PUBLISHED PAPERS
ANTIMICROBIAL ACTIVITY OF SPHAGNETICOLA TRILOBATA (L.) PRUSKI, AGAINST SOME HUMAN PATHOGENIC BACTERIA AND FUNGI

KUMUDINI INDIRA TOPPO, SHUBHA GUPTA, DEEPAK KARKUN*, SHIRISH AGRAWAL AND ANIL KUMAR*
1Department of Botany, Govt. V.Y.T. P.G. Autonomous. College Durg, (C.G.), 491 001, INDIA
Microbial Culture Laboratory, Department of Biotechnology, Govt. V. Y. T. P.G. Autonomous, College Durg - 491 001, INDIA
2Chief Conservator of Forest, Govt. of Chhattisgarh Raipur - 492 001
e-mail: aiumum_ashley@yahoo.co.in

KEYWORDS
Antimicrobial activity
Medicinal plants
Human pathogenic bacteria and fungi

Received on :
18.03.2013

Accepted on :
24.05.2013

*Corresponding author

ABSTRACT
The side effect and quick microbial adaptation to resist synthetic antibiotic has compelled researcher to find out compound from natural sources are free from side effect and resistance. In this connection the present study has been carried out for assessment of antimicrobial activities of methanolic and aqueous extracts of leaf, stem, root and flower of Sphagnetica trilobata (L.) Pruski, against bacteria namely Pseudomonas aeruginosa (MTCC - 7296), Staphylococcus aureus, (MTCC - 7443), Salmonella typhi (MTCC - 733), Mycobacterium tuberculosis (MTCC-300) and fungal organisms namely Microsporum canis (MTCC –2820), Epidermophyton floccosum (MTCC-613), Trichophyton rubrum (MTCC-296) and Aspergillus candidus (MTCC-1989). The zone of inhibition (ZOI) for the methanolic extract of leaf of S. trilobata was found 8.99 ± 0.46mm, 16.92 ± 0.58mm and 12.93 ± 0.28mm against S. aureus, S. typhi and P. aeruginosa respectively. The ZOI for the methanolic extract of flower was found 23.79 ± 0.27mm, 19.66 ± 0.94 mm and 23.60 ± 0.92mm against S. aureus, S. typhi and P. aeruginosa respectively. Besides, the ZOI for methanolic extract of both root and stem was found 09.19 ± 0.34 and 08.66 ± 0.43mm against S. aureus only. The highest zone of inhibition (23.79mm) was found in the methanolic extract of flower against S. aureus. The ZOI for methanolic and aqueous extract of leaf and methanolic extract of root was found 17.73 ± 0.46mm, 15.66 ± 0.63mm and 16.19 ± 0.33mm respectively against Epidermophyton floccosum. The ZOI for methanolic extract of leaf was found11.33 ± 0.34mm against Trichophyton rubrum while the ZOI for aqueous extract of leaf was found 13.73 ± 0.49 mm against Microsporum canis. The highest zone of inhibition (17.73mm) was found in the methanolic extract of leaf against Epidermophyton floccosum. Above findings may be exploited for application against respective pathogenic microorganism and modern drug formulation.

INTRODUCTION
The pathogenic bacteria and fungal infection is a cosmopolitan problem and the situation is more critical especially in the third world countries where in most cases lack of adequate sanitation and primary health care programs make it difficult and expensive to combat diseases. A number of higher plants have been used for centuries as remedies for human diseases. Currently studies pertaining to the use of botanicals in management of pathogens and related diseases are highly focused (Koche, 2013; Toppo, 2013; Mathad, 2013; Mathad, 2013; Mahapatra, 2013; Bish, 2013).

This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effects (Orner and Elminia, 2003; Saadabi et al., 2009). About 80% of individuals from developed countries use traditional medicines which have compounds derived from medicinal plants (Ibninoza et al., 2009). Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. In addition, certain antibiotics present undesirable side effects such as nausea, depression of bone marrow, thrombocytopenic purpura and agranulocytosis leading to the emergence of previously uncommon diseases (Marchese and Shito, 2001; Poole, 2001). This has given scientists the impetus to search for newer and alternative microbial compounds from medicinal plants (Aliero and Afolayan, 2006). Plant extracts and phytochemicals with antimicrobial properties are of great significance in therapeutic treatments viz. Parthenium hysterophorus (Asteraceae) possess luteolin (Zhou et al., 2011c), paeonolide and pahenin (Zhou et al., 2011d)and Chrysanthemum indicum (Asteraceae) contains terpenoid, flavonoids, oxygenated terpenes, sesquiterpenes and the antimicrobial activity of such compounds have been established by Sassi, et al. (2008).

The review of literature revealed that considerable contributions have been made on medicinal plants by many workers (Dadka et al., 2013; Dandapat et al., 2013; Kullu et al., 2013; Kumar et al., 2013; Kumar et al., 2013a; Mahato et al., 2013; Tabassum et al., 2013; Toppo et al., 2013; Sahu et al., 2013).

Sphagnetica trilobata (L.) Pruski. (Previously accepted name, Wedelia trilobata L.) is a member of Asteraceae, (Meena, et
its common name is "Wedelia" or trailing daisy. It is a creeping, perennial herb, stem rooting at the nodes, leaves short petiolate, opposite—decussate, ovate, lobed, irregularly toothed, capitula heterogamous, receptacle convex, ray florets are golden yellow in colour (Hossain and Hassan, 2005). *Sphagneticoila trilobata* is native to the tropics of Central America and has naturalized in many wet tropical areas of the world, West Indies, Hawaii, South Florida, India, and Bangladesh (Hossain and Hassan, 2005). It has been historically used as traditional folk medicinal plant for the treatment of various ailments, (Li et al., 2012). Coe et al. (1996) have reported that fruits, leaves and stem are used in childbirth and in the treatment of bites and stings, fever and infection. Leaves are used in the treatment of kidney dysfunction, cold, wounds and amenorrhoea and dysmenorrhea (Tsai et al., 2009; Govindappa et al., 2011; Meena et al., 2011). It was reported that numerous potential bioactive molecules such as sesquiterpenes, diterpenes, (Kaurenoic acid, triterpenes lactones, luteolin and volatile oil, with antioxidant, anti-inflammatory, antimicrobial, hepato-protective activity, insecticidal, larvicidal and tripanocidal activity, anticancer, anti-tumoural activity have been isolated from various parts of the plant (Taddei and Rosas-Romero, 1999; Zhang, et al., 2004; Huang, 2006; Zhang, 2008; Maldini et al., 2009; Wu and ). The antimicrobial activity of *Sphagneticoila trilobata* was reported by many earlier workers, Taddei and Rosas Romero (1999), Utrakoont et al. (2009), Govindappa et al. (2011) and Chethan et al. (2012).

The test organism *Pseudomonas aeruginosa* is a Gram-negative, aerobic, bacillus, non-spore forming bacterium, widespread in nature, inhabiting soil, water, plants and animals (including humans) (Palleroni, 2008). It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs) (Bitosri, 2012) and bacteremia. It was the third and fifth most common cause of hospital-acquired urinary tract infections in the USA and Europe, respectively (Anonymous, 1996; Bouza et al., 2002). *Mycobacterium tuberculosis*, a small, highly aerobic, nonmotile bacillus, a causative agent of tuberculosis (Kassim and Ray, 2004), typically attacks lungs. One third of the world’s population is thought to have been infected with *M. tuberculosis* with new infections occurring at a rate of about one per second (WHO, 2010). *Salmonella typhi* is a Gram-negative, rod-shaped, non-spore-forming, predominantly motile with peritrichous flagella. It is the only one that is pathogenic exclusively for humans, in whom it causes typhoid or enteric fever. It is estimated that more than 33 million cases of typhoid fever occur annually causing more than 500,000 deaths (Khan et al., 2008). It remains a serious problem in India (Kumar et al., 2001; Saha et al., 2002). *Staphylococcus aureus* is a Gram-positive, coccal, non-motile, non-spore forming facultative anaerobes bacterium. It is often found as a commensal associated with skin, skin glands, and mucous membranes, particularly in the nose of healthy individuals (Crossley and Archer, 1997). *S. aureus* is one of the main causes of hospital and community-acquired infections (nosocomial) which can result in serious consequences (Diekema et al., 2001).

*Microsporum canis* is a zoophilic dermatophyte which is basically animal pathogens, Cats and Dogs are the main sources of infection. It is a common agent of ringworm in animals but it is also frequently associated with human infection (English, 1972). This species invades hair, skin and rarely nails. Both macro and micro conidia are produced. *Trichophyton rubrum* is an anthropophilic fungus, which infection is restricted to man only, mainly associated with community life. It is a dermatophyte becoming more prevalent among urban populations, due mainly to the "modern" way of life such as the wearing of occlusive shoes, which maintain heat and humidity (Philpot, 1977). It frequently causes chronic infections of skin, hair and nails, especially in toe webs, soles and palms. This genus produces smooth walled macroconidia and microconidia. *E. ictococcus* is another anthropophilic dermatophyte. Its infection usually occur on the skin and nails. It is not known to invade hair. *E. ictococcus* is transmitted between individuals by contact, particularly in community swimming pool areas, common showers and gym facilities. This genus is a common cause of *tinea pedis* and *tinea cruris* (eczema marginatum of Hebrae) affecting inginal areas, particularly in males, although some infections do occur in females (Howard et al., 1983). It does not produce microconidia. *Aspergillus candidus* is a pathogenic fungus. It is characterized by white, typically globose conidial head; A. candidus represents a potential respiratory hazard for grain workers (Tracyk and Dutchkiewicz, 2000). It has been claimed to be involved in a wide range of human infections including invasive aspergillosis (Ribeiro et al., 2005), aspergillosis, otomycosis (Yasin et al., 1978), brain granuloma and onychomycosis (Comere and Eastman, 1975; Piraccini, 2002).

The pathogenic feature of considered bacteria viz. *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Mycobacterium tuberculosis* and fungus viz. *Microsporum canis*, *Trichophyton rubrum*, *E. ictococcus* and *Aspergillus candidus* is of very high intensity not only for human but also for cattle. A large number of human populations are suffering from pathogenicity of these microbes. The scientific community is struggling with these microbes since very long period but even today above bacteria and fungi are a serious challenge for us. Many antibiotics have been discovered against such pathogens but unfortunately after sometimes all develop resistance against antibiotics. Another aspect is toxicity of antibiotics with lot of side effect. So this is the need of the hour to search suitable molecule from natural resource to combat with above mentioned pathogen and that too without toxicity and minimum side effect. Thus present work has been undertaken with the objective to ascertain the antibacterial and antifungal activity of extracts obtained from different parts of *Sphagneticoila trilobata* and constantly screened for their possible pharmacological value.

**MATERIALS AND METHODS**

**Collection and identification of plants**

The plant *Sphagneticoila trilobata* was collected from Durg District (20°23 NL and 22°02’NL and 80°48’EL and 81°57’EL) occupies geographical area of 8537km². The area which was selected for the collection of the plant materials for the present study was 50km². Around the district headquarters, the identification and authentication of the plants was carried out.
<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of organism</th>
<th>Sphagnum sp.</th>
<th>S. trilobata</th>
<th>M. M.</th>
<th>A. M.</th>
<th>Leaf</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>8.66 ± 0.43</td>
<td>9.19 ± 0.34</td>
<td>8.99 ± 0.46</td>
<td>23.79 ± 0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Salmonella typhi</td>
<td>-</td>
<td>-</td>
<td>16.92 ± 0.58</td>
<td>19.66 ± 0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas aeruginosa</td>
<td>-</td>
<td>-</td>
<td>12.93 ± 0.28</td>
<td>23.60 ± 0.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mycobacterium tuberculosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M-Methanol extract, A-Aqueous extract

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of organism</th>
<th>Sphagnum sp.</th>
<th>S. trilobata</th>
<th>M. M.</th>
<th>A. M.</th>
<th>Leaf</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Microsporum canis</td>
<td>-</td>
<td>13.73 ± 0.49</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Epidermophtyton floccosum</td>
<td>-</td>
<td>15.66 ± 0.63</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Trichophyton rubrum</td>
<td>-</td>
<td>16.19 ± 0.33</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Aspergillus flavus</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

M-Methanol extract, A-Aqueous extract

Preparation of plant extract

The root, stem, leaves and flowers of *Sphagnum trilobata* was washed thoroughly three times with running tap water and once with distilled water and then shade dried for seven days, coarsely powdered and used for extraction. The powdered plant material was extracted with solvents methanol and distilled water. The water of the plant material and solvent were 1:10 and it was subjected to Soxhlet extraction unit (MSW, India) for about 48h. The solutions were used further in the determination of the antibacterial and antifungal analysis.

Microorganism used

The human pathogenic fungus used for this study were *Staphylococcus aureus* (MTCC-7443), *Salmonella typhi* (MTCC-733), *Pseudomonas aeruginosa* (MTCC-7296), *Mycobacterium tuberculosis* (MTCC-300) and four human pathogenic fungi considered for the study were *Microsporum canis* (MTCC-2820), *Epidermophtyton floccosum* (MTCC-613). *Trichophyton rubrum* (MTCC-296) and *Aspergillus candidus* (MTCC-1989), obtained from Microbial Type culture collection and Gene Bank of IMTECH Chandigarh, India. All these pathogenic organisms were selected for the study on the basis of their clinical importance.

Antimicrobial activity

The antimicrobial activity was evaluated by agar disk diffusion method accepted by NCCLS which is a modification described by Bauer et al., 1966. The disk of 6.0mm of Whatman filter paper no. 1 was saturated with plant extracts and allowed to dry. The impregnated disks were then placed on to the surface of a suitable solid agar medium like Nutrient Agar (Himedia, India) for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and Lowenstein Jensen Medium (Himedia, India) for *Mycobacterium tuberculosis*, Potato Dextrose Agar (Himedia, India) for *Microsporum canis*, Sabouraud Dextrose Agar (Himedia, India) for *Trichophyton rubrum* and *Epidermophtyton floccosum*, Czapek Yeast extract Agar (Himedia, India) for fungus *Aspergillus candidus*, The bacteria seeded plates containing *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* were incubated for 24h at 37°C and plates containing *Mycobacterium tuberculosis* was incubated for three weeks at 37°C. Fungal seeded plates were incubated for 72h at 25°C, except *Trichophyton rubrum* which is incubated at 30°C for 72h in the incubator (Coslab, India). The microbial growth was determined by measuring the diameter of zone of inhibition in millimetre (Das et al., 2010).

RESULTS AND DISCUSSION

The antibacterial activity of *Sphagnum trilobata* was found significant against three bacteria, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. The methanolic extract of leaf showed 8.99 ± 0.46mm, 16.92 ± 0.58mm, 12.93 ± 0.28mm and flower extract of methanol showed 23.79 ± 0.27mm, 19.66 ± 0.94mm and 23.60 ± 0.92mm zone of inhibition against *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa* respectively. The methanol extract of stem and root showed zone of inhibition 8.66 ± 0.43mm and 9.19 ± 0.34mm against *Staphylococcus aureus* only. All extracts were not effective against *Mycobacterium tuberculosis*. Aqueous extract of all parts of the plant were not effective against all tested bacteria. (Table 1, Fig. 1).

In our study, the antifungal activity of *Sphagnum trilobata* was found significant against three fungal organisms *Microsporum canis*, *Trichophyton rubrum* and *Epidermophtyton floccosum* in leaf and the root extract. The methanolic extract of leaf and root and aqueous extract of leaf showed 17.73 ± 0.46mm, 16.19 ± 0.33mm and 15.66 ± 0.63mm zone of inhibition against *Epidermophtyton floccosum*. The methanolic extract of leaf showed 17.33 ± 0.34mm zone of inhibition against *Trichophyton rubrum* and aqueous extract of leaf, showed 13.73 ± 0.49mm zone of inhibition against *Microsporum canis*, all extracts were not effective against *Aspergillus candidus* (Table 2, Fig. 2).

Some previous literature related to antibacterial activity of n-hexane extract of *Sphagnum trilobata* are available against *Bacillus subtilis*, *Mycobacterium smegmatis*, *S. aureus*, *S. epidermidis*, *E. coli*, *Proteus vulgaris*, *P. aeruginosa*, *Salmonella paratyphi* and *Shigella sonnet*. The aqueous extract was inactive against the tested bacteria (Taddi et al., 1999).
The antibacterial activity of ethanol extract of leaf and stem of Sphagnetocila trilobata against E. coli, S. typhi, P. aeruginosa, S. aureus and K. pneumoniae, Xanthomonas oryzae and X. axonopodis was reported by Govindappa et al. (2011), but we found antibacterial property of methanolic extract of leaf of Sphagnetocila trilobata against Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa and methanolic extracts of stem and root of Sphagnetocila trilobata against Staphylococcus aureus only. Methanolic extract of flower of Sphagnetocila trilobata was reported against Staphylococcus aureus only by Chehan et al. (2012), but we found effect against Salmonella typhi and Pseudomonas aeruginosa also. In the case of fungi, Govindappa et al. (2011) reported the methanolic extract of leaf, stem and flower of Sphagnetocila trilobata exhibited less activity on the species of Fusarium and Aspergillus, but in this study, first time we are reporting significant antifungal property of Sphagnetocila trilobata against Epidermophyton floccosum in methanolic and aqueous extract of leaf and in methanolic extract of root. Both the leaf and root part were found effective against Epidermophyton floccosum. The plant was also found effective against Trichophyton rubrum in methanolic extract of leaf and Microsporum canis in aqueous extract of leaf. All are the dermatophytes. Findings of Taddei and Rosas Romero (1999) have not showed any biological activity of Sphagnetocila trilobata against Trichophyton rubrum in n-hexane extract but in our study we reported the significant antifungal activity against Trichophyton rubrum in methanolic extract of leaf. Uttrakoon et al. (2009) reported the efficacy of essential oil extracted of Sphagnetocila trilobata leaves on the growth of Aspergillus flavus but we found antifungal activity in aqueous and methanolic extract of leaf against Epidermophyton floccosum, in aqueous extract of leaf against Microsporum canis, in methanolic extract of leaf against Trichophyton rubrum. On the basis of our significant findings we conclude that there is an urgent need of study of action of
specific ingredients of *Sphagnetocila triobita* against particular microorganism for pharmaceutical application.

**REFERENCES**


ANTIMICROBIAL ACTIVITY OF PARTHENIUM HYSTEROPHORUS LINN. AND MORINGA OLEIFERA LAM AGAINST SOME HUMAN PATHOGENIC BACTERIA AND FUNGUS

Shubha Gupta¹, Kumudini Indira Toppo¹, Deepak Karkun¹ and Anil Kumar²

1 Department of Botany, Govt. V.Y.T. PG. Autonomous College Durg, (C.G.), 491001, India.
2 Microbial Culture Laboratory, Department of Biotechnology, Govt. V.Y.T. PG. Autonomous College Durg (C.G.), 491001, India.

Corresponding Author Email: aimum_aishley@yahoo.co.in
Received: 10 January, 2013 Revised: 20 March, 2013 Accepted: 23 April, 2013

ABSTRACT: Antimicrobial effects of Parthenium hysterophorus Linn. (Asteraceae), and Moringa oleifera Lam. (Moringaceae), were studied on bacteria Salmonella typhi (MTCC-733), Staphylococcus aureus (MTCC-7443), Pseudomonas aeruginosa (MTCC-7296) and a fungus Microsporum canis (MTCC-2820). All four parts, root, stem, leaves and flowers of the plants were extracted with methanol and distilled water. The methanol extract of the flowers of Parthenium hysterophorus exhibited significant antimicrobial activity against all four human pathogenic microorganisms but the methanol extract of the stem showed only antifungal activity against Microsporum canis. The aqueous extract of the flowers of Moringa oleifera showed significant antibacterial activity against Salmonella typhi and considerable activity against Staphylococcus aureus. While the aqueous extract of leaves exhibited significant antibacterial activity against Pseudomonas aeruginosa only. The aqueous extract of stem and methanol extract of the leaves of Moringa oleifera showed significant antifungal activity against Microsporum canis. In the antibacterial activity the highest zone of inhibition (24.33±1.94) mm was found in methanol extract of flower of P. hysterophorus against Staphylococcus aureus and in antifungal activity, the highest zone of inhibition (26.80±0.70) mm was found in the methanol extract of leaves of M. oleifera against Microsporum canis. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal values, which have yet to be discovered; large number of plants is constantly screened for their possible pharmacological value and particularly for antimicrobial properties.

INTRODUCTION

Medicinal plants are an important source for the therapeutic remedies of various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century [1-3]. Since time immemorial, different parts of Medicinal plants have been used to cure specific ailments in India. Primarily interest in herbal drugs stems from the belief that green medicine is safe and dependable, compared to costly synthetic drugs which are invariably associated with adverse effects [4]. Researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that they have developed to antibiotics[5]. Thus Indian medicinal plant-based industry is growing fastly for pharmaceuticals, phytochemicals, nutraceuticals, cosmetics and other valuable products. Parthenium hysterophorus Linn. (Family-Asteraceae), commonly known as Congress grass, Congress weed, Carrot weed and Wild feverfew, and is now considered as one of the most feared noxious weed [6]. The plant is used in the treatment
of ulcerated sores, wounds, fever, anaemia and heart troubles. A decoction of the root is used in treatment of dysentery [7]. It is applied externally on skin disorders and decoction of the plant is often taken internally as a remedy for a wide variety of ailments [8]. It is also reported as promising remedy against hepatic amoebiasis [9].

The chemical analysis has indicated that all the plant parts including trichomes and pollen contain particularly sesquiterpene lactones such as parthenin, hystein and dihydroparthenin. Leaves contain about 5% parthenin[10]. Histamine was found (0.585%) in the aerial parts and (0.35%) in the roots parts [11].

*Moringa oleifera* is the most widely cultivated species of a monogenic family, the *Moringaceae*, commonly known as “Drumstick”, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [12,13]. *Moringa oleifera* is traditionally used for treatment of anemia [14,15], cholera,[16,15], asthma, bronchitis, catarh, headaches, abnormal blood pressure, hysteria, pimples, psoriasis, semen deficiency, sore throat, sprain, tuberculosis and aphrodisiac. [18], rheumatism and gout [19,18]. Also used as a tonic, diuretic and chologogue, useful in scurvy and they have also strong antibacterial and antimalarial properties [17, 20, 21]. The plant was reported to contain various amino acids, fatty acids, vitamins, and nutrients and other specific components that have been reported to have hypotensive, anticancer, and antimicrobial activity [22]. are, 4-((4′-o-acetyl-a-L-rhamnosyloxy)benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl isothiocyanate, Niazipenic, Pterygospermin, benzyl isothiocyanate, 4-(a-L-rhamnopyranosyloxy) benzyl glucosinolate.

Enteric or diarrhoeal infections are major public health problems in developing countries. Enteric bacteria comprised of *Salmonella* sp., *Shigella* sp., *Proteus* sp., *Klebsiella* sp., *E. coli*, *Pseudomonas* sp., *Vibrio cholerae* and *S. aureus* which are major etiologic agents of sporadic and epidemic diarrhoea both in children and adults [23]. Recently, it has been demonstrated that many human pathogenic bacteria have developed resistance against several synthetic drugs indicating need to search for alternative medicine [24,25,26].

*Pseudomonas aeruginosa* is a gram negative, aerobic, rod belongs to the family *Pseudomonadaceae*, more than half of all clinical isolates produce the blue green pigments phycocyanin. *Pseudomonas* has a characteristic sweet odour. These pathogens are widespread in nature, inhabiting soil, water, plants and animals (including humans). *Pseudomonas aeruginosa* has become an important cause of infection, especially in patients with compromised host defense mechanisms. It is the most common pathogen isolated from patients who have been hospitalized longer than one week. It is a frequent cause of nosocomial infections such as pneumonia, urinary tract infections (UTIs) [27] and bacteremia. Pseudomonal infections are complicated and can be life threatening [28].

*Salmonella typhi* is a Gram-negative enteric bacillus belongs to family *Enterobacteriaceae*. It is a motile, facultative anaerobe that is susceptible to various antibiotics infections of *S. typhi* leads to the development of typhoid or enteric fever. This disease is characterized by the sudden onset of a sustained and systematic fever, severe headache nausea and loss of appetite. Other symptoms include constipation or diarrhoea, enlargement of the spleen, possible development of meningitis and general malaise [29]. Although antibiotics have markedly reduced the frequency of typhoid fever in the developed world, it remains endemic in developing countries [30].

*Staphylococcus aureus* is a facultative anaerobic Gram-positive coccobacillary bacterium. It is frequently found as part of the normal skin flora on the skin and nasal passages. It is estimated that 20% of the human population are long-term carriers of *S. aureus* [31]. It can cause a wide variety of diseases in human and other animals through either toxin production or penetration. Staphylococcal toxins are a common cause of food poisoning, as they can be produced by bacteria growing in improperly-stored food items. Each year, some 500,000 patients in American hospitals construct a staphylococcal infection [32].

*Microsporum canis* is a dermatophyte fungus which belongs to phylum Ascomycotina, and family *Arthrodermataceae* [33]. Colonies that are flat, spreading, white to cream-coloured, usually have a bright golden yellow to brownish yellow reverse pigment, [34]. Both macro and microconidia are produced but the predominant conidial structures are macroconidia. The macroconidia are multi septate with thick wall and rough surface *Microsporum canis*
Table 1: Antibacterial Potential of The Plants Extracts

<table>
<thead>
<tr>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Typhi</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Parthenium hysterophorus</th>
<th>Moringa oleifera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>stem</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mean</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SE±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>mean</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>t</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SE±</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M-Methanol extract, A-Aqueous extract, Conc. 400 µl, (Zone of inhibition in mm).

91
Table 2. Antifungal Potential of The Plants Extracts

<table>
<thead>
<tr>
<th>Organism</th>
<th>S.No.</th>
<th>Parthenium hysterophorus</th>
<th>Moringa oleifera</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Root</td>
<td>stem</td>
<td>leaf</td>
</tr>
<tr>
<td></td>
<td>M A</td>
<td>M A</td>
<td>M A</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>- 12.66</td>
<td>- 9.66</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>- 13.00</td>
<td>- 8.66</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>- 11.00</td>
<td>- 10.00</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>- 13.00</td>
<td>- 9.66</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>- 12.33</td>
<td>- 9.33</td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td>- 12.40</td>
<td>- 9.46</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>- 4.55</td>
<td>- 3.52</td>
</tr>
<tr>
<td>SE±</td>
<td></td>
<td>- 0.30</td>
<td>- 0.22</td>
</tr>
</tbody>
</table>

M-Methanol extract, A-Aqueous extract, Conc: 400 µL (Zone of inhibition in mm).

causes ringworm of the scalp and skin in children and has been occasionally reported as the cause of nail infections [36]. *Microsporum canis* is the most commonly encountered species in human infections and is distributed worldwide. No race in any geographical location is totally free from dermatophytoses [37].

**MATERIALS AND METHODS**

**Collection and identification of plants:** Plants were collected from Durg District Latitude (20° 23' N and 22° 02' N) and the Longitude (80° 48'E and 81° 57'E) occupies geographical area of 8537 sq k.ms. in January to April 2011. The area which was selected for the collection of the plant materials for the present study was 50 sq k.ms. around the district headquarters. The identification and authentication of the plants was carried out at Department of Botany, Govt. V.Y.T.P.G. Autonomous College, Durg, C.G. India. Confirmation of taxonomic identity of the plants was achieved by comparison with voucher specimens kept at the Herbarium of Botany Department.

**Solvent Extraction:** All parts of plants selected for the study were collected, sterilized, shade dried, powdered, and then powder was extracted with methanol and water solvent in Soxhlet extractor for 48h. After extraction, solvent was preserved in airtight bottle for further use.

**Collection of microorganisms:** Standardized strains were obtained from the (MTCC) Microbial Type Culture Collection and Gene Bank Institute of Microbial Technology Sector 59-A, Chandigarh-160036, INDIA. Three bacteria, *Salmonella typhi* (MTCC-733), *Staphylococcus aureus* (MTCC-7443), *Pseudomonas aeruginosa* (MTCC-7296) and a fungal strain *Microsporum canis* (MTCC 2820), were used for experiment and maintained on suitable culture media at 4°C.

**Antimicrobial activity of plant extract:** The antimicrobial activity was performed by using a modified agar disk diffusion method [38]. In this method, 6 mm sterilized filter papers disks (Whatmann No. 1) saturated with plant extract of desired concentrations were used. The impregnated discs are then placed onto the surface of a suitable solid agar medium. The media has been pre-inoculated with test organisms and was incubated for 24 h at 37°C (bacteria) and 48h at 25°C (fungi) [39]. After incubation, zone diameter is measured in millimetres.
RESULT AND DISCUSSION

The antibacterial activity of *P. hysterophorus* was found significant against all three bacteria. The flower extract of methanol showed zone of inhibition 19.66 mm (t=-3.34), 24.33 mm (t=-3.78) and 15.13 mm (t=-3.89) respectively against *S. typhi*, *S. aureus* and *P. aeruginosa*. The aqueous extract of flower of *M. oleifera* exhibited zone of inhibition 8.93 mm (t=-3.50) and 7.60 mm (t=-2.45) respectively against *S. typhi*, *S. aureus*. The aqueous extract of leaves of *M. oleifera* was also exhibited zone of inhibition 9.19 mm (t=-3.67) against *P. aeruginosa*. The root, stem and leaves extracts of *Parthenium hysterophorus* and the root and stem of *M. oleifera* was not found effective for antibacterial activity. The antifungal activities of both plants were also investigated against fungus *M. canis*. The methanol extract of flower and
stem of *P. hysterophorus* showed zone of inhibition 9.46 mm (t=3.52) and 12.40 mm (t=4.55) respectively. The aqueous extract of stem and methanol extract of the leaf of *M. oleifera* exhibited zone of inhibition 22.33 mm (t=3.80) and 26.80 mm (t=3.04) respectively. The root and leaf extract of *P. hysterophorus* and root and flowers of *M. oleifera* was not found effective for antifungal activity. Many earlier workers evaluated the antimicrobial properties for different parts of *P. hysterophorus* and *M. oleifera* plant against different human pathogenic microorganisms. In 2011, Khan et al.[40] have reported that *P. hysterophorus* was found to be possessing antimicrobial activity against Gram negative bacteria such as *S. aureus*, *E. coli*, *K. pneumonia*, Gram positive bacteria *M. luteus*, *B. subtilis* and fungi *A. niger*, *A. flavus*, *A. fumigates*, *F. solani*. In another study, which was carried out on [41] the aerial parts of *P. hysterophorus* was found with antimicrobial activity against bacteria, *S. aureus*, *E. coli*, *P. aeruginosa*, and fungi *A. niger*, *C. albicans*, *F. oxysporum*. Another study [42] also exhibited the antimicrobial property of *P. hysterophorus* against bacteria *S. pyphi*, *B. subtilis*, *E. faecalis*, *Klebsiella pneumonia*, *Sclerotinia parastephi* and fungi *F. oxysporum*, *F. eridiforme*, *F. moniliforme*, *Curtararia sp. , Stenophyllum sp. S. rolfsii*. Our finding is to be attributed to above author with novelty of anti-[Microsporum canis](#) properties of *P. hysterophorus*. Chloroform and ethanol extract of *M. oleifera* leaf, was reported to possess antimicrobial property against some wide range of pathogens. *M. oleifera* leaves chloroform extract having antibacterial property against *Escherichia coli*, *P. aeruginosa, S. aureus, S. pyogenes* and antifungal activity against *A. niger, C. albicans* [15]. *M. oleifera* leaf ethanol extract also exhibited broad spectrum antibacterial property against *E. coli*, *P. aeruginosa, S. aureus, E. aerogenes* [43] and antifungal activities against dermatophytes such as *Trichophyton rubrum*, and *Trichophyton mentagrophytes*. [44]. The steam distillate of *M. oleifera* has been shown to possess antimicrobial property against some bacteria i.e more inhibition was observed in *E. coli* (73.43%) followed by *S. aureus* (70.34%), *K. pneumoniae* (51.80%), *P. aeruginosa* (49.16%), *B. subtilis* (45.67%), and fungi *A. niger* (46.51%) followed by *A. oryzae* (26.31%), *A. terreus* (23.07%), *A. natalus* (16.21%). [45]. Again our finding is affirmative to previous author but antifungal property of aqueous extract of stem and methanol extract of leaf of *Moringa oleifera* against pathogenic fungus *M. canis* (MTCC-2820) is the novelty of our work.

To establish antimicrobial property of specific metabolites of both plants against *S. pyphi* (MTCC-733), *S. aureus* (MTCC-7443), *P. aeruginosa* (MTCC-7296) and *M. canis* (MTCC-2820) are required for further study.

REFERENCES

ANTIMICROBIAL ACTIVITY OF ACHYRANTHES ASPERA AGAINST SOME HUMAN PATHOGENIC BACTERIA AND FUNGI

Shubha Gupta¹, Kumudini Indira Toppo¹, Deepak Karkun¹, Anil Kumar²,³, Nihar Mishra¹, Rakesh Dasgupta¹ and Tripti Thakur¹

¹Department of Botany, Govt. V.V.I. PG. Autonomous College, BURG-491001, India
²Microbial Culture Laboratory, Department of Biotechnology, Govt. V.V.I. PG. Autonomous College, BURG-491001, India

The search for compounds with antimicrobial activity has gained increasing importance in recent times, due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic-resistant microorganisms. However, there has also been a rising interest in the research for natural products from plants for the discovery of new antimicrobial and antioxidant agents in the last three decades. Present investigation revealed the antimicrobial properties of methanolic and aqueous extracts of leaf, stem, root and flower of Achyranthes aspera against bacteria namely Pseudomonas aeruginosa (MTCC-796), Staphylococcus aureus (MTCC-7443), Salmonella typhi (MTCC-733), Mycobacterium tuberculosis (MTCC-360) and fungi namely Microsporum canis (MTCC-2830), Epidermophyton floccosum (MTCC-413), Trichophyton rubrum (MTCC-2396) and Aspergillus candidus (MTCC-1989). The methanolic extract of flower of Achyranthes aspera showed zone of inhibition against Salmonella typhi, Pseudomonas aeruginosa and Staphylococcus aureus. The highest zone of inhibition (10.99 mm) was found against Salmonella typhi. Both the extract of root (methanolic and aqueous) and methanolic extract of stem was found effective against Epidermophyton floccosum, whereas only aqueous extract of root and stem was found effective against Microsporum canis. The aqueous extract of leaf and flower and methanolic extract of stem was found with potential antifungal property against Aspergillus candidus. Present finding pave the path for further investigation for identification of specific molecule against specific organism.

INTRODUCTION

The age-old Indian system of medicine have been neglected mainly because of the rapid expansion of the allopathic system of medical system. This is despite the fact that our country has a long history of local health traditions, which are backed by thousand of years.
sculptures left behind by practitioners of this system of medicine. Over 7000 different species of plants found in different ecosystems are said to be used for medicinal purposes in our country. The traditional Indian system of medicine can be broadly classified into the empirical forms of folk medicine, which are village-specific, region-based, and community-based. The other system, called in Ayurveda, Siddhva and Unani systems of medicine, is said to be more complicated and elaborated with theoretical and research findings. It is also said to be documented in thousands of regional manuscripts. About 1,500 medicinal manuscripts are said to be available in libraries and private collection in India and abroad, but only a few hundred of these books are made available to students and teachers of Indian medicine (Pandit and Boher, 2004). Traditional medical practices have been known for centuries in many parts of the world (Seifwara, 1984). However, these practices vary from country to country. In Indian medicinal system, the Ayurveda roots, barks and leaves of different plants are employed as medicine to cure various diseases. These medicinal plants produce a number of secondary metabolites which are important for its medicinal values. But, these traditional practices need scientific authentication. Now a day, the scientific community is actively involved in studying the medicinal properties such as antifungal, anti-inflammatory, antioxidants etc., of these plants. According to WHO, 80% of population depend on traditional medical practices for their primary health care needs. In developing countries like India 65% of the population in the rural area uses traditional form of medicine to meet their primary health care needs (Anonymous, 1992). Many investigators have demonstrated the antimicrobial activity of some higher plants (Achyrocline and Agynis, 1987; Rocin and Ron, 1989; Mahria et al., 1992) and quite a number of chemical compounds of plant origin have been shown to possess antimicrobial activities (Carroll et al., 1992).

Achyrocline aspera Linn (Amaranthaceae) found throughout tropical Asia, Africa, Australia and America. It is commonly found as a weed on waysides and waste places (Bunar, et al., 2003) an erect, annual herb, 3-8 meter in height, with a woody base. Stem is ribbed, branched, nodes bulged, leaves are elliptic, pubescent on both sides, ovate to ovalate (Zafar, 2009) with long cylindrical tip root, numerous greenish-white flowers in terminal spikes up to 7.5 cm long, bracts and bracteoles persisting, ending in a spine. Fruit is void and sterile. Seeds are sub cylindrical, round at the base, reddish brown and shining. Achyrocline aspera is commonly known as Chinchla or Patamengh in various text of Ayurveda, used to treat various ailments. Whole plant is useful for therapeutic purpose (Cingaro and Kulkarni, 2012). Traditionally, the plant is used in asthma, cough, night blindness and cutaneous diseases. It is diuretic, purgative and laxative (Nabirani, 2009), useful in osteoma, dropsy, piles, boils and sores of skin etc., in pneumonia and in bowel complaints (Chopra et al., 1958). It is also used as in bites of poisonous snakes and reptiles (Rangjani, 2006). Anonymous, (2007), reported the plant is useful in liver complaints, rheumatism, scabies and other skin diseases. In India, A. aspera has been widely used to treat urinary disorders (Sahom and Gosai, 2006) and in management of various gynecological disorders (Khan and Khan, 2005). Phytochemical investigations of this plant were carried out by different authors (AI, 1995). The seeds of Achyrocline aspera
Antimicrobial Activity of *Achyranthus Aspera* contains saponins A (D-Gluconic acid) and B (L-D- galactopyranosyl ester of D-Glucuronic acid), (Haribunan & Rangaswami, 1970; Ali, 1993). Rastogi and Mehrotra, (2004) reported 10-ericosanone, 10-octadecanone & 4-triterpenoic acid from the plant. Banerji and Chaudhry in 1970, reported ceylanone from the roots and whole plant of *Achyranthus Aspera*. Achyranthine and B-horatine which are water soluble alkaloids were isolated from the whole plant (Anonymous, 2005). Seven chemical compounds viz., phenanthroquinone, hydrogenquinone, spathanol, nerol, s-ionone, assone and sequin were isolated from the volatile oil of the leaves of *Achyranthus Aspera*. Hydroquinone (37.7%) was found as the chief constituent (Rameshwar, 2007).

*M. tuberculosis* (Mycobacteriaceae) a small, highly acrobic, nonmotile, bacillus. It has an unusual, waxy coating on its cell surface (primarily mycolic acid), which makes the cells impervious to Gram staining, so acid-fast detection techniques are used instead. *M. tuberculosis* is a common and in many cases lethal, infectious disease caused by *M. tuberculosis* (Kumar et al., 2007). Tuberculosis typically attacks lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit their saliva through the air (Konstantinov, 2010). *Staphylococcus aureus* (Staphylococcaceae) is a Gram-positive, coccoid, non-motile, non-spore forming facultative anaerobic bacteria that form grape-like clusters. *Staphylococcus aureus* species can cause many infections in the skin, nose, throat, vagina, and gastrointestinal tract, most of which are minor and not life threatening (Slutman and Nahhas, 2017). *S. aureus* is a human pathogen, one of the main causes of hospital and community-acquired infections (nosocomial) which can result in serious consequences (Volkmann et al., 2001). *S. aureus* infections can spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and by contact with objects such as towels, sheets, clothing, or orthopedic equipment used by an infected person.

*Pseudomonas aeruginosa* (Pseudomonalaceae) is a Gram-negative, aerobic, bacillus, non-spore forming, bacterium. *P. aeruginosa* infects healthy tissues rarely, but, when defenses are compromised, it can infect virtually all tissues. This explains why most infections are nosocomial (Morrison and Weinol, 1984). It is typically a positive result to the oxidase and catalase test. It is a metabolically versatile bacterium capable of surviving in aquatic, plant, animal and human-associated habitats (Westman et al., 2010; Pullen et al., 2008). Khan et al. (2007). *P. aeruginosa* thrives in hospital environments, and is a particular problem in this environment since it is the second most common cause of infection in hospitalized patients (nosocomial infections).

*Salmoneilla typhi* (Enterobacteriaceae) is a Gram-negative, rod-shaped, non-spore forming, predominantly motile with peritrichous flagella. *S. typhi* is the only one that is pathogenic exclusively for humans in whom it causes typhoid or enteric fever (Zhang et al., 2008). Epidemiologic classification of *Salmoneilla* is based on the host preferences. The first group includes host-restricted serotypes that infect only humans such as *S. typhi* (Gray and Fedorka-Cray, 2002; Boyen et al., 2008). *S. typhi*, which is only transmitted from human to human, is most common in developing nations where access to safe drinking water may be limited and waste disposal and treatment may be inadequate (Volkmann et al., 1997).
Microsporum canis is a dermatophyte which belongs to phylum Ascomycota, and family Arthrodermataceae (Simpanya, 2000). Zoophilic M. canis (Cats and Dogs) is common infecters in humans are acquired from infected dogs or cats. No one in any geographical location is totally free from dermatophytes (Rippon, 1988). Microsporum canis causes ringworm of the scalp and skin in children and has been occasionally reported as the cause of nail infections (Deopik et al., 2010). Colonies that are flat, white to cream-coloured, with a dense cottony granular surface, golden yellow to brownish yellow reverse pigment. Both macroconidia and micro conidia are produced but the predominant conidial structures are macro conidia with multi septate thick-walled and rough surface (Emmons, 1984).

Trichophyton rubrum is a dermatophyte which belongs to phylum Ascomycota and family Arthrodermataceae (Simpanya, 2000) is an anthropophilic dermatophyte, (exclusively infects humans) and distributed worldwide (Rippon, 1988). It is the most common cutaneous agent of dermatophytosis. Infections are more common in adult males than in children and women. Trichophyton differs from Microsporum and Epidermophyton by having cylindrical, cigar-shaped, thick-walled and smooth macroconidia. The colony surface is downy, white to buff colored. Reverse colony color is deep red (Deopik et al., 2009).

Epidermophyton floccosum is a dermatophyte which belongs to phylum Ascomycota, and family Arthrodermataceae (Simpanya, 2000). Humans are the primary host of Epidermophyton floccosum. It is widespread in most countries of the world (Rippon, 1988). E. floccosum is transmitted between individuals by contact, particularly in community swimming pool areas, common showers, and gym facilities. Its infections usually occur on the skin of the toes, limbs, soles of feet, palms of hands, and nails (Deopik et al., 2010). Epidermophyton floccosum is differentiated from Microsporum and Trichophyton by the absence of macroconidia and does not infect hair (Simpanya, 2000). Colonies are frequently granular, sJha-like in texture and range from olive to yellow-brown. Reverse culture color is deep yellowish-brown. E. floccosum macroconidia are smooth-walled, broadly club-shaped with rounded ends, usually containing less than six cells and are found singly, or in clusters.

Aspergillus candidus is a pathogenic fungus which belongs to phylum Ascomycota, and family Trichocomaceae. A. candidus has been claimed to be involved in a wide range of human infections including invasive aspergillosis (Rippon, 1988), pulmonary aspergillosis, aspergillosis, otomycosis (Yoshin et al., 1973), brain granulomas and otomycoascariosis (Cormier and Eastman, 1975; Pirri, 2002). It also represents a potential respiratory hazard for grain workers (Trzeciak and Dutkiewicz, 2000). A. candidus is moderately xerophilic, (Lucas & Magas 1991) and widely distributed in nature and develops upon vegetation in the later stages of decay. It has also been isolated from birds, either healthy or with lesions (Sharma et al., 1971). It is characterized by white, typically globule conidial head, producing globule smooth, thin walled conidia.

MATERIAL AND METHODS
Collection and identification of plants
Achyranthes aspera was collected from Durg District of Chhattisgarh (Latitude 20° 33’ N and
Antimicrobial Activity of Achyranthes aspera

22°02'N and Longitude 80°54' E and 8°57'E) occupies geographical area of 8537 sq km. The area which was selected for the collection of the plant materials for the present study was 50 sq km around the district headquarter. The identification and authentication of the plants was carried out at Department of Botany, Govt. V.T.P.G. Autonomous College, Durg, C.G. India and later also confirmed by Botanical Survey of India, Kolkata.

Preparation of plant extract

The root, stem, leaves and flowers of Achyranthes aspera was washed thoroughly three times with running tap water and once with distilled water and then shade dried for seven days, coarsely powdered and used for extraction. The powdered plant material was extracted with solvents methanol and distilled water. The ratio of the plant material and solvent were 1:1 and it was subjected to Soxhlet extraction unit (MSW, India) for about 48 hrs. The extracts were used for chemical analysis and determination of the antibacterial and antifungal property.

Microorganism used

The human pathogenic bacteria used for this study were Staphylococcus aureus (MTCC-7443), Salmonella typhi (MTCC-733), Pseudomonas aeruginosa (MTCC-2796), Mycobacterium tuberculosis (MTCC-300) and four human pathogenic fungi considered for the study were Microsporum canis (MTCC-2820), Epidermophyton floccosum (MTCC-613), Trichophyton rubrum (MTCC-296) and Aspergillus candidus (MTCC-1989), obtained from Microbial Type Culture Collection and Gene Bank of IMTECH, Chandigarh, India. All these pathogenic organisms were selected for the study on the basis of their clinical importance.

Antimicrobial Activity

The antimicrobial activity was evaluated by agar disk diffusion method accepted by NCCIL which is a modification described by Bauer et al., 1966. The disk of 6.0mm of Whatman filter paper no. 1 was saturated with plant extracts and allowed to dry. The impregnated disks are then placed on to the surface of a suitable solid agar medium like Nutrient Agar (Himedia, India) for Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Listeria monocytogenes (Himedia, India) for Mycobacterium tuberculosis, Potato Dextrose Agar (Himedia, India) for Microsporum canis, Sabouraud Dextrose Agar (Himedia, India) for Trichophyton rubrum and Epidermophyton floccosum. Czapek Yeast extract Agar (Himedia, India) for fungi Aspergillus candidus. The bacteria seeded plates containing Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi were incubated for 24 hrs at 37°C and plates containing Mycobacterium tuberculosis was incubated for three weeks at 37°C. Fungal seeded plates were incubated for 72 hrs at 25°C, except Trichophyton rubrum which was incubated at 30°C for 72 hrs in incubator (Conlab, India). The microbial growth was determined by measuring the diameter of zone of inhibition in millimetre (Das et al., 2010).

RESULT AND DISCUSSION

From our study we found that the Achyranthes aspera showed less antibacterial but strong antifungal activity. The antibacterial activity of Achyranthes aspera was found significant against Salmonella typhi at 35% p. against Pseudomonas aeruginosa at 10% p.
and non-significant against *Staphylococcus aureus*. The methanolic extract of flower showed 08.99±0.21 mm, 07.19±0.08 mm, 07.13±0.08 mm zone of inhibition against *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* respectively. Only methanolic extract of flower of *Achyranthes aspera* exhibited antimicrobial property and it was not found in extract prepared in other solvents. *Mycobacterium tuberculosis* was highly resistant and did not showed any zone of inhibition. (Table No.01, Fig.1a & Fig.1b).

In our study, we found good antifungal property of *Achyranthes aspera* against three fungal organisms *Microsporum canis*, *Epidermophyton floccosum* and *Aspergillus candidus* in leaf, stem, flower and root extract. Both the methanolic and aqueous extract of root showed 13.33±0.33 mm and 10.92±0.56 mm zone of inhibition respectively while the methanolic extract of stem showed 12.06±0.46 mm zone of inhibition against *Epidermophyton floccosum*. The aqueous extract of leaf and flower showed 09.19±0.33 mm and 17.91±0.33 mm respectively and the methanolic extract of stem showed 14.06±0.50 mm zone of inhibition against *Aspergillus candidus*. Where in only the aqueous extract of stem and root showed 12.66±0.27 mm and 04.66±0.53 mm zone of inhibition respectively against *Microsporum canis*. The plant was found ineffective against *Trichophyton rubrum* (Table No.02, Fig.2a & Fig.2b).

Many previous workers have reported the antimicrobial properties in different parts of *Achyranthes aspera* against different human pathogenic microorganisms. In previous literature, Gupta et al. (2010) reported the antibacterial activity of ethanol extract of leaves of *Achyranthes aspera* against *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The antibacterial property of methanolic extract of whole plant of *Achyranthes aspera* was reported against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Aeromonas salmonia*, *Pseudomonas aeruginosa*, *Salmonella typhi* *Proteus mirabilis* and *Staphylococcus aureus* by Sharma et al. in 2011. Periyasamy et al. (2010) investigated the antibacterial activity of chloroform, ethyl acetate, methanol and aqueous extract of leaves of *Achyranthes aspera* against *Escherichia coli*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Ethanol extract of root and aerial parts of *Achyranthes aspera* was investigated for its antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, and *Proteus vulgaris* by Kumar, et al. in 2009. The methanolic extract of leaf showed a strong inhibitory activity against the *Staphylococcus aureus* (Londhekar et al., 2011). But we found the antibacterial activity of methanolic extract of flower of *Achyranthes aspera* against *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The aqueous extract of all parts were not effective against all tested organism.

In the case of fungi, Londhekar et al. (2011) reported antifungal activity of leaf extract of *Achyranthes aspera* against *Microsporum canis* but in our study we found root extract was effective, not the leaf extract, thus our finding differs from the study of Londhekar et al. (2011). Mishra et al., in 2012 have reported the antifungal activity of *A. aspera* root against *Trichophyton rubrum*, whereas in our study we found the root extract is effective against *Microsporum canis* as well as *Epidermophyton floccosum*. Islam et al., (2009) and Sharma et al. (2011) have also reported the efficacy of *Achyranthes aspera* leaf against *Aspergillus niger* and *Aspergillus flavus*, but we found the antifungal activity of leaf, stem,
Antimicrobial Activity of Achyranthes Aspera

The flower and flower parts of *Achyranthes aspera* against another pathogenic species of *Aspergillus* i.e., *Aspergillus candidus*, Flammoli. *et al.* (2009), did not found any activity with the aqueous extract of the leaves against tested fungal strains, but we found that aqueous extract of the leaves was also effective against *Aspergillus candidus*. Khara *et al.* (2012) reported the high potency of *Achyranthes aspera*, from high altitude area, against fungal strains *Aspergillus flavus*, *Aspergillus niger*, and dermatophytes *Microsporum gypseum*, *Trichophyton mentagrophytes*, and *Trichophyton rubrum*, as the same way we found *Achyranthes aspera* also equipotent against *Aspergillus candidus*, (another pathogenic species of *Aspergillus*) and dermatophytes *Microsporum canis* and *Epidermophyton floccosum*. Apart from above findings, several investigators have also reported that the different parts of the plant *Achyranthes aspera* exhibited equipotent activity against fungal strains *Candida albicans* *Aspergillus flavus*, *Cryptococcus neoformans*, *fungium*, *Pentospora*, *Phytophthora*, and *Sclorenum sp.* (Girigovan, and Kalakurthy, 2012; Kumar, et al. 2003; Kaur, et al., 2005), whereas Dangi, *et al.* (2012) carried out the antimicrobial activity against pathogenic fungi *Candida albicans* and *Aspergillus niger*. In our study we found antifungal activity of all parts of *Achyranthes aspera* against some another human pathogenic fungal strains viz. *Mucor canis*, *Epidermophyton floccosum* and *Aspergillus candidus*. On the basis of our finding and previous literature we conclude that *Achyranthes aspera* having potent antibacterial and antifungal property but it is variable in different solvents from different parts and probably the efficacy is also variable seasonally. The seasonal variation might be due to variation of secondary metabolite production in different season. So our comparative analysis for whole year in different solvents on the basis of polarity is required for wide spectrum pathogenic bacteria and fungus, beside identification of specific active ingredient against specific organism.

Table 01: Showing zone of inhibition (in mm) by *Achyranthes aspera* against four bacteria at 40μl conc. in two solvents.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of organism</th>
<th>Stemp</th>
<th>Root</th>
<th>Leaf</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.713+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td><em>Salmonella typhi</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.592+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.21</td>
</tr>
<tr>
<td>3</td>
<td><em>Proteus mirabilis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.719+</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.08</td>
</tr>
<tr>
<td>4</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M-Methanol extract, A-Aqueous extract
Fig. 1a: Showing Zone of Inhibition (in mm) of *Achyranthes aspera* against four bacteria at 100 μl Conc. in two solvents.

Fig. 1b: Showing zone of inhibition by flower (MeOH) extract of *Achyranthes aspera* for various bacterial strains.

Against *S. typhi*  
Against *P. aeruginosa*  
Against *S. aureus*  

Table-02: Showing zone of inhibition (in mm) by *Achyranthes aspera* against four fungal species at 400 μl Conc. in two solvents.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of organism</th>
<th>Strain</th>
<th>Root</th>
<th>Leaf</th>
<th>Flower</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>A</td>
<td>M</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td><em>Mucor plumbeus</em></td>
<td>12.66± 0.29</td>
<td>-</td>
<td>10.66± 0.63</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td><em>Epidermophyton floccosum</em></td>
<td>12.06± 0.46</td>
<td>-</td>
<td>13.33± 0.33</td>
<td>10.92± 0.36</td>
</tr>
<tr>
<td>3</td>
<td><em>Trichophyton rubrum</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td><em>Aspergillus candidus</em></td>
<td>14.00± 0.50</td>
<td>-</td>
<td>-</td>
<td>09.19± 0.33</td>
</tr>
</tbody>
</table>

M-Methanol extract, A-Aqueous extract
Antimicrobial Activity of Achyranthes Aspera

Fig 2a: Showing Zone of Inhibition (in mm) of Achyranthes aspera against four fungal strains at 400 μg conc. in two solvents.

Fig 2b: Showing zone of inhibition by different extracts of Achyranthes aspera for various fungal strains.

REFERENCES


Antimicrobial Activity of Achyranthes aspera


Drug Research Institute, Ludhiana and National Institute of Science Communication 
and Information Resources, New Delhi, 5, 7-8.
Rameshwar, RD (2007). Essential oil constituents of Achyranthes aspera leaves. Indian 
Perfumer, 51(1): 33-34.
some medicinal plants reported in the literature. Phytother Res. 3:117-125.
North Cachar Hills district of Assam, northeast India. Journal of Ethnobotanical 
Sidelined, 2: 33-35.
Achyranthes aspera Linn. Procured from Himachal Pradesh, Punjab and Haryana, India. Research 
Journal of Chemical Sciences, 1(9): 80-82.
Environmental and Ecotoxicology, 5(4): 204-208.
Revista Iberoamericana de Micologia, 6:9, 1-48030 Bilbao (Spain).
Ungung Pandang, Indonesia: high-risk groups and high-risk behaviours. Tropical 
Bacterial Infections in Animals. IV ed. (Ed. Gyle, C.L., Pressey J.F., Songer J.G., Thoen 
Zhang, X.L., Juta, V.T. and Pan, Q, (2008). Salmonella Typhi: From a Human Pathogen to a 
Vaccine Vector. Cellular & Molecular Immunology, 5, 91-97.