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Research Article

Alectra parasitica A. Rich. – An Unexplored Parasitic Plant with Potential as Antimicrobial Agent

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Abstract

The present study was carried out to evaluate antimicrobial activity of three different extracts of plant - *Alectra parasitica* A. Rich. The samples were tested against 7 bacterial strains and 2 fungal strains by well diffusion method. The antibacterial activity was tested against human pathogenic strains i.e. *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi*, *Pseudomonas aeroginosa* and *Streptococcus pneumoniae* while, antifungal activity was tested against *Aspergillus niger* and *Candida albicans*. The results showed that, *A. parasitica* acetone and ethanol extracts showed significant antimicrobial activity against microorganism tested. The water extract exhibited no antimicrobial activity against microorganism tested. Comparatively it was showed that, *A. parasitica* acetone extract posses more antibacterial as well as antifungal activity than ethanol extract.

INTRODUCTION

Microorganisms are causative agents of almost all kinds of acute and chronic diseases. The plant based antimicrobials have enormous therapeutic potential (Robbers *et al*., 1996; Barbour *et al*., 2004; Devi & Lawrence, 2014). Higher plants have traditionally been used in folk medicine showing inhibition against bacteria, fungi and yeasts (Hulin *et al*., 1998). *Alectra parasitica* A. Rich is an unexplored parasitic plant locally known as Nirgunda (family-Scrophulariaceae) parasite on the roots of *Vitex negundo* L. This plant is indigenous to India and first reported by Kamble & Pradhan (1988) from Akola district of Maharashtra. It has been used in the treatment of leprosy, tuberculosis, paralysis, swellings, fever, expulsion of intestinal worms and constipation for centuries in traditional Ayurvedic medicinal practices, remaining strictly confined to a limited areas (Anonymous, 1986; Chopra *et al*., 1956; Rangari, 2006; Saxena & Saxena 2009; Sikarwar *et al*., 2007). Only Vaidoos and mendicants practiced with it and it has not been known sufficiently to practitioners of indigenous medicine in other parts of the country. Properties and uses of this drug as known to local people were also recorded. It is felt that this may prove to be a medicinal plant of economic importance (Awasthi *et al*., 2008; George *et al*., 2011).

Alectra parasitica A. Rich is effective in the treatment of various infectious diseases. Kakpure & Rothe (2012) reported the presence of alkaloids, carbohydrates, sterols, glycosides, saponin, flavonoids, quinone, coumarins and phenolics compounds in *A. parasitica* which is useful for treating different diseases and infections as having a potential of providing useful drugs of human use. Earlier, only Saxena & Vyas (1993) reported different extracts of *A. parasitica var. chitrakutensis* (Rau.) R. Prasad possesses antibacterial activity and none of the extracts of the samples shows antifungal activity.

Despite the intense uses of *A. parasitica* as medicinal plant and the researches concerning the pharmacological importance of this plant, the thorough knowledge of antimicrobial activity is still scarce. So, in the present study an attempt has been made the laboratory evaluations to assess the antimicrobial properties of *Alectra parasitica* A. Rich.

MATERIALS AND METHODS

The antimicrobial activity of *Alectra parasitica* A. Rich in three different extracts i.e. acetone, ethanol and water extracts were carried out by using well diffusion method described by (Mukharjee, 2002; Satish *et. al*., 2008 and Nitha *et. al*., 2012).

Collection and identification of plant materials: The plant *Alectra parasitica* A. Rich was collected from Popatkhed, Dhargad, Patur, Shahanur and Shirla forest areas of Akola district, Maharashtra. The collected plants were identified with the help of standard floras (Kamble & Pradhan, 1988; Naik, 1998; Singh *et. al.* 2001) and herbarium specimens were deposited in Herbarium of Department of Botany, Shri Shivaji College, Akola and the collected whole plant materials was shade dried and grinding into a powder, packed in polythene bags until further use.

Preparation of extracts: The dried plant powdered material of *A. parasitica* (100 g) was extracted with acetone, ethanol and water. The flask kept it on rotary- shaker at 150 rpm for 24 hrs. After 24 hours, the supernatant was filtered through Whatman filter paper No. 41. The acetone and ethanol solvent suspension was completely evaporated using vacuum while, water suspension was boiled in water bath and evaporated. Then, the residues obtained was dissolved in 1% $DMSO_4$ (Dimethyl Sulphoxide) and used for further study.

Bacterial cultures: Bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India. All the bacterial cultures were maintained in nutrient agar and stored at 4^0C.

Antimicrobial Assay: The antimicrobial assay was performed by well diffusion method. Nutrient Broth was prepared in tubes as the media for test bacteria. The bacterial inoculums were spread evenly on the surface of the nutrient agar plates using a sterilized cotton swab. For agar well diffusion method, wells were prepared in the plates with the help of a cork-borer (0.6 cm). 100 µl of the test compound was introduced into the each well. The plates were incubated overnight at 37 ºC for 24 hrs each bacterial strain. Amoxicillin, Gentamycin and Chloramphenicol standard antibiotics were used as a positive reference while, DMSO was used as a negative control.

For fungi, Sabouraud's Dextrose Agar for *Candida albicans* and *Aspergillus niger* were prepared in plates as the media. Fungal strain *Candida albicans* plates were incubated at 37°C for 48 hrs while, *Aspergillus niger* plates were incubated at RT 25-30°C for 72 hrs. Clotrimazole, Ketoconazole and Nystatin standard antibiotics were used as a positive reference while, DMSO was used as a negative control. The antimicrobial activities were then assessed by measuring the diameter of the growth–zone of inhibition in millimeters (including well diameter of 6 mm) for the test organisms comparing to the standard antibiotics. The diameters of zone of inhibition (in mm) are presented in results.

Table-1: Composition of bacterial maintenance Medium (Nutrient agar)

S. N.	Ingredients	Quantity
1	Peptic digest of animal tissue	5 gm/ L
2	Yeast extract	1.5 gm/ L
3	Beef extract	1.5 gm/ L
4	Sodium Chloride	10 gm/ L
5	pH	7.5
6	Agar-agar	15 gm/ L
7	Distilled water	1000ml

Table-2: Composition of fungal maintenance Medium (SDA)

S. N.	Ingredients	Quantity
1	Peptone (Meat & Casein)	10 gm/ L
2	Dextrose monohydrate	20 gm/ L
3	Agar- Agar	15 gm/ L
4	pH	5.8
5	Distilled water	1000 ml

RESULTS AND DISCUSSION

Naturally occurring substances of plant origin have been reported to inhibit the growth of microorganisms. Bacterial infection seems especially controllable due to good hygiene and the availability of effective antibacterial drugs (Dhale & Mogle, 2011). In the present study, the antimicrobial activity of *Alectra parasitica* A. Rich of acetone, ethanol and water extracts were tested by using well diffusion method against 7 bacterial and 2 fungal strains.

The antibacterial activity was tested against the following human pathogenic strains *viz. Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Proteus vulgaris, Salmonella typhi,*

Table 3 - Antibacterial activity of *A. parasitica* showing zone of inhibition in mm.

S. N.	Organisms	Acetone extract	Ethanol extract	Water extract	AMXC	GNTM	CRMP
1	*S. aureus*	16	17	--	18	18	21
2	*Bacillus subtilis*	15	12	--	12	25	28
3	*Escherichia coli*	07	06	--	15	24	22
4	*Proteus vulgaris*	18	13	--	07	26	23
5	*Salmonella typhi*	15	13	--	14	25	23
6	*P. aeroginosa*	11	09	--	19	28	24
7	*S. pneumoniae*	16	14	--	10	25	23

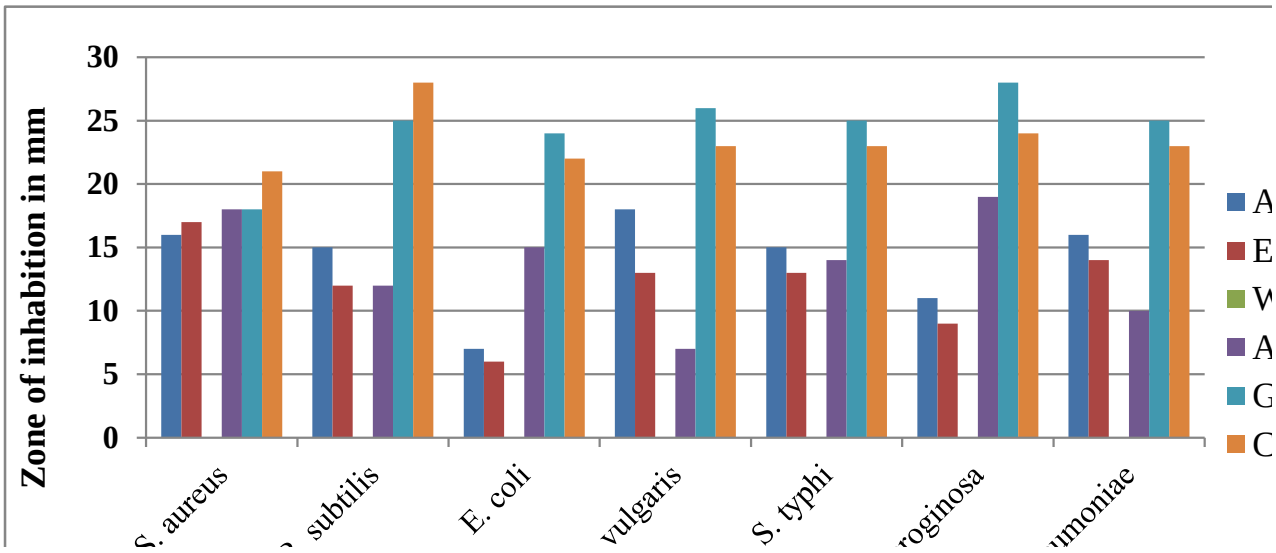
Where, AMXC = Amoxicillin, GNTM = Gentamycin and CRMP = Chloramphenicol.

Table 4 - Antifungal activity of *A. parasitica* showing zone of inhibition in mm.

S.N.	Organisms	Acetone extract	Ethanol extract	Water extract	CTMZ	KTCZ	NYST
1	*Aspergillus niger*	12	15	--	10	14	27
2	*Candida albicans*	18	12	--	08	18	22

Where, CTMZ = Clotrimazole, KTCZ = Ketoconazole and NYST = Nystatin.

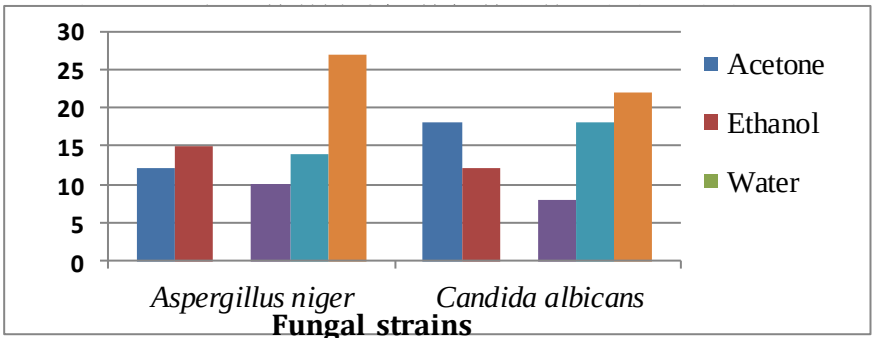
Graph 1 – showing antibacterial activity of *A. parasitica* showing zone of inhibition in mm.



Pseudomonas aeroginosa and *Streptococcus pneumoniae* while, antifungal activity was tested against *Aspergillus niger* and *Candida albicans.* The results were compared with the antibacterial activity of three standard antibiotics Amoxicillin, Gentamycin, Chloramphenicol and antifungal activity of three standard antibiotics Clotrimazole, Ketoconazole and Nystatin respectively.

It was found that, acetone and ethanol extracts showed significant antibacterial as well as antifungal activity of *A. parasitica* against the above mentioned microorganism. The water extract of *A. parasitica* exhibited no antimicrobial activity against microorganism tested (Table-3 & 4). The results showed that, *A. parasitica* acetone extract posses more antibacterial activity than ethanol extract.

Graph 2 – showing antifungal activity of *A. parasitica* showing zone of inhibition in mm.



The significant and highest antibacterial activity (zone of inhibition 18 mm) was shown by acetone extract of *A. parasitica* against *Proteus vulgaris*, successively zone of inhibition 16 and 15 mm were shown by *Staphylococcus aureus*, *Streptococcus pneumonia* and *Bacillus subtilis*, *Salmonella typhi* respectively. Whereas, the minimum zone of inhibition 07 mm was shown by acetone extract against *E.coli*. However, ethanol extract shown moderate zone of inhibition 17, 14, 13, 12, 09 & 06 mm against *Staphylococcus aureus*, *Streptococcus pneumonia*, *Proteus vulgaris*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeroginosa* and *E.coli* respectively. The highest antibacterial activity (zone of inhibition 28 mm) was shown by standard antibiotic Gentamycin against *Pseudomonas aeroginosa* and Chloramphenicol against *Bacillus subtilis* while, least antibacterial activity (zone of inhibition 07 mm) was shown by standard antibiotic Amoxicillin against *Proteus vulgaris.* The detailed results are depicted in (table-3 & graph-1).

The highest antifungal activity (zone of inhibition 18 mm) was shown by acetone extract of *A. parasitica* against *Candida albicans* while, the least (zone of inhibition 12 mm) was showed by both acetone and ethanol extract of *A. parasitica* against *Aspergillus niger* and *Candida albicans* respectively. Water extract was not seen the antifungal activity against tested organisms. The highest antifungal activity (zone of inhibition 27 mm) was shown by standard antibiotic Nystatin against *Aspergillus niger*, while least antifungal activity (zone of inhibition 8 mm) was shown by standard antibiotic Clotrimazole against *Candida albicans* (table-4 & graph-2).

However, the earlier workers Saxena & Vyas (1993) reported different extracts of *A. parasitica var. chitrakutensis* (Rau.) R. Prasad possesses antibacterial activity and none of the extracts of the samples shows antifungal activity. This antimicrobial activity is due to the presence of different secondary metabolites (aromatic substances) which can synthesize by plants (Borde *et al*., 2013; Mogle, 2013). In the present study both the acetone and ethanol extracts shows the significant antifungal activity. This is due to the higher solubility of the active compounds into acetone and ethanol solvents (Kakpure & Rothe, 2012; Devi & Lawrence, 2014). So, the result obtained in the present study showed that *A. parasitica* is effective against several bacterial infection and fungal pathogens.

The results obtained in the present study are in agreement to a certain degree with the traditional uses of the plant. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. From the above results it can be concluded that, *A. parasitica* whole plant powder extracts have great potential as antimicrobial compounds against microorganisms and that they can be used in the treatment of various infectious diseases caused by resistant microorganisms. *Alectra parasitica* A.

significant antibacterial as well as antifungal activity and so this plant may be serve as leads for the development of new pharmaceuticals that address hither to unmet therapeutic needs. However, further investigation on isolation and characterization of the active principles of this plant extracts responsible for the antimicrobial activity is necessary and it would give a comprehensive evidence of bioactive potential of this medicinal plant.

The millenarian use of this plant in folk medicine suggests that it represents an economic and safe alternative to treat infectious diseases.

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