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Phytochemical screening and antibacterial activity of leaf extract of *Martynia annua, L.* and *Premna latifolia, Roxb.*

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Abstract
Objective: To evaluate phytochemical screening and antibacterial activity of aqueous, ethanol, acetone and petroleum ether extract of leaves of *Martynia annua.* and P*remna latifolia.*
Methods: The qualitative phytochemical screenings were carried out by standard biochemical assays. The antimicrobial activity of the plant was determined by agar well diffusion method carried out by microdilution techniques against two gram negative human pathogenic bacteria.
Result: The preliminary phytochemical analysis of *Martynia annua* and *Premna latifolia* leaves indicated the presence of alkaloids, flavonoids, phytosterols, glycosides, phenolic compounds and tannin in all the solvents except aqueous extract whereas amino acids was absent in all the solvent. Out of the four extracts evaluated the ethanolic leaf extract of both the study plants showed promising activity against four pathogenic bacteria with maximum zone of inhibition (30mm).The acetone and petroleum ether were found to be less effective and showed moderate zone of inhibition against all the tested microorganisms The poor response was obtained with aqueous extract which showed less activity against all the tested microorganisms.
Conclusion: The leaves of *Martynia annua.* And *Premna latifolia* might represent a new phytoconstituents and antimicrobial source with stable, biologically active components that can establish a scientific base for modern medicine

Keywords: Martynia annua, Premna latifolia, phytochemical, antimicrobial.

Introduction
Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. They have been used as remedies and for health care preparations [27]. The value of medicinal plants to the mankind is very well proven. India harbors about 15 percent (3000 – 3500) medicinal plants, out of 20,000 medicinal plants of the world. About 90 percent of these are found growing wild in different climatic regions of the country. It is estimated that 70 to 80% of the people worldwide rely chiefly on traditional health care system and largely on herbal medicines [28].The medicinal plants and their derivatives have long been recognized as an important source of therapeutically effective medicines as they contain secondary metabolites which are potential sources of drugs. Furthermore, increasing reliance of the medicinal plants in the industrialized countries has been traced to the extraction and development of several drugs and chemotherapeutic from these plants as well as from traditionally used rural herbal remedies [30]. During the past 20years, at least one novel compound from higher plants has been marketed every 2.5 years [8].
Plants offer a large range of natural compounds belonging to different molecular families which possess interesting biological activities which attracted several researchers to their elucidation to provide knowledge that will lead to advancement in medicine [4]. It is imperative that any crude drug for pharmacological or pharmaceutical use needs to be subjected to scrutiny for botanical identity. The role of phytochemical analysis are sought at this juncture to provide a set of diagnostic features of the drug which will help to a considerable extent to ascertain the botanical identity of the drug [6]. Phytochemicals are non-nutritive plant chemicals that have protective or diseases preventive properties. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases [22].

Throughout the world, antibiotics are used to treat all microbial infections; however, bacteria are gradually becoming resistant against antibiotics [15]. This alarming situation calls for an accelerated search for novel antibacterial drugs and therapeutic agents [26]. The presence of antimicrobial agents in plants has opened new avenues for the discovery of novel natural products that can serve as substitutes for antibiotics [9]. Although plant extracts have great potential in treating of infectious diseases caused by resistant microorganisms 24, but it is surprising to note that less than 5% world plant species have been analyzed so far as potential medicine, while rests of the 95% of plants are still to be analyzed [23].

Martynia annua, Linn. belonging to family Pedaliaceae is a potential medicinal plant, which has not been investigated sufficiently so far. It is an erect, branched, glandular hairy large herb distributed throughout road sides, rubbish heaps and waste places. The plant is ethnomedicinally used for the treatment of tuberculous, sore throat and some venomous bites.

Premna latifolia, Roxb. belonging to family Verbenaceae is a medicinal plant. It is an annual herbs or shrubs commonly grows in tropical and subtropical regions. The plant is ethnomedicinally used for the treatment of diuretic, dropsy, fevers, liver complaints, rheumatism, asthma, cough, boils, scrofulous diseases, headache, diarrhoea, beriberi, stomachic, eye lotion, vaginal irritation, sore eyes, toothache and snake bites.

Materials and Methods

Plant materials

Martynia annua and *Premna latifolia* were collected from Nellithurai Beat, Karmadai range, Western Ghats, Coimbatore District of Tamilnadu, India. The plant materials were identified with the help of standard local floras (Flora of Presidency of Madras and Fyson).

Preparation of powder

Fresh leaves were collected from the plant part and shade dried. Leaves were ground into a coarse powder and used for further investigations. Extractions were carried out using four solvents viz., Aqueous, Ethanol, Acetone and Petroleum ether.

Extraction procedure

Coarsely powdered plant material were extracted using water, ethanol, acetone and petroleum ether through Soxhlet apparatus. The collected extracts were used for preliminary screening and anti-microbial activity. The obtained crude extract was stored at 4°C.

Preliminary screening of Qualitative Phytochemical analysis

The condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, reducing sugars, catechins, anthroquinones, flavonoids, terpenoids, sugars, phenols, saponins, tannins and aminoacids. The presence of phytochemicals from aqueous, ethanol, acetone, and petroleum ether extract of alel the samples was qualitatively determined[5]Phytochemical screening of different successive solvent extract was carried out by following the methods of Brindha (1981), Harborne (1998), Lala (1993), Trease and Evans (2002), Kokate *et al*. (2004), Edeoga *et al*. (2005), Khandelwal (2008) and Siddiqui *et al*. (2009).

Determination of antibacterial activity

a) Microorganisms used

The pathogens used in this present study are *Bacillus subtilis, Klebsiella pneumonia, Proteus mirabilis and Staphylococcus aureus.* The gram positive *(Bacillus subtilis, Staphylococcus aureus)* and gram negative bacteria *(Klebsiella pneumonia*, *Proteus mirabilis*) were precultured in nutrient broth over ight. These cultures were then grown in Muller Hinton agar medium. The cultures were collected from Nandha College of Arts & Sciences. Erode, Tamil Nadu, and were maintained on nutrient broth (NB) at 37^0C.

b) Antibacterial activity

Antibacterial assay was carried out by Agar well diffusion method using microorganisms cell suspension whose concentration was equilibrated to 0.5 McFarland standards. The antibacterial activity was tested against leaf extracts of *Martynia annua* and *Premna latifolia.* The inoculums of microorganisms were prepared from bacterial culture. About 15-20 ml of Muller-Hinton agar medium was poured in the sterilized glass petridishes and allowed to solidify. One drop of each strain was spread over the medium by a sterile rod. Wells of 5 mm in diameter and about 2 cm apart were punctured in the cultured media using sterile cork borer. About 1 ml of plant extract was added to the wells. Inoculated plates were incubated at 37^0C for 24 hrs. Antibacterial activities were evaluated by measuring the diameters of inhibition zones. The Minimum Inhibitory Concentration (MIC) of water, ethanol, acetone and petroleum ether extract was determined as the lowest concentration of the plant extract inhibiting the visible growth of organism.

Results

Phytochemical Screening

The present study was carried out in *Martynia annua and Premna latifolia and the study* revealed the presence of medicinally active constituents. The phytochemical active compounds of *Martynia annua* and *Premna latifolia* were qualitatively analysed for leaves and the results are presented in Table 1. The preliminary phytochemical analysis of *Martynia annua* leaves indicated the presence of alkaloids, flavonoids, phytosterols, glycosides, phenolic compounds and tannin in the solvents acetone, petroleum ether and ethanol. Alkaloids, flavonoides, saponins and tannins were present in aqueous extract. The phytochemical screening of *Premna latifolia* leaves showed the presence of alkaloids, saponins, carbohydrates, proteins and quinines in all the extracts whereas amino acids was absent in all the solvents of both the test plants.

Table 1: Phytochemical analysis of the leaf extracts of *Martynia annua, L. and Premna latifolia, Roxb*

S.No	Phyto constituents	Tests	Aqueous		Ethanol		Acetone		Petroleum ether	
			M	P	M	P	M	P	M	P
1.	Alkaloids	Mayer's test	+	+	+	+	+	+	+	+
2.	Glycosides	Borntrager's test	+	+	+	+	+	+	+	+
3.	Saponins	Froth forming test	+	+	+	+	+	+	+	+
4.	Phenolic compounds	Lead acetate test	+	+	+	+	+	+	+	+

5.	Tannins	Fecl3 test	+	+	+	+	+	+	+	+
6.	Phytosterols	Libermann Buchard test	-	-	+	+	+	+	+	+
7.	Carbohydrates	Fehilings test	+	+	+	+	+	-	+	+
8.	Proteins	Biuret test	+	+	+	+	+	+	+	+
9.	Amino acids	Barfoed's Test	-	-	-	-	-	-	-	-
10.	Flavanoids	Alkaline test	-	-	+	-	+	-	+	+
11.	Quinones	Quinone test	-	-	-	-	+	+	+	+
12.	Terpenoids	Terpenoid test	+	+	-	-	+	+	+	+

Antimicrobial activity

The antibacterial activity of the tested extracts of *Martynia annua* and *Premna latifolia* showed significant reduction in bacterial growth in terms of zone of inhibition. The leaf extracts showed dose dependent activity i.e., while increase in the concentration of extract, the zone of inhibition is also increased (Table 2). In the present study, maximum growth of inhibition (30 mm) was observed in *Martynia annua* ethanolic leaf extracts at 100 µg/mL against *Staphylococcus aureus*. It was followed by *Klebsiella pneumonia* (29 mm), *Bacillus subtilis* and *Proteus mirabilis* (28 mm). Similarly, acetone, aqueous and petroleum ether extracts showed maximum growth inhibition (29 mm) at the concentration of 100 µg/mL against *Staphylococcus aureus*, *Bacillus subtilis* and *Proteus mirabilis* which were followed by *Klebsiella pneumonia* (28 mm). Ethanol extract at the concentration of 25,50,75 µg/ mL also showed significant activity (23 - 27 mm) of zone of inhibition against all the tested organisms the acetone, petroleum ether and aqueous extracts of *Martynia annua* leaf were found to be less effective and showed moderate zone of inhibition against all the tested microorganisms.

The results of the present study showed that, leaf extracts of *Premna latifolia* especially ethanol extract possess bioactive compounds with antibacterial activity against many pathogens. Ethanol extract showed activity against all the tested microorganisms with maximum zone of inhibition (29mm). The poor response was obtained with aqueous extract which showed less activity against all the tested microorganisms. Comparitively *Martynia annua* was found to be efficient against all the four pathogens, whereas *Premna latifolia* was found to be less efficient.

Table 2: Antibacterial activity of leaf extracts of Martynia annua,L. and Premna latifolia, Roxb. against human pathogenic microorganisms

S.No	Plant extracts	Concentration(µg/ml)	Zone of growth inhibition(mm)							
			Bacillus subtilis		*Klebsiella pneumonia*		*Proteus mirabilis*		*Staphylococcus aureus.*	
			M	P	M	P	M	P	M	P
1.	Ethanol	25	24	20	26	21	23	21	25	25
		50	26	20	26	21	26	21	26	26
		75	27	20	27	22	27	22	28	26
		100	28	21	29	22	28	23	30	27
2.	Acetone	25	7	5	6	6	10	10	7	6
		50	7	6	7	6	12	10	8	6
		75	8	6	9	6	12	11	9	7
		100	9	6	11	7	14	11	16	8
3.	Petroleum ether	25	9	9	9	9	10	10	12	12
		50	13	12	10	10	12	10	13	12
		75	11	10	11	11	13	11	15	13
		100	21	18	13	11	16	12	19	14
4.	Aqueous	25	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
		50	Nil	Nil	Nil	Nil	6	Nil	7	Nil
		75	7	4	6	5	8	6	8	6
		100	8	6	7	6	9	7	10	6

Discussion

The pharmacological action of crude drugs and other therapeutic uses are due to their therapeutically active constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds or secondary metabolites of plants which serve as defense mechanism against predation by many microorganisms, insects and herbivores. So, the preliminary phytochemical analysis revealed pronounced importance because the crude drugs possess varied composition of secondary metabolites [19, 20].

In the present study, phytochemical screening of all four extracts showed significant indication about the presence of metabolites. Alkaloids, Saponinis, Tannins, Amino acids, Flavanoids and Terpenoids, were found to be present in the all the sequential extracts of *Martynia annua* and *Premna latifolia* leaf. It is suggested that the ethanol extract of leaf revealed a significant scope to develop a novel broad spectrum of antimicrobial drug formulation and can be used to carry out further pharmacological evaluation to be used as antibacterial agents/drugs. The results of the present study also supplement the folkloric usage of the studied plants which possess several known and unknown bioactive compounds with bio-activity. By isolating and identifying these bioactive compounds new drugs can be formulated to treat various diseases and disorders [25].

Plant rich in tannins have antibacterial potential due to their basic character that allows them to react with proteins to form stable water soluble compounds thereby killing the bacteria by directly damaging its cell membrane [11]. Flavonoids are a major group of phenolic compounds reported for their antiviral [7], antimicrobial [21]. and spasmolytic properties [2]. Alkaloids isolated from plant are commonly found to have antimicrobial properties [1]. The antimicrobial activity of the leaf extracts of *Martynia annua* and *Premna latifolia* as recorded in present study might therefore be attributed to the presence of above phytochemicals i.e. flavonoids, terpenoids, amino acids, glycosides, tannins, amino acids and carbohydrates [3]. From the above study, it is concluded that the leaves of *Martynia annua* and *Premna latifolia* might represent a new antimicrobial source with stable, biologically actjive

components that can establish a scientific base for the use in modern medicine. Further studies are needed to isolate and characterize the bioactive principles to develop new antimicrobial drugs from *Martynia annua* and *Premna latifolia.*

Conclusion

The plant screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. These findings suggested that *Martynia annua* and *Premna latifolia* leaves could be a potential source of natural antioxidant having great importance as therapeutic agent and preventing oxidative stress related degenerative diseases. The leaves of *Martynia annua* and *Premna latifolia* can provide lead molecules which could be useful substrate for the synthesis of new broad spectrum antibiotics for the treatment of infections caused by the organisms. Further purification, identification and characterization of the active compounds would be our priority in future studies.

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