Phytoconstituents and antimicrobial activity of *Walsura trifoliata* (A. JUSS.) Harms.

J. Josphin Mini, Natarajan Gajendran
Department of Plant Biology and Plant Biotechnology, Presidency College, Chennai-600005, India
josphinmini@gmail.com, gajend6an@yahoo.com

Abstract

Background/Objectives: *Walsura trifoliata* is known for its ethno botanical uses but only scant data is left with scientific proof; moreover, globally there is a spur in documenting and establishing database for available medicinal plants. We report here the medicinal uses of *W. trifoliata*.
Methods: The crude extract obtained from various parts of the plant viz. leaves, bark and root using hexane, ethyl acetate and methanol solvents were investigated. The shade dried plant parts were powdered and subjected to solvent extraction. The crude extract obtained after evaporating the solvent was dissolved in dimethyl sulphoxide before any test or analysis. The pathogenic bacteria used were *Bacillus subtilis*, *Salmonella pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and the pathogenic fungi were *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans*, *Botrytis cinerea*, *Trycophyton rubrum* and *Trichophyton mentagrophytes*.
Results: Maximum antibacterial activity of methanol root extract (16 mm inhibition zone with 5 mg/ml) was observed with *Staphylococcus aureus* on Muller Hinton Agar medium. However, the bark extract of the solvent imposed only moderate activity on the bacterium (12 mm inhibition zone with 5 mg/ml). Leaf extracts from all solvent systems recorded poor inhibition in the given concentration. *Candida albicans, Trycophyton rubrum* and *Trichophyton mentagrophytes* showed minimum inhibitory concentration of 0.31 mg/ml with methanol root extract. The minimum inhibitory concentration was greater than 2 mg/ml in hexane extract of leaf, bark and root. In the present study the antimicrobial activity was significant in methanol root extract of *Walsura trifoliata*.
Conclusion: The methanol extract obtained from the root of *Walsura trifoliata* has significant antimicrobial activity due to the phytochemical components present in the root.

Keywords: *Walsura trifoliata,* Phytoconstituents, Antibacterial, Antifungal.

1. Introduction

More than 80% of the population in developing countries depends on plants for their healthcare [1]. There has been a spur in the systematic screening of plants using traditional knowledge with the purpose of discovering new bioactive principles. Traditions of collecting, processing and applying plants and plant-based medications have been handed down from generation to generation. Traditional medicine is an important source of alternative medicine; in addition, it has its roots in the heritage of indigenous people. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, much attention has been paid to those compounds isolated from plants of known medicinal value [2]. The bioactive phytochemical constituents such as alkaloids, flavonoids, phenolics, essential oils, tannins and saponins are usually responsible for the medicinal properties [3],[4]. Hence a vast number of medicinal plants, wild herbs and shrubs are always under phytochemical investigations [5],[6].

Microbial infections pose health problems throughout the world. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [7]. Herbal plants can be an alternative but a cheap source of medicine to conventional antibiotics against common bacterial infections [8]. So there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases [9].

Walsura trifoliata (Meliaceae) is a tree species, densely foliaceous, bark brown smooth, leaves trifoliate, flowers pale yellow, berries globose, tomentose, orange yellow, seeds solitary enveloped in white juicy aril. The wood is used in making furniture [10],[11],[12],[13]. The plant is well reputed in traditional system of medicine and used by tribal people to treat various diseases such as skin allergies, astringent and diarrhoeia [14]. But not much laboratory studies were made confirming the traditional usage [15]. The present study is to investigate the antimicrobial activity of *Walsura trifoliata* leaf, bark and root in hexane, ethyl acetate and methanol extracts.

2. Materials and Methods

2.1. Plant Collections

Walsura trifoliata (A. Juss.) Harms. was collected from Kambakkam, Chithur District of Andra Pradesh, India. The identity was confirmed by Dr. D. Narasimhan, Centre for Floristic Research, Department of Botany, Madras Christian College, Chennai, India.

2.2. Method of extractions

The leaves, bark and root were carefully removed, shade-dried and powdered. The powders were extracted three times by cold percolation method separately with 9 L of hexane, ethyl acetate and methanol at room temperature for 48 h. The filtrates were dried under reduced pressure at 40 °C and the extracted powder was stored in a refrigerator at 2–8 °C for use in subsequent experiments. The extracted powders were dissolved in dimethyl sulphoxide (DMSO), subjected to phytochemical analysis and antimicrobial activity [16].

2.3. Phytochemical analysis

The preliminary analysis for phytoconstituents of the powdered solvent extracts was done by following conventional protocol [17],[18].

3. Antimicrobial activity test

3.1. Antibacterial activity by disc diffusion method

The antimicrobial activity was tested as per the standard reference method [19]. The micro organisms used for this purpose include; *Bacillus subtilis* (MTCC 441), *Salmonella pyogenes*, *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* ATCC 15380*, Pseudomonas aeruginosa* (ATCC 15380) and the pathogenic filamentous fungi *Aspergillus flavus*, *A. niger* MTCC 1344, *Candida albicans* 46/01, *Botrytis cinerea*, *Trycophyton rubrum 57/0, Trichophyton mentagrophytes* 66/01. All micro organisms used in the test were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Hi-media, Mumbai). The test cultures (100 µl of suspension containing 10^8 CFU/ml bacteria) were swabbed on the top of the solidified media and allowed to dry for 10 min. The tests were conducted at three different concentrations of the crude extract (1.25, 2.5, 5.0 mg/disc). The discs (6 mm in diameter) were impregnated with 20 µl of the extracts. The loaded discs were placed on the surface of the medium and left 30 min at room temperature for compound diffusion. Streptomycin (10 µg/disc) was used as positive control. The plates were incubated for 24 h at 37 °C. Zone of inhibition was recorded in millimetres and the experiment was repeated twice.

3.2. Antifungal assays using broth micro dilution method

The filamentous fungi were grown on Sabouraud Dextrose Agar (SDA) slants at 28 °C for 10 days and the spores were collected using sterile double distilled water and stored at 4 °C until usage. The antifungal activity was performed according to the standard reference method [20]. The extracts were dissolved in 2% dimethyl

sulfoxide (DMSO) with water. The initial concentration of the extract was 2 mg/ml. The initial test concentration was serially diluted two-fold in 96 well plates. Each well was inoculated with 5 µl of suspension containing 10^4 spore/ml of fungi. The antifungal agent Fluconazole was included in the assay as positive control; MIC was defined as the lowest extract concentration showing no visible fungal growth after incubation time.

4. Results and Discussion

Indian system of traditional medicine provides a number of medicinal plants to treat various diseases. Traditional knowledge and literature on medicine play an important role in the discovery of novel leads from medicinal plants [21]. The present study validates the antimicrobial activity of *Walsura trifoliata* leaf, bark and root in hexane, ethyl acetate and methanol extracts. In phytochemical analysis, presence of tannins, steroids, phenols, saponins and alkaloids were found in leaves, bark and root extracts obtained using hexane, ethyl acetate and methanol. Flavonoids were found in hexane, ethyl acetate and methanol root extract (Table. 1). The leaves, bark and root in hexane, ethyl acetate and methanol extracts were used against twelve microorganisms in this study. The antibacterial activity was shown in Table 2. Significant activity was found in all the root extracts. Moderate activity was found in all the bark extracts. Activity was less in leaves extracts. Only the methanol extract of leaves showed activity. Dabur *et al*., [22] reported that the methanol extracts of *Acacia nilotica* and *Justicia zeylanica* exhibited good antimicrobial activity.

Table 1. Phytochemical analysis of Walsura trifoliata

Plant Parts and extract		Phytoconstituents						
		Carbohydrate	Flavonoids	Tannins	Phenols	Steroids	Saponins	Alkaloids
Young Leaves	Hexane	-	-	+	+	+	+	+
	EA	-	-	+	+	+	+	+
	Me	-	-	+	+	+	+	+
Mature Leaves	Hexane	-	-	+	+	+	+	+
	EA	-	-	+	+	+	+	+
	Me	-	-	+	+	+	+	+
Bark	Hexane	-	-	+	+	+	+	+
	EA	-	-	+	+	+	+	+
	Me	-	-	+	+	+	+	+
Root	Hexane	-	+	+	+	+	+	+
	EA	-	+	+	+	+	+	+
	Me	-	+	+	+	+	+	+

EA; Ethyl acetate, Me; Methanol extract. (+ : present, - : absent)

The ethanol, methanol and petroleum ether extracts were more effective than dichloromethane, and aqueous extract in *Vitex leucoxylon* [23]. Methanol extract of *T. fassoglensis* had an antimicrobial effect against four out of the five bacterial strains tested i.e. *B. cereus, S. aureus, E. coli and B. subtilis*, whereas, the petroleum-ether extract showed no antibacterial activity [4]. In the present study maximum inhibitory zone (16 mm) was observed in 5 mg/ml methanol extract of the root against *S. aureus.* 2.5 mg/ml methanol extract showed 14 mm inhibitory zone against *S. aureus*. 14 mm inhibitory zone was also observed in 5 mg/ml methanol extract of the root against *B. subtilis*. Comparing to Gram-positive and Gram-negative bacteria, the plant extract inhibited the growth of Gram-positive bacteria significantly.

The methanol extracts of *Combretum caffrum* and *Salix capensis* effectively inhibited the growth of both the Gram-positive and the Gram-negative bacteria, *Schotia latifolia* extract inhibited the bacteria with the exception of two Gram-negative bacteria namely *E. coli* and *K. pneumoniae* [24]. Similar results were also reported by other workers [25],[26], whereby the majority of the antibacterial activity observed was in the methanol extracts. The aqueous extract of *Juniperus oxycedrus* had no antimicrobial effect against the test

microorganisms whereas the methanol extract had inhibitory effects on the growth of 57 strains of 24 bacterial species in the genera of *Acinetobacter*, *Bacillus*, *Brevundimonas*, *Brucella*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Pseudomonas*, *Staphylococcus* and *Xanthomonas* [27]. As per the earlier reports and the present investigations, significant antibacterial activity was found in the methanol extract.

Table 2. Antibacterial activity of crude extract of Walsura trifoliata young leaves, matured leaves, bark and root.

Name of the extract		Concentration (mg/ml)	Zone of Inhibition (mm)					
			B.s	***S.p***	***S.a***	***E.c***	***K.p***	***P.a***
Young Leaves	Hexane	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	Ethyl acetate	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	Methanol	1.25	-	-	-	-	-	-
		2.5	-	-	8	-	-	-
		5	8	8	10	-	-	-
Matured Leaves	Hexane	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	Ethyl acetate	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	8	8	-	-	-
	Methanol	1.25	-	-	-	-	-	-
		2.5	-	-	8	-	-	-
		5	8	10	10	8	-	-
Bark	Hexane	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	-	-	-	-
	Ethyl acetate	1.25	-	-	-	-	-	-
		2.5	-	-	8	-	-	-
		5	-	8	10	-	-	-
	Methanol	1.25	-	8	-	-	-	-
		2.5	-	8	10	8	-	8
		5	8	10	12	10	10	8
Root	Hexane	1.25	-	-	-	-	-	-
		2.5	-	-	-	-	-	-
		5	-	-	8	-	-	-
	Ethyl acetate	1.25	-	-	-	-	-	-
		2.5	-	8	10	-	-	8
		5	8	10	12	-	8	10
	Methanol	1.25	9	10	12	-	8	8
		2.5	10	11	14	8	11	10
		5	14	12	16	8	10	12
Streptomycin	(µg/ml)	10	22	20	20	16	18	8

The minimum inhibitory concentration for antifungal activity was given in Table 3. The MIC 0.031 mg/ml was observed in the methanol extract of the root against *C. albicans, T. rubrum* and *T. mentagrophytes.* The methanol root extract was active against *A. flavus* and *B. cinerea* at the concentration of 0.062 mg/ml. The extract from the bark and root in ethyl acetate showed moderate activity. 0.25 mg/ml of bark ethyl acetate extract was active against *A. niger* and *B. cinerea.* 0.125 mg/ml of bark ethyl acetate extract was active against *T. rubrum* and *T. mentagrophytes*. The leaves showed very less activity. The highest activity was obtained with both methanol and water extracts of bitter kernels, which showed broad spectrum antimicrobial activity against Gram-positive, Gram-negative and *Candida* strains [28]. In the present investigation, the methanol extract from the root of *W. trifoliata* showed significant antimicrobial activity.

Table 3. Antibacterial activity of crude extract of Walsura trifoliata young leaves

Tested Fungi	Minimum inhibitory concentration(2mg/ml)											
	Young leaf			Mature leaf			Bark			Root		
	Hex	E.A	Meth	Hex	E.A	Meth	Hex	E.A	Meth	Hex	E.A	Meth
Aspergillus niger	>2	1	1	>2	0.5	0.5	1	0.5	0.5	0.5	0.25	0.125
Aspergillus flavous	>2	1	1	>2	1	0.5	>2	0.5	0.5	1	0.5	0.062
Candida albicans	>2	1	0.5	1	1	0.25	1	0.5	1	1	0.5	0.031
Botrytis cinerea	>2	0.5	0.5	>2	1	0.5	1	0.25	0.5	0.5	0.25	0.062
T.rubrum	>2	0.5	0.5	1	0.5	0.25	0.5	0.125	0.125	0.5	0.125	0.031
T.mentagrophytes	>2	1	0.5	1	0.5	0.25	1	0.5	0.125	0.5	0.125	0.031

Hex: Hexane, E.A: Ethyl.acetate, Meth: Methanol

5. Conclusion

The antimicrobial constituents of plant set an explanation for the usage of herbs in traditional folk medicine. The result of the present study showed that the extracts of *W. trifoliata* contain varied phytochemical components which have potential applications against human pathogens. Further purification of crude extracts for active ingredients especially from the root of *W. trifoliata* warrants our attention.

6. References

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