used as standard. The capability of extracts to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = [(Abs control- Abs test) / Abs control] 100

Where Abs control is the absorbance of the control reaction and Abs test is the absorbance in the presence of the sample. The antioxidant activity of the methanolic leaf extract was expressed as IC50 and compared with standard. The IC50 value was defined as the concentration (in μg/ml) of extracts that scavenges the DPPH radicals by 50%.

Reducing Power Assay

Reducing power ability of extracts was determined by method of Lu and Foo [11]. 1 ml of plant extract solution with different concentration was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (10g/l), and then the mixture was incubated at 50^0 C for 20 minutes. 2.5 ml of trichloroacetic acid was added to the mixture, and then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $Fecl_3$ (1g/L) and absorbance measured at 700nm in UV-Visible Spectophotometer. Butylated hydroxyl anisole (BHA) was used as standard and phosphate buffer used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.

Result and Discussion

Seven plants – *Chonemorpha fragrans* (Moon) Alston., *Chilocarpus malabaricus* Bedd., *Madhuka longifolia* (Koenig) J.F.Macbr., *Pittosporum neelgherrense* Wightt.,, *Raphidophora pertusa*(Roxb.) Schott., *Fagraea ceilanica* Thunb., and *Rauvolfia tetraphylla* L., were used traditionally to treat different type of diseases such as skin diseases, dysentery, diarrhoea, rheumatism, mumps etc., and thus were the focus of the current study. Preliminary phyto-chemical analysis of selected plant extracts revealed the presence of tannins, saponins, flavonoids, terpenoids and cardiac glycosides and is summarized in Table-2.

Table 2: Preliminary phytochemical analysis of selected medicinal plants

Sl. No.	Medicinal Plants	Tannins	Phlobatannins	Saponins	Flavonoids	Terpenoids	Cardiac glycosides
	Chonemorpha fragrans	-	-	+	+	+	+
2	*Chilocarpus malabaricus*	+	-	+	+	-	+
3	*Madhuka longifolia*	-	-	-	-	+	+
4	*Pittosporum neelgherrense*	-	-	+	+	+	+
5	*Raphidophorapertusa*	+	-	+	+	+	+
6	*Fagraea ceilanica*	-	-	+	+	-	+
7	*Rauvolfia tetraphylla*	+	-	-	+	-	+

+ present, - absent

Flavonoids are known to possess antimicrobial and antioxidant activity [12]. Tannins are known for their antibacterial activity [13]. These phyto-chemicals could be responsible for their antimicrobial property and antioxidant activity. Out of 7 plants tested for antimicrobial activity, 6 plant species showed antibacterial activity by inhibiting both tested bacteria. The results of the antibacterial activity of plant extracts tested by disk diffusion method are shown in Table-3.

Table 3: Antibacterial activity of different extracts of selected medicinal plants

Sl No.	Medicinal Plants	*E.coli*		*Staphylococcus aureus*	
		Methanol extract	Diethyl ether extract	Methanol extract	Diethyl ether extract
1	*Chonemorpha fragrans*	7 mm	10 mm	7 mm	13 mm
2	*Chilocarpus malabaricus*	-	13 mm	-	16 mm
3	*Madhuka longifolia*	-	-	-	20 mm
4	*Pittosporum neelgherrense*	16 mm	10 mm	7 mm	19 mm
5	*Raphidophora pertusa*	8 mm	11mm	6 mm	10 mm
6	*Fagraea ceilanica*	15 mm	10 mm	8 mm	14 mm
7	*Rauvolfia tetraphylla*	-	10 mm	-	16 mm
	Chloramphenicol	29 mm		30 mm	
	Cephoperazone	8mm		9 mm	

Methanol and diethyl ether extract of *Pittosporum neelgherrense* showed significant antibacterial activity followed by *Fagraea ceilanica,* as compared to other plant extracts. *Raphidophora pertusa* exhibited better inhibition activity against *E.coli* than with *Staphylococcus aureus, while Chonemorpha fragrans* was more effective against *Staphylococcus aureus* than *E.coli.* In case of *Chilocarpus malabaricus* and *Rauvolfia tetraphylla* only diethyl ether extract possessed activity. All selected plants showed activity against both the bacteria but *Madhuka longifolia* was able to inhibit only *Staphylococcus aureus* with good zone of inhibition. Among the two standards used as positive control chloramphenicol has shown significant activity against both bacteria and cephoperazone has shown lesser inhibition zone. All our plant extracts have proved better activity than cephoperazone. *Madhuka longifolia* has possessed inhibition activity nearer to chloramphenicol. These antimicrobial activities of selected plants may be due to the presence of phytochemicals like flavonoids, tannins and saponins [14, 15]. Methanol extract of seven plants were screened for their total phenolic content Table-4 and antioxidant ability. This was necessary because phenolic compounds present in the plants are mainly responsible for their antioxidant activity. The antioxidant potential of methanol extract is due to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers [16]. Among the seven selected plants, *Chonemorpha fragrans* was found to contain larger amount of phenolic compounds as compared to

Table 4: Total Phenolic Content of selected medicinal plants

MedicinalPlants	Total Phenolic Content (mg GAE/gm)
Chonemorpha fragrans	88 ±0.121
Chilocarpus malabaricus	25 ±0.010
Madhuka longifolia	71±0.018
Pittosporum neelgherrense	25 ±0.022
Raphidophora pertusa	26.5±0.021
Fagraea ceilanica	55±0.196
Rauvolfia tetraphylla	28±0.028

other plants (Figure 1) and is expressed as gm equivalent to gallic acid. In the present investigation, DPPH radical inhibition capacity of methanolic extract of selected plant was done as they have found to contain phenolic compounds and was compared with ascorbic acid. It was observed that the methanolic extract of *Madhuka longifolia* had higher scavenging activity as compared to

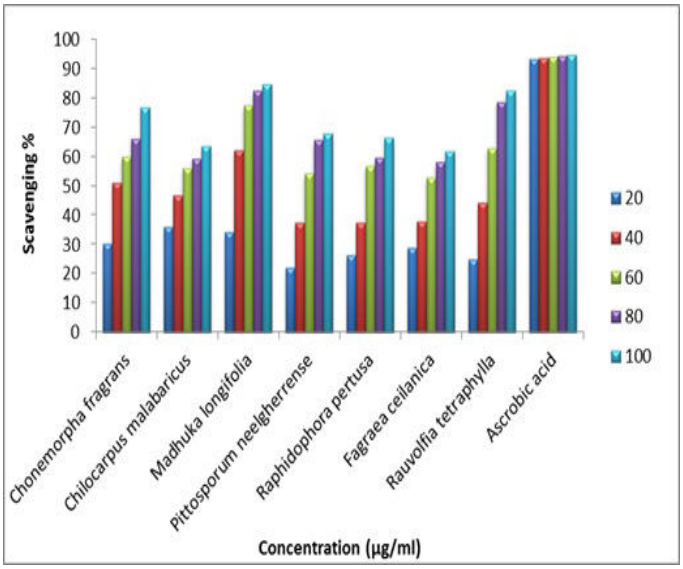

Figure 1:DPPH redical scavenging assay of selected medicinal plants

other extracts. At a concentration of 100 µg/ml, it scavenged about 84.42 % of DPPH radicals. At the same concentration ascorbic acid scavenged 94.87% of DPPH radicals. DPPH radical scavenging ability of selected plants are shown in Figure 1 and IC 50 values are presented in Table-5. DPPH radical scavenging property of methanol extract of selected medicinal plants is due to the presence of antioxidant molecules having hydrogen donating ability [17]. The ability of test plant extracts to reduce Fe^{3+} to Fe^{2+} was determined.

Table 5: IC_{50} values of the DPPH radical scavenging activities of selected medicinal plants

MedicinalPlants	IC Value (µg/ml)
Chonemorpha fragrans	50 µg/ml
Chilocarpus malabaricus	41 µg/ml
Madhuka longifolia	30 µg/ml
Pittosporum neelgherrense	56 µg/ml
Raphidophora pertusa	60 µg/ml
Fagraea ceilanica	58 µg/ml
Rauvolfia tetraphylla	40 µg/ml

Chonemorpha fragrans showed significant reducing ability as compared to BHA. *Madhuka longifolia* and *Pittosporum neelgherrense* showed considerably good reduction activity than other plants (Figure-2).

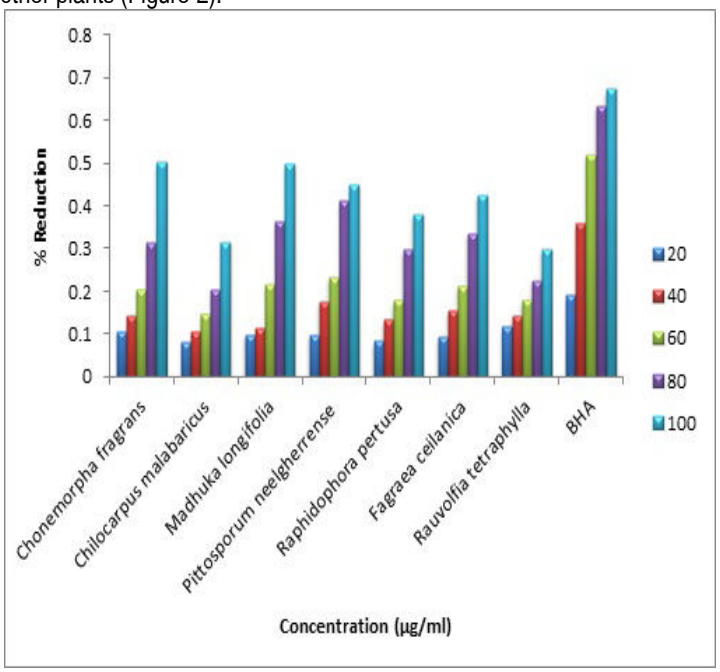

Figure 2: Reducing Power assay of selected medicinal plants

This reducing ability is due to the presence of reductants. Thus, the results indicated that the presence of reductants in the test plant extracts at different concentrations can act to break the free radical chain formation. These reductants are found to be present in larger quantities in *Chonemorpha fragrans* than other tested plants.

Conclusion

The present work proved the antibacterial and antioxidant activity of seven traditionally used medicinal plants. The results of the present study suggest that the tested plant samples have moderate to potent activity and the presence of certain phyto-chemicals are responsible for bioactivity. Present study might be useful for further screening and development of novel potential therapeutic agents in future.

References

[1]. Sumitra Chanda, Yogesh Baravalia. Screening of some plant extracts against some skin diseases caused by oxidative stress and Microorganisms. African Journal of Biotechnology 2010; 9(21): 3210-3217.

[2]. Cerutti P, Shah G, Peskin A, Amstad P. Oxidant carcinogenesis and antioxidant defense. Ann NY AcadSci 1992; 663: 158–66.

[3]. Motamed SM, Naghibi F. Antioxidant activity of some edible plants of the Turkmen Sahra region in northern Iran. Food Chem 2010; 119: 1637-1642.

[4]. Myachi Y. Skin diseases associated with oxidative injury, In: Fuchs J, Parcker L (ed). Oxidative Stress in Dermatology. Marcel Dekker, New York, 1993, pp. 323-331.

[5]. David R, Bickers, Mohammad Athar. Oxidative Stress in the Pathogenesis of Skin Disease, Journal of Investigative Dermatology 2006; 126: 2565-2575.

[6]. Krishnaraju AV, Rao TVN, Sundararaju D. Assessment of bioactivity of Indian Medicinal Plants using Brine shrimp (Artemiasalina) lethality assay. Int J ApplSciEng 2005; 2: 125-134.

[7]. O guyemi AO. In: SofawaraA.ed Proceedings of a conference of African medicinal plants. Ife-Ife: Univ life, 1979, pp. 20-22.

[8]. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disc method. American Journal of Clinical Pathology 1966; 45: 493-496.

[9]. Singleton VL, Rossi JA. Colorimetry of total phenolic substances. US: American Chemical Society Symposium series. 1965; 26: 47-70

[10]. Sunil K, Dinesh K,Manjusha, Kamal S, Nidhan S,Bhoodev V. Antioxidant and free radical scavenging activity of *Citrulluscolocynthis*(L.) Schrad. Methanolic fruit extract. Acta pharm 2008; 58 :215-220.

[11]. Lu Y, Foo Y. Antioxidant activities of polyphenols from sage (Salvia officinalis.) Food Chem2000; 75: 197-202.

[12]. Adedapo AA, Mogbojuri OM, Emikpe BO. Safety evaluations of the aqueous extract of the leaves of *Moringaoleifera*.J Med Plants Res 2000;3(8):586–591.

[13]. Callow RK. Steroids. Proc. Royal, Soc London Series A, 1936, pp.157-194.

[14]. Scalbert A. Antimicrobial properties of tannins. Phytochemistry, 1991, 30, pp. 3875-3883.

[15]. Field JA, Lettinga G. Toxicity of tannic compounds to microorganisms. Plants Polyphenols. Synthesis, Properties, Significance. Basic Life Science 1992; 59: 673-692.

[16]. Hakkim FL, Shankar CG, Girija S. Chemical composition and antioxidant property of holy basil (*Ocimum sanctum* L.) leaves, stems and inflorescence and their in vitro calluscultures. J Agri and Food Chem 2007;55: 9109–9117.

[17]. Chen CW, Ho CT. Antioxidant properties of polyphenols extracted from green tea and black tea. Journal of Food Lipids1995; 2: 35-46.