

Antibacterial And Phytochemical Analysis Of Root Extract Of *Alysicarpus Vaginalis* Dc. Against Respiratory Tract Pathogens

Prashant Arya¹, J. P. Mehta¹ and Sanjay Kumar²

¹Department of Botany and Microbiology, H.N.B. Garhwal University Srinagar (Garhwal)

²Department of Botany and Microbiology, Gurukul Kangri University (Haridwar)

Email- prashantarya09@gmail.com

Email-sanjay.gku@gmail.com

ABSTRACT: Present day infectious diseases pose serious problems to health and are the main cause of morbidity and mortality worldwide. The uses of herbs in treatment of human and animal diseases have long been established. Most plant extracts have been shown to possess antimicrobial agents active against microorganisms *in vitro*. These plants contain medicinal properties which make them potent to cure or prevent diseases. Nature has been a prime source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. The Himalayan natives have a great wealth of medicinal plants and traditional medicinal knowledge. Monitoring of susceptibility of herbal drugs for bacterial pathogens is important for appropriate choice of treatment. The present study was to evaluate the antibacterial activity and phytochemical analysis of various roots extracts of *Alysicarpus vaginalis* (Chukalai) against selected common respiratory tract pathogens i.e. *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442, *Klebsiella pneumoniae* MTCC 4030 and *Pseudomonas aeruginosa* MTCC 2474. Plants root were collected from different parts of Garhwal Himalaya and authenticated at Botanical Survey of India, Northern Regional Center Dehradun. Properly washed with water and shade dried Root of the plant at room temperature. The plant material were crushed and extracted according to polarity, low to high in petroleum ether (PET), chloroform (CHCl₃), methanol (MeOH) and Water (H₂O) by using Soxhlet apparatus. The antibacterial activity was examined by agar well diffusion method and the minimum inhibitory concentration (MIC) by twofold serial dilution method. Erythromycin was used as positive control to determine the sensitivity of the strains and Di Methyl Sulphoxide (DMSO) used as negative control. The results showed that methanolic extract was more active than other extracts in its antibacterial activity. The zone of inhibition exhibited by methanol extract against tested microorganisms ranged between 06 mm to 13 mm, respectively. MIC values were recorded between 3.12 mg/ml to 25 mg/ml for all the organisms. The Phytochemical screening for Methanol (MeOH) extract of *A. vaginalis* has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpenes, saponins and tannins. This investigation supports a good response to the use of *A. vaginalis* in herbal medicine and as a base for the development of new drugs and phytomedicine in foundation for its use in treatment of respiratory infectious diseases.

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Keywords- Antibacterial activity, Agar well diffusion method, MIC, Phytomedicine, Respiratory tract pathogens, *Alysicarpus vaginalis*.

INTRODUCTION

Our earth is full of medicinal plants. These medicinal plants having therapeutical properties are very useful in curing various diseases. Almost all our present medicines exhaled from medicinal plants. Approximately, 8000 species of medicinal plants are used as different systems of medicines in India. India is blessed with huge biodiversity due to different climatic zones, in which numerous medicinal plants were reported. The Indian state of Uttarakhand, located in central Himalayan region, is richly gifted

with a large variety of plant species, many of which have medicinal properties. Medicinal plants play an important role in the lives of people in Uttarakhand by providing basic health care and employment to the farmers (Alam & Kop, 2005). The Central Himalayan Region covers the new state of India, provides excellent opportunities for studying the Traditional Knowledge Systems (TKS). The Indian Himalayan region alone supports about 18,440 species of plants (Angiosperms: 8000 spp., Gymnosperm: 44 spp., Pteridophytes: 600 spp., Bryophytes: 1736 spp.,

Lichens: 1159 spp. and Fungi: 6900 spp.) of which about 45% are having medicinal properties. According to (Samant *et al.*, 1998) out of the total species of vascular plants, 1748 spp. species are medicinal. Uttarakhand is a store house of a rich variety herbs and medicinal and aromatic plant species. Medicinal plants produce a wide variety of compounds which in addition to give them characteristic pigments, odour and flavor characteristics may also have antimicrobial properties (Cowan, 1999; Sanjay *et al.*, 2010, Kumar *et al.*, 2014). For thousands of years, traditional plant derived medicines have been used in most parts of the world and their use in fighting microbial disease is becoming the focus on study (Bhavnani & Ballow, 2000; Chariandy *et al.*, 1999). Intensive studies on extracts and biologically-active compounds isolated from medicinal plants have played an essential role in drug discovery in last decade. Various parts of such plants like root, tubers, bark, flowers, leaves and seeds are used for medicinal purposes. More so, many of these plants have been known to synthesize active secondary metabolites such as phenolic compound found in essential oils with established potent insecticidal (Kambu *et al.*, 1982) and antimicrobial activities, which really has formed the basis for their applications in some pharmaceuticals, alternative medicines and natural therapies (R'ios & Recio, 2005; Reynolds 1996; Lis-Balchin & Deans, 1997). As if a comparable study is made between antibiotics and pathogen resistance it is concluded that antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. But, overuse of antibiotics has become the major factor for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms (Harbottle *et al.*, 2006). Thus, in the light of the evidence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance.

Respiratory tract infections are a major cause of illness and death and are common in intensive care unit (ICU) (Cardoso *et al.*, 2007). Respiratory diseases, including allergies, asthma and chronic obstructive pulmonary disease (COPD) are a major public health burden worldwide. The prevalence of these diseases is increasing and there is a continued need for new and improved therapies. The most frequent causal agents of these infections are *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. (Ibrahim *et al.*, 2000)

Alysicarpus vaginalis

The genus *Alysicarpus* belongs to family Fabaceae. Commonly called as Chukalai, there are approximately 78 species in their genus with 20

species are reported in India (Haines, 1978). They commonly found in open grass land, crop fields, and way sides, ranging an altitude 560 to 1000m amsl, Srinagar, Pauri, Chauras and Kirtinagar in Garhwal Himalaya, almost throughout India, ascending to 1000m Afghanistan, Pakistan and tropical America. The genus comprises annual Prostrate Herbs, perennial. Glabrous or a line of hairs on stem, erect or diffuse at 30cm long, branched Leaves, flowers small, mostly callus 5 mm, usually binate at each node of rachis. Flowering period started from September to November and fruiting period is October to February (Gaur, 1999). Several species of *Alysicarpus* has been used in indigenous system of medicine an anti-inflammatory in stomachache, and also an antidote to snake bite. It is also used in skin diseases and as a diuretic. The leaves are used in fever, jaundice and leaf paste is applied externally on skin allergy (Shankarnarayan, 1988). The root of *A. vaginalis* traditionally use for treatment of asthma cold cough and other Respiratory tract infection in Garhwal (Adhikari *et al.*, 2010).

MATERIALS AND METHODS

Plant Material - Plant was collected from two different districts of Uttarakhand, Srinagar and Srikot (Pauri Garhwal) Chauras and Kirtinagar (Tehri Garhwal) Uttarakhand and authenticated at Botanical Survey of India (BSI), Northern Regional Center Dehradun Accession No.115354. Collected plant Root material was washed jet properly, dried under shade at room temperature and crushed to small pieces by using pestle and motor.

Preparation of Extract - Plant extracts were prepared by immersing 200g of powdered plant material in 600 ml of four different solvents according to polarity low to high i.e. petroleum ether (PET), Chloroform(CHCl₃), methanol (MeOH) and water (H₂O), loaded in Soxhlet assembly and extracted for 72 h through successive method (Ahmed *et al.*, 1998). Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvent in vacuum evaporator at 30°C. Residues were stored at 4°C until further use. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200mg/ml for agar well diffusion method.

Test Microorganisms - The five bacterial strains causing respiratory infections used in this study were *Klebsiella pneumoniae* MTCC 4030, *Pseudomonas aeruginosa* MTCC 2474, *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655, *Streptococcus pyogenes* MTCC 442. These Bacterial strains were procured from Institute of Microbial Technology (IMTECH), Chandigarh.

Preparation of Inoculums - Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiment were prepared by transferring

a loopful of cells from stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37°C.

Antibacterial testing – The antibacterial activity of different extracts was determined by agar well-diffusion method (Ahmed *et al.*, 1998). 0.1 ml of 12-16 h incubated cultures of bacterial species were mixed in molten Mueller Hinton Agar medium no. 173 (Hi media Pvt. Ltd., Mumbai, India) and poured in pre-sterilized petri plates. A cork borer (6 mm diameter) used to punch wells in solidified medium and filled with extracts of 45 µl of 200 mg/ml final concentration of extracts. DMSO was used as negative control. The efficacy of extracts against bacteria was compared with the broad spectrum antibiotic erythromycin (positive control). The plates were incubated at 37°C for 24 h in BOD incubator and the diameter of the zone of inhibition was measured in millimeter. Each sample was assayed in triplicate and the mean ± SD values were observed. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the wells.

Determination of Minimum Inhibitory Concentrations (MICs)

Two-fold serial dilution method was used to determine the minimum inhibitory concentrations (MICs) against selected bacterial organisms (Aboaba *et al.*, 2006). Methanol extract was diluted double fold (2:2) with nutrient broth in a series of six test tubes. Concentration of 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/ml of crude Methanol extract were prepared separately and dissolved in 1 ml of DMSO. An aliquot of 1 ml of bacterial suspension (1.5×10^6) was inoculated into each tube. Control tubes were inoculated with same quantity of sterile distilled water. All tubes were incubated at 37°C for 24 h. The lowest concentration that did not permit any visible growth when compared with control was considered as the minimum inhibitory concentration. The MICs was considered as the lowest concentration that could not produce a single bacterial colony. The contents of all tubes that showed no visible growth were cultured on Mueller-Hinton agar, incubated at 37 °C for 24 h.

Phytochemical screening – Major Phytocompound, in the crude Root extracts of *A. vaginalis* were subjected to Phytochemical screening to decide the presence of bioactive components by using standard qualitative methods (Trease and Evans, 1996).

Test for alkaloids - Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. The formation of precipitate with colour change indicated the presence of alkaloids (Moffat *et al.*, 1986).

Test for flavonoids - methanolic extract of plant material to the test solution a mixture of zinc dust and

concentrated hydrochloric acid was added drop wise. After few minutes change in colour of the sample was observed it indicated the presence of flavonoids.

Test for glycosides - Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with Ba(OH)₂. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H₂SO₄ and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

Test for Steroids - The extract mixed with 3 ml CHCl₃ (Chloroform) and 2 ml conc. H₂SO₄ (Sulphuric acid) was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

Test for Sugars- The extract was taken and a mixture of equal parts of Fehling's solutions I and II previously mixed was added and heated. The colour of precipitate of cuprous oxide showed the presence of reducing sugar.

Test for Saponins - The extracts (0.5 mg) were boiled with water (10 ml) for two Minutes in a test tube and cooled. The mixture was vigorously shaken and then left for 3 minutes. The Formation of 1 cm layer of foam indicates the presence of saponins (Arthur and Chan, 1962).

Test for Tannins - Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute H₂SO₄ follow a yellow brown precipitate indicates the presence of tannins.

RESULTS AND DISCUSSION

Peer reviewed published reports on *A. vaginalis* focus only on taxonomy, diversity and ethanobotanical aspects (Jain *et al.*, 2009). Although some research communications are available on antibacterial activity of *A. vaginalis* but it is limited in number. Further, most of the studies have been conducted just to find out the zone of inhibition against some common bacterial pathogens, but have not investigated the minimum inhibition concentration (MICs) and zone of inhibition. Many of the present findings on these extracts are in agreement with previous workers (Khan *et al.*, 2011). reported that whole plant extract of *A. vaginalis* use to performed antibacterial activity using Kirby Bauer disk diffusion test and broth micro dilution against clinically important bacterial pathogens two gram positive *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778) and three gram negative *Pseudomonas aeruginosa* (ATCC 9027), *E. coli* (ATCC 35218), *S. typhimurium* (ATCC 13311) but they could not found antibacterial activity of extract of *A. vaginalis* (Silva *et al.*, 2015)

This study investigated *in vitro* antibacterial activity of crude root extract of *A. vaginalis* from different solvent. The data characterizing the antibacterial

activity of crude extract of *Alysicarpus vaginalis* root are presented in Table 1. The study indicated that the Methanol extracts exhibited a higher degree of antibacterial activity as compared to water, chloroform and petroleum ether extracts.

The maximum inhibition by MeOH extract was found against *Streptococcus pneumoniae* (13 mm), *Klebsiella pneumoniae* (12 mm) and *Staphylococcus aureus* (11 mm), respectively. The minimum activity was found against *Streptococcus pyogenes* (6 mm) followed by H₂O, CHCl₃ and PET.

In case of *S. pneumoniae* crude methanolic extract of *A. vaginalis* give 13 mm zone of inhibition its same as

commercial use broad spectrum antibiotic erythromycin. But in other case the positive control (erythromycin) was found little more effective as compared to *A. vaginalis* extracts. Erythromycin is a macrolide antibiotic with wide spectrum antimicrobial nature. For respiratory tract infections, it has better coverage of microorganisms especially for atypical organisms including mycoplasma and legionellosis. DMSO (Di methylsulphoxide) use as a negative control, they give no zone of inhibition against test organism.

Table 1: The inhibition zones diameters of various root extracts of *Alysicarpus vaginalis*.

Microorganism	Diameter of the inhibition zone (mm)				Positive control (Erythromycin)	Negative Control DMSO
	PET	CHCl ₃	MeOH	H ₂ O		
<i>Staphylococcus aureus</i>	11 ± 0.52	9 ± 0.86	11 ± 0.86	8 ± 0.73	14 ± 0.51	0
<i>Streptococcus pneumoniae</i>	10 ± 0.75	12 ± 0.5	13 ± 0.32	8 ± 0.97	13 ± 0.91	0
<i>Streptococcus pyogenes</i>	11 ± 0.86	10 ± 0.76	10 ± 0.57	6 ± 0.57	15 ± 0.72	0
<i>Klebsiella pneumoniae</i>	10 ± 0.57	11 ± 0.5	12 ± 0.5	10 ± 0.45	12 ± 0.12	0
<i>Pseudomonas aeruginosa</i>	9 ± 0.41	8 ± 0.86	12 ± 0.84	8 ± 0.97	6 ± 0.57	0

Values are Mean ± SD of three replicates; Cork borer diameter: 6 mm;

PET- Petroleum Ether.

CHCl₃ - Chloroform.

MeOH- Methanol.

H₂O- Water.

The results of MICs showed that they ranged from 3.12 to 25 mg/ml (Fig. 2). Root extract of *A. vaginalis* presented similar MICs against *S. aureus* and *K. pneumoniae* (6.25 mg/ml) respectively. Moreover, methanolic extract of this plant manifested a better MIC against *S. pneumoniae* (3.12 mg/ml) and maximum MIC value recorded against *S. pyogenes* (25 mg/ml). Therefore, the activity observed for *A. vaginalis* provides a rationale for its use in treatment of respiratory infectious diseases.

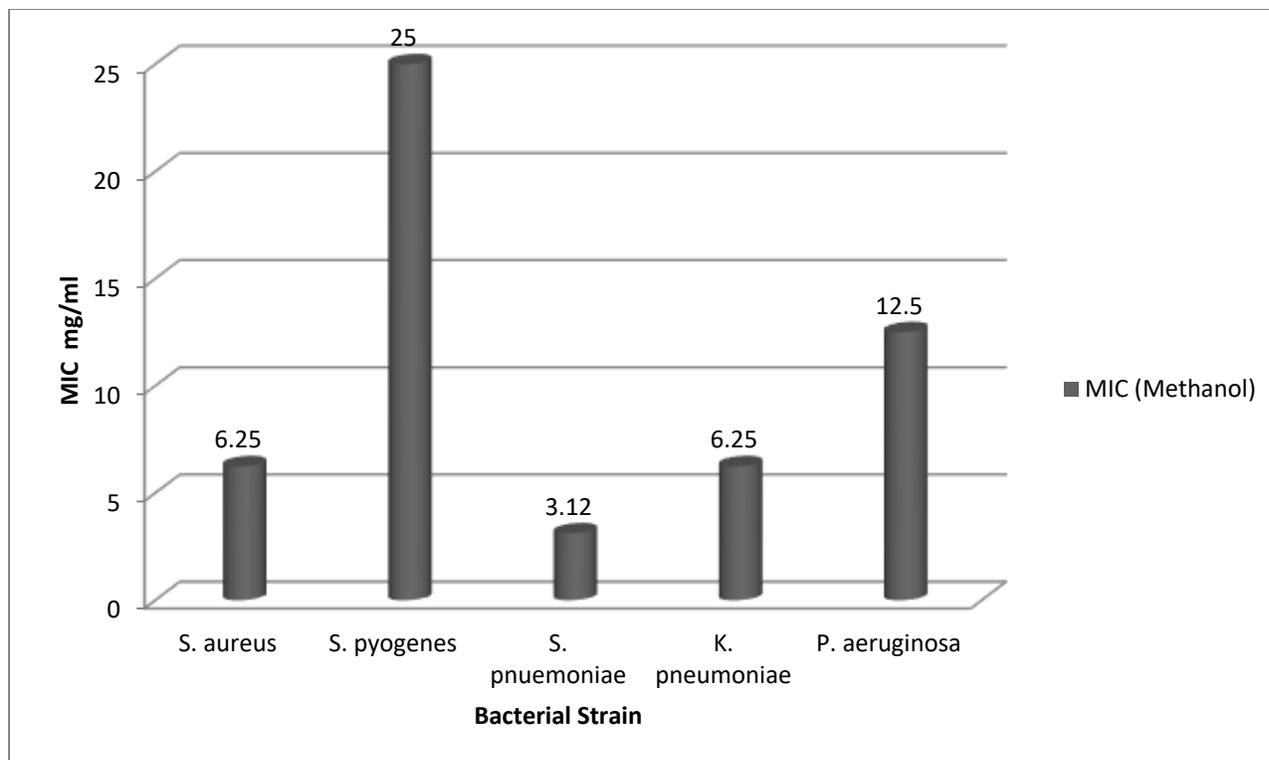


Figure-2: Minimum inhibitory concentrations (MICs) mg/ml of methanol extract of *A. vaginalis*. The inhibition is noted at 3.12 mg/ml against *S. pneumoniae*, 6.25 mg/ml against *S. aureus* and *K. pneumoniae*, 12.5 mg/ml against *P. aeruginosa* and 25 mg/ml against *S. pyogenes*.

Moreover, *A. vaginalis* displayed a basis for use of extract in treatment of respiratory diseases in human beings which could be caused by *K. pneumoniae*, *P. aeruginosa*, *S. aureus*, *S. pneumoniae* and *S. pyogenes*.

Some preview report on study of *A. vaginalis* showed performed antioxidant and in vitro antibacterial activity against the test organism. (Rattanata *et al.*, 2014) But it did not inhibit growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Shigella* spp (Chah *et al.*, 2006). *A. vaginalis* was reported to have antioxidant and antiproliferative activity (Rathi *et al.*, 2010). Some other worker reported the Chemical Constituents of *A. vaginalis* is Caffeic acid, Catechol, Cumarinic acid, Gentisic acid, Gallic acid, P-hydroxybenzoic acid Syringic acid, Vanillic acid, Salicylic acid, Acetic Acid, Ethyl acetate. (Shahin and Ahmad, 2012).

The Phytochemical screening of root of *A. vaginalis* methanolic extracts has shown that plant contains flavonoids, glycosides, alkaloids, steroids, terpenes, sugars, saponins and tannins which are very important constituents when looking for pharmacologically active Phytochemical in the plant. These secondary metabolites and compounds could be responsible for its antibacterial property against tested microorganisms. Previous pharmacological studies exposed the role of *A. vaginalis* in some Herbal drugs for treatment of common cold, asthma, and cough moreover; *A. vaginalis* displayed a basis for use of extract in treatment of respiratory diseases in human beings which could be caused by *P. aeruginosa*, *S. aureus*, *S. pneumoniae*, *S. pyogenes* and *K. pneumoniae*.

Phytoconstituents	Solvent			
	Petroleum Ether (PET)	Chloroform (CHCl ₃)	Methanol (MeOH)	Water (H ₂ O)
Alkaloids	+	+	+	+
Flavonoids	-	+	+	-
Glycosides	+	-	-	+
Steroids/ Terpenes	-	-	+	-
Sugars	-	-	-	+
Saponins	+	+	+	+
Tannins	+	+	+	+

+ = Present - = Absent

This study good supports the traditional use of *A. vaginalis* and indicated that it contains some major bioactive compounds inhibiting the growth of microorganisms there by proving very effective source of derived drugs. It is recommended that further research should be carried out to investigate the bioactive component of this plant. The need for establishment of standard dosage cannot be over emphasized. This is necessary to investigate the toxicity level of extract resulting from over dosage or from any of phytochemical component present in plant material.

CONCLUSION

Based on these results, it is concluded that this study demonstrate the root of *A. vaginalis* (methanolic extract) have potent antibacterial activity against selected highly pathogenic respiratory tract pathogens which might be due to the Phytochemical present in these parts. Moreover, the result indicates a strong possibility of developing safe potent and cheap Antibacterial agent from root of *A. vaginalis*. There is future aspects of this study the identification and purification of active compound(s) those responsible for antibacterial activity of *A. vaginalis*. The conclusion indicates that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Root of *A. vaginalis* could be a source of new antibiotic compounds.

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