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Evaluation of Antimicrobial Activity of Root Extracts of *Abitulon indicum*

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Abstract

Antimicrobial activity of *Abitulon indicum* roots was studied against seven pathogenic bacteria and three fungal strains by agar well diffusion method. Antimicrobial activity was recorded for hexane, chloroform, methanol, ethanol and aqueous extracts. Alcohol (ethanol and methanol) extracts exhibited the highest degree of antimicrobial activity compared to aqueous, chloroform and hexane extracts. *Pseudomonas aeruginosa* was turned out to be the most susceptible bacterium to the crude root chemical constituents, using the standard Tetracycline and Clotrimazole. Minimum inhibition concentration values of hexane, chloroform, methanol, ethanol and aqueous extracts were determined by the agar dilution method and ranged between 62.5 and 1,000 µg. The study suggested that the root extracts possess bioactive compounds with antimicrobial activity against the tested bacteria and fungi, revealing a significant scope to develop a novel broad spectrum of antimicrobial drug formulation from *Abitulon indicum*.

Keywords: alcohol extract, bioactive compounds, microorganisms, MIC

Introduction

Plants represent a valuable source of natural products and have the capacity to produce a large number of organic photochemicals, such as secondary metabolites, which serve as plant self defense mechanisms against pests and pathogens. Natural products have played an important role in treating and preventing human ailments. It is estimated that over 50% of all drugs (and their derivatives and analogs) in clinical use, are higher plant derived natural products. According to the World Health Organization (WHO), about 80% of the people in developing countries still rely on traditional medicine for their primary health care and about 85% of such medicines involve the use of plant extracts. According to recent estimates from WHO, more than 3.5 billion people in the developing world rely on plants as source of medicine for various ailments. Over 20,000 plants have medicinal values and many plants are yet to be explored for their potential. In addition, many of the existing synthetic drugs cause various side effects. Hence, drug development from plant based compounds could be useful in meeting the demand for newer drugs with minimal side effects (Srivastava *et al.,* 2000).

A. indicum L. (Indian Abutilon, Indian mallow) is a small shrub in the Malvaceae family, native to tropic and subtropical regions (Kirtikar and Basu, 1980). It is often used as a medicinal plant and widely used in pharmacological disorders and ailments. Traditionally, it is used in inflammation, piles, gonorrhea treatment and as an immune stimulant. The root and bark are used as a diuretic, anthelmintic, pulmonary sedative and for fever release (Kashmiri *et al.,* 2009). The root is used for diuretic disorders and can be taken for the relief of hematuria (Thongsiri, 2001). It is also effective in the treatment of leprosy. The root infusion is prescribed as a cooling medicine (fever) and is considered useful in strangury and haematuria.

The root is true, 1.2-1.5 cm in diameter, cylindrical with smooth surface, yellow in color with strong fragrance and saltish in taste. The antimicrobial activities of the crude extracts of leaves (Prabahar *et al.,* 2009), whole plant (Geda *et al.,* 1978; Naqvi *et al.,* 1991), flowers (Mateen *et al.,* 2011), roots (Bhakuni *et al.,* 1971; Valsaraj *et al.,* 1997) were studied, but so far the literature on antimicrobial activity of ethanol and benzene extracts of *A. indicum* roots is scarce.

Materials and methods

Chemicals, media and antibiotics

The organic solvents (hexane, chloroform, methanol, ethanol and dimethyl sulphoxide -DMSO), were obtained from Rankem company, India. Nutrient broth, Nutrient agar and Saboraud dextrose agar were obtained from Hi-media, Mumbai, India. The antibacterial agent Ciprofloxacin was obtained from Axiom Laboratories Ltd., India.

Root collection

The roots of *A. indicum* L. were collected from Sudikonda forest, East Godavari district, Andhra Pradesh. The specimen was authenticated by Prof. Vatsavaya S. Raju, Plant Systematics Lab, Kakatiya University, Warangal, and voucher specimen was deposited in the Herbarium, Botany department (BDH), Andhra University, Visakhapatnam.

Root extract

The dugout roots were cleaned and dried in shade (25-28 °C) for a month. The dried roots were grounded using a mechanical grinder. Sequential extraction was done using hexane, chloroform, followed by methanol and finally

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ethanol. The filtrates were concentrated by removing the solvents under reduced pressure at 40 °C using a rotary evaporator. The concentrated crude extracts were labeled and stored at 4 °C (Rao *et al.,* 2010).

Simultaneously, the aqueous extract of the root was prepared by adding boiled water to the root obtained powder in a beaker, on water bath, with occasional stirring, for 4 hrs. The aqueous extract was then filtered and reduced under pressure. At the time of testing, known quantity of crude extract (100 mg/ml) was dissolved in DMSO.

Microbial strains and growth conditions

Seven bacterial strains namely *Bacillus subtilis* (MTCC 2763), *Escherichia coli* (MTCC 2960), *Klebsiella pneumoniae* (MTCC 4032), *Pseudomonas aeruginosa* (MTCC 6642), *Proteus vulgaris* (MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Streptomyces pneumoniae* (MTCC 1935), as well as three fungal strains *Aspergillus niger* (MTCC 4360), *Candida albicans* (MTCC 4748) and *Saccharomyces cerevisiae* (MTCC 4742) were procured from IMTECH, Chandigarh, India. Broth and agar were prepared according to the manufacturer's instructions.

Before testing, the bacterial suspension was transferred to nutrient broth and cultured at 37 °C. Inoculates were prepared by adjusting the turbidity of the medium to match the 0.5 MC farland standard. The fungal cultures were maintained on Saboraud dextrose agar, incubated at 25 °C for 4 days. The fungal growth was harvested and washed with sterile normal saline and finally suspended in 100 ml of sterile normal saline; the suspension was stored in refrigerator till used.

Determination of antimicrobial activity

Antibacterial and antifungal activity of *Abitulon indicum* L. root extracts were determined using agar well method (Owk *et al.,* 2014). For susceptibility test, 100 µl of inoculums, equivalent to 10 CFU, were mixed with 6 ml of nutrient agar (to ensure even distribution of bacteria) and poured immediately into the sterile petri plates. The petri plates were left for 10 minutes in order for agar to solidify. A sterilized 6 mm borer was used to make wells in the centre of the divided areas. About 50 µl of each extract were then pipette into the wells. Petri plates with bacteria and test extracts were incubated at 37 °C for 16-18 hr after which the inhibition zone (IZ) was measured using an antibiotic zone reader scale (HiAntibiotic ZoneScale-c).

For the antifungal activity, the same method as for bacteria was adopted and Saboraud dextrose agar was used. The inoculated medium was incubated at 25 °C for two days for *Candida albicans*, *Saccharomyces cerevisiae* and three days for *Aspergillus niger*. About 500 µg of clotrimazole were dissolved in 1 ml of sterile de ionized water. About 10 µl of 0.5 mg/ml clotrimazole (equivalent to 5 µg dose) and 10 µl of DMSO were pipette into wells. For bacteria, multidrug antibiotic disc was used (Axiom Laboratories Ltd., India). Each experiment was conducted in triplicates and diameter of the IZ surrounding each well was recorded.

The extracts that exhibited IZ were subjected to minimum inhibition concentration (MIC) assay by using serial two-fold dilution (Krishna Rao *et al.,* 2013). A quantity of 0.6 g of each extract was dissolved in 300 ml sterile nutrient broth which yields initial concentration of 2,000 µg/ml. Subsequently, two-fold serial dilution was made from the stock to obtain 1,000, 500, 250, 125 and 62.5 µg/ml concentrations. One ml of standardized inoculum of each test organism was introduced into each extract nutrient broth mixture and then incubated at 37 °C. The lowest concentration inhibiting growth was regarded as the MIC of the extracts. For the fungi, the inoculated medium was incubated at 25 °C for two (*C. albicans*, *S. cerevisiae*) or three (*A. niger*) days.

Statistical analysis

Each experimental data from triplicates was subjected to one way ANOVA using Minitab version 15. A significant level of 0.05 was used for all statistical analyses.

Results and discussions

The antimicrobial activity of the five different solvent extracts of *A. indicum* revealed that the ethanol extract had significant activity against all the tested microorganisms, followed by methanol extract, while the chloroform and hexane extracts possessed moderate activity and the aqueous extract had the weakest activity. The results of the present study were significant at level of $p>0.05$.

Ethanol extract exhibited the highest inhibitory zone against *P. aeruginosa*, followed by *P. vulgaris*, while methanol extract exhibited the maximum inhibitory effect against *E. coli* and *S. griesus*, and hexane extract showed the high zone of inhibition against *K. pneumoniae* and *P. aeruginosa*. The chloroform and aqueous extracts showed maximum inhibitory zone against *S. cerevisiae* (Table 1). Ethanol extract evinced significant antimicrobial activity with standard antibiotics tetracycline and clotrimazole.

From the MIC values (Table 2), it was observed that *P. aeruginosa, P. vulgaris* and *A. niger* were the least sensitive to ethanol extract at concentration of 62.5 µg/ml, whereas methanol extract was found to have the smallest MIC value against *E. coli* and *S. griesus*.

Alcohol extract of *A. indicum* roots produced the most consistent level of inhibition of microbial growth. The results indicated that most of the active constituents responsible for exerting antimicrobial action are expected to be soluble in alcohol. The preliminary phytochemical investigation revealed the presence of alkaloids, amino acids, anthraquinone, carbohydrates, cardiac glycosides, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids (Table 3). The combined activities of these secondary metabolites produced a synergic effect, thus increasing the antimicrobial potency of alcohol extracts of *A. indicum*.

Hexane and chloroform extracts had low antimicrobial effect on the tested organisms. This may be due to the presence of only few compounds extracted into the solvent with not enough inhibitory activity against tested pathogens.

Prabahar *et al.* (2009) reported that ethanol extracts of *A. indicum* leaves showed antimicrobial activity against Gram-negative organisms *P. aeruginosa, P. vulgaris, E. coli, Shigella sonnei* and Gram-positive organisms such as *B. subtilis, B. megatherium, S. leuka, S. aureus*, while ethanol extracts of flowers was found to be effective against *S. aureus, P. aeruginosa,*

Table 1. Antimicrobial activity of different extracts of *Abitulon indicum* roots

Organism tested	Zone of inhibition (in mm) ± SE					Standard drugs/ controls	
	Methanol extract	Ethanol extract	Aqueous extract	Chloroform extract	Hexane extract	Tetracyclin/ clotrimazole	DMSO
B. subtilis	19±0.6	20±0.7	14±0.1	16±0.0	12±0.4	18^{T}	Nil
E. coli	20±0.6	18±0.6	Nil	15±0.6	15±1.1	22^{T}	Nil
K. pneumoniae	18±0.1	17±0.2	18±0.0	14±1.5	16±0.2	24^{T}	Nil
P. aeruginosa	17±0.4	24±0.1	12±0.5	16±0.3	16±0.2	23^{T}	Nil
P. vulgaris	18±1.4	22±0.5	Nil	12±0.2	14±0.5	22^{T}	Nil
S. aureus	19±0.0	18±0.4	Nil	16±0.0	14±0.4	24^{T}	Nil
S. griesus	20±0.3	20±0.1	Nil	14±0.2	12±0.4	22^{T}	Nil
A. niger	16±0.5	19±0.0	10±0.3	Nil	Nil	18^{C}	Nil
C. albicans	14±0.2	16±0.3	20±0.6	12±0.4	13±0.0	23^{C}	Nil
S. cerevisiae	19±1.1	21±0.3	21±0.7	18±0.5	12±0.4	20^{C}	Nil

All the values of inhibitory activity for the extracts tested were significant at 0.05 levels

Table 2. MIC obtained with different extracts of *Abitulon indicum* roots

Organism tested	Methanol extract	Ethanol extract	Aqueous extract	Chloroform extract	Hexane extract
B. subtilis	250	250	1,000	1,000	>1,000
E. coli	62.5	500	>1,000	1,000	1,000
K. pneumoniae	500	500	500	1,000	125
P. aeruginosa	500	62.5	>1,000	500	125
P. vulgaris	500	62.5	>1,000	1,000	1,000
S. aureus	250	500	>1,000	1,000	1,000
S. griesus	62.5	250	>1,000	>1,000	>1,000
A. niger	1,000	62.5	>1,000	>1,000	>1,000
C. albicans	1,000	1,000	250	1,000	>1,000
S. cerevisiae	250	125	125	500	>1,000

Table 3. Preliminary phytochemical constituents of *Abitulon indicum* root extracts

Phytochemical constituents	Chloroform extract	Methanol extract	Ethanol extract	Aqueous extract
Alkaloids	+	+	+	+
Aminoacids	+	+	+	+
Anthraquinone	-	-	-	-
Carbohydrates	-	+	+	+
Cardiac glycosides	+	+	+	-
Flavonoids	+	+	+	+
Glycosides	+	+	+	-
Phenols	-	+	+	+
Saponins	+	+	+	+
Steroids	-	+	+	-
Tanins	-	+	+	+
Terpenoids	-	-	+	-

Salmonella typhi, S. paratyphi, P. vulgaris, K. pneumonia, E. coli, Shigella sonnei (Mateen *et al.*, 2011). These are in accordance with the present study results, as ethanol extract of roots showed antimicrobial activity against *B. subtilis, E. coli, K. pneumonia, P. aeruginosa, P. vulgaris, S. aureus*, *S. griesus, A. niger, C. albicans* and *S. cerevisiae*. Poonkothai (2006) reported the ethanol and chloroform leaf extract showed activity against *B. subtilis, S. aureus, K. pneumonia, P. aeruginosa, E. coli, S. typhi*, while Gurumurthy *et al.* (2011) stated petroleum ether, chloroform and methanol extracts showed high antimicrobial activity against *S. aerus, P. aureus, K. pneumoniae* and less activity found against *E. coli*.

The finding of the present study also showed methanol, ethanol, aqueous, chloroform and hexane extracts were efficient against all tested microorganisms except *E. coli, P. vulgaris, S. aureus, S. griesus* for aqueous extract and *A. niger* for both chloroform and hexane extracts of *A. indicum* roots.

Conclusions

A. indicum is already considered as medicinal plant. The plant is said to be a source of many bioactive principles acting against some human ailments; the root extracts analyzed hereby exhibited high degree of antimicrobial activity against all tested bacteria and fungal strains. The present study also suggested that root posses' bioactive compounds responsible for exerting antimicrobial action against infectious diseases caused by bacteria and fungi. Therefore, it is concluded that alcohol extracts of *A. indicum* roots brings to light the scope to develop a novel broad spectrum of antimicrobial drug formulation.

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