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Original Article

ANTIMICROBIAL ACTIVITY OF *WITHANIA SOMNIFERA* AND *CALOTROPIS PROCERA ON PATHOGENIC STRAINS*

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ABSTRACT

Objectives: The present study was planned to investigate the antimicrobial activity with Minimum Inhibitory Concentration of different part of *Withania somnifera* and *Calotropis procera***.** Therefore, the preliminary successive solvent extraction and chemical test revealed the presence of secondary metabolites in various extracts, and provide us clue for further investigation.

Methods: Different solvents i.e., Chloroform, and Ethanol, were used for extraction of active secondary metabolites by Soxhlet method. Percentage of yield[4], and MIC by gradient plate technique [13]. Statistical analysis done by the standard error by mean (SEM), in which n=3.

Results: The significant antimicrobial activity found in *Calotropis procera* stem extract with ethanol was 1.3±0.046 mg/ml to 2.8±0.009 mg/ml on bacterial strain and 10.4±0.013 mg/ml to 13.3±0.015 mg/ml MIC values with±SEM. In *Withania somnifera*, it was an average. The highest percentage of yield was observed in Withania Somnifera with Ethanol and Chloroform i. e from 2.78% to 4.83%. Various numbers of secondary metabolites were extracted from 3 to 5 in *Withania somnifera* and *Calotropis procera* combindly.

Conclusion: Ethanol has been shown as a best solvent to yield, extracted components and antimicrobial effects. In CRE and CSE extracts, only root extracts has been shown best inhibitory effects on all concerned microbes, while chloroform were also good solvents to extract secondary metabolite components.

Keywords: *Withania somnifera, Calotropis procera*, Secondary metabolites, Antimicrobial activity, yield, phytochemical estimation, Solvents.

INTRODUCTION

The value of medicinal plants depends upon the use of plants as raw materials in the pharmaceutical industry. People living in rural areas from their personal experience know that these traditional remedies are a valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses. Several medicinal compounds have been successfully isolated from plants and presently being used for the treatment and management of different diseases. The discovery of new drugs through natural products is the single most successful strategy which led the invention of several modern drugs. Through the use of the plants, human beings have taken advantage of the medicinal compound known as secondary metabolites present in the leaves, stems, roots and sap of the plants. The secondary metabolites such as saponins, alkaloids, flavonoids, glycosides, anthraquinone, steroids and tannins from plants has also been used in the modern system of medicines for their extensive therapeutic values [1, 2].

Withania somnifera (Ashawagandha) of family Solanaceae is commonly known as "Indian Winter cherry" or "Indian Ginseng". In Ayurveda Ashwagandha is considered a 'Rasayana' herb. This herb is also considered an adaptogen which is an herb that works to normalize physiological function. In India, this herb, *W. somnifera* holds a position of importance similar to Ginseng in China and is commonly known as Ashwagandha, Winter-cheery, *Withania,* Ashwagandha, in Sanskrit, means ''horse´s smell''. This name originated because of the odor of its root which resembles that of the sweaty horse. The species name somnifera means "sleep-making" in Latin, indicating that to it are attributed sedating properties, but it has been also used for sexual vitality.

Calotropis procera (called Akond in Bengali, Swallow wort in English, Akundia in Hindi), a wild growing plant is well known for its medicinal uses in traditional system of medicine for the treatment of a variety of disease conditions that include leprosy, ulcers, tumors, piles and disease of spleen, liver and abdomen. The methanol extract of leaves of *Calotropis procera* Linn was subjected to the potential antioxidant and antibacterial activities. The root bark and leaves of *Calotropis procera* are used by various tribes of central India as a curative agent for jaundice [2]. The plant has been reported to pass diverse biological activities. Different parts of the plant have been found to possess proteolytic, antimicrobial, larvicidal and anticancer activities [3]. C*alotropis procera* is small, erect and compact shrub, which has been known to possess analgesic antitumor, antihelmintic, antioxidant, hepatoprotective, antidiarrheal, anticonvulsant, antimicrobial, estrogenic, antinociceptive and antimalarial activity [3].

Through the therapeutic value of this plant has been investigated, but the detailed phytochemical studies in relation to its antimicrobial activity have not been investigated. Therefore, keeping the above facts in view, the present study has been planned to investigate the antimicrobial activity of leaf, and root of *Withania somnifera* and *Calotropis procera.*

MATERIALS AND METHODS

Collection of plant

The whole plant was collected from Govt. Nursery of Modinagar, Dist. Ghaziabad, U. P, India, and has been authenticated by NBRI, Lucknow, India (Voucher/Specimen no. is NBRI-SOP-202) a voucher specimen was deposited in the department.

Extraction

The plant's part (stems, leaves, roots) was separated from each other and washed carefully under running tap water followed by distilled water. These were shade dried under room temperature for one week and pulverized to a fine powder, using a sterilized mixer grinder. The 300 gm powder of each plant's part extracted by the Soxhlet method using Chloroform, Ethanol as solvents on the basis of their polarity of 4.1and19.4.

Yield

The percentage yields were calculated using following formula [4]:

$$\text{Yield in \%} = \frac{\text{Quantity of extraction in gm}}{\text{Quantity of dry powder of plant in gm}} \text{x100}$$

Qualitative Phytochemical investigations of extract

A qualitative chemical test was conducted with the aim to check the presence of various phytochemical constituents in different parts of plant extracts. The phytochemical analysis was conducted using the test developed by Kokate *et al.* (2013) and the presence of different phytochemicals in the extract was listed in the table which as further used for qualitative investigation.

Antimicrobial activity

Collection of strain

The strain of Bacterial pathogens *Corynebacterium diphtheriae*: MTCC 116, *Pseudomonas aeruginosa*: MTCC 10462, *Bacillus thuringiensis*: MTCC 10484, *Bacillus anthracis:* MTCC 10095, *Salmonella typhi*: MTCC 3231, *C. pneumoniae*: MTCC 7162 and Fungal pathogens like *A. fumigatus*: MTCC 4163, *C. neoformans*: MTCC 6333, *C. albicans*: MTCC 7253, was collected from innovative lifesciences, lucknow and *C. vaginitis:* Clinical isolate, *B dermatitidis* clinical isolate. Gaurang Homeo Clinic, Aliganj, Sector-1, Lucknow 226010, Uttar Pradesh.

Preparation of bacterial/Fungal strains and culture conditions

The obtained cultures of *S. typhi, C. pneumoniae, B. thurengenesis, B. anthracis, P. aeruginosa* were maintained on nutrient agar and *A. fumigates, C. neoformans, C. albicans, C. vaginitis, B. dermatitidis* on Potato Dextrose agar medium by making slants and the stock cultures of were transferred at monthly intervals. A single colony was transferred in sterile 100 ml of nutrient broth/potato dextrose media and incubated at 37 °C/25 °C in a shaker at 140 rpm for 14 h. Culture of bacteria and fungi was recovered by centrifugation and were suspended in sterile distilled water; the concentration of the pathogen was optimized by maintaining OD to 0.1 at 600 nm [5].

Screening of bioactive compounds against various microbes

The method used to screen plant extracts before determination of MIC, was agar disc diffusion method, in which 10 ml of nutrient agar media poured in a sterile Petri dish, 100 µl of test organisms were spread on the surface of media, wells were prepared with help of sterile borer and wells were aseptically filled by 50 µl of plant extract along with positive (Antibacterial compound Tetracycline and Antifungal compound Fluconazole at 50 µg/ml and 100 µg/ml respectively) and negative control (Autoclaved Distilled water). Plates were incubated aerobically at 37 °C/25 °C for 72 h. The diameter of zones of inhibition was measured. The initial concentration of MIC was 10 mg/ml for bacterial strains and 20 mg/ml for fungal strains.

Minimum inhibitory concentration (MIC) determination

MIC is carried out by double agar double diffusion method. 10 ml molten agar medium (Nutrient agar for bacteria and Potato dextrose for Fungal strains) was poured into the plate without antibiotic and was allowed to harden. After the hardening of agar (2-5 min), the plate was set flat on the desk and 10 ml medium containing the antibiotic (10 mg/ml) was added. It was allowed to harden for 15 to 20 min. Using 100 µl fresh cultures (grow overnight) of concern microbe and spread with the help of inoculation spreader, microbes were spread out over the surface of the medium, taking care not to tear the agar. Later it was incubated for approximately 72 h. The plate was observed in the pattern of microbial growth. Tetracycline (Microxpress) was taken for bacterial strains as positive control and Fluconazole (Microxpress) was used as standard compound as positive control for fungal strains.

S. No.	Name of the secondary metabolite	Method-1	Method-2
1	Alkaloid	Hager's method	Tannic acid method
2	Saponin	Foam test	
3	Steroids/Terpenoids	Salkowaski reaction	
	Flavonoids	Shindona test	Sodium hydroxide test
5	Tannins	Lead Acetate test	
6	Card. Glycosides	Kellar Killani test	Beljet's test
7	Anthroquinone	Confirmation test given in kokate.(Pharmacology Hand Book)	

Analysis of data

Size of petri plate was 100 mm. Concentration of antibiotic/extracted drug-10 mg/ml, and total medium was poured on a single plate was 10 ml. So 100 mg drugs have been poured into the 100 mm plate as a higher to lower concentration. Every 1 mm of plate surface area has been considered as 1 mg/ml concentration of drug for inhibition for bacterial strains and 2 mg/ml= 1 mm was considered for fungal strains, the distance was measured from the top end and the concentration of the compound was calculated as MIC. Statistical analysis has done by the standard error by mean (SEM), in which n=3.

RESULTS AND DISCUSSION

In the present study, chloroform and ethanol extracts of stem, leaf, roots of *Withania somnifera* and *Calotropis procera* were evaluated for the presence of phytochemical constituents as well as their antimicrobial activity was tested. Successive solvent extraction values in various organic solvent were observed as chloroform than ethanol (table 1).

The results of the phytochemical analysis were carried out in extracts of *Withania somnifera and Calotropis procera* medicinal plant. The experiment showed the presence of secondary metabolites such as alkaloid, glycosides, flavonoids, saponins, tannins, steroids and anthraquinone. Hence the phytochemical screening reveals that chloroform extracts show high no. of secondary metabolites, while ethanol has shown average extracted secondary metabolites. Thus the preliminary screening analysis is helpful in the direction of bioactive components in discovery and development of new drug (table 2)

Table 1: Yield of *Withania somnifera* and *Calotropis procera*

S. No.	Solvent	Quantity (mg)	Yield (%)	Appearance
1	WLC	2415	4.83	Light brown
2	WRC	1390	2.78	Cream
3	CSE	1945	3.89	Cream
4	CRE	1615	3.23	Cream

Table 2: Phytochemical study on *Withania somnifera* and *Calotropis procera*

S. No.	Code	Alk	Sap	Std	Flv	Tan	Cd. Gly	Antq	No. of S. M
1	WLC	--	++	++	++	--	--	++	4
2	WRC	++	++	++	--	++	--	++	5
3	CSE	--	++	++	++	--	--	++	4
4	CRE	++	++	++	--	--	--	--	3

The antimicrobial activity study showed that *Calotropis* root extract and *Calotropis* stem extract with ethanol gives excellent inhibitory effect against all experimental pathogen, while *withania* leaf extract with chloroform given average If we discussed about *Calotropis* stem ethanol extract inhibitory effects, representing excellent results against multi drug resistant strain *P. aeruginosa, S. typhi, B. thruengenesis, B. anthracis, A. fumigates and C. vaginitis, C. neoformans, C. albicans* and *B. dermatidis.* On the other hand it is showing average effects *against C. pneumoniae* (bacterial cell).

Withania leaf chloroform and *Withania* root extract inhibitory effects, representing average inhibitory effects against *B. thruengenesis, B. anthracis, S. typhi, Pseudomonas aeruginosa,, C. pneumoniae* (bacterial cell), *C. neoformans, A. fumigates, C. albicans C. vaginitis* and *B. dermatidis* (fungal cell).

Table 3: MIC of *Withania somnifera and Calotropis procera* through Gradient Plate technique on Pathogenic microbial Strains with SEM Mg/ml MIC value with mean±SEM (n=3)

S. No.	Bacterial Strains	WLC	WRC	CSE	CRE	+ve Control (µg/ml)	-ve Control
1	*P. aeruginosa*	7.5±0.061	6.8±0.091	1.3±0.046**	1.5±0.061**	36±3.31	-
2	*S. typhi*	8.6±0.012	9.2±0.019	1.5±0.027**	2.1±0.015**	25±3.53	-
3	*B. thruengenesis*	9.3±0.012	7.2±0.014	2.8±0.009**	1.7±0.010**	27.5±6.12	-
4	*B. anthracis*	8.9±0.011	7.8±0.007	2.3±0.012**	1.2±0.011**	21±4.30	-
5	*C. pneumoniae*	9.0±0.018	9.4±0.009	8.9±0.010	2.5±0.0073**	26±2.44	-
	Fungal Strains						
1	*A. fumigatus*	18.2±0.009	19.4±0.012	12.3±0.013**	13.4±0.011**	65±5.00	-
2	*C. neoformans*	16.4±0.015	17.3±0.013	10.5±0.014**	12.2±0.015**	72±4.63	-
3	*C. albicans*	15.9±0.012	18.6±0.011	13.3±0.015**	14.5±0.016**	36±5.09	-
4	*C. vaginitis*	19.2±0.014	15.6±0.015	10.4±0.013**	12.3±0.012**	46±4.30	-
5	*B. dermatidis*	18.5±0.010	13.6±0.020	12.4±0.013**	13.5±0.013**	60±2.50	-

*=good, **=very good, SEM=±standard error with mean,-ve control= Double distilled water,+ve control= Tetracycline, (-) = No inhibition concentration.

CONCLUSION

Ethanol has been shown as a best solvent to yield, extracted components and antimicrobial effects. In CRE and CSE extracts, only root extracts has been shown best inhibitory effects on all concerned microbes, while chloroform were also good solvents to extract secondary metabolite components.

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CONFLICT OF INTERESTS

Declared None

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