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

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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 resistancy. 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 *Sphagneticola 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 floccossum* (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 floccossum*. The ZOI for methanolic extract of leaf was found17.33 ± 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 floccossum*. 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; Bisht, 2013).

This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effects (Omer and Elnima, 2003; Saadabi *et al.,* 2009). About 80% of individuals from developed countries use traditional medicines which have compounds derived from medicinal plants (Igbinosa *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), parthenolide and parthenin (Zhou *et al.,* 2011d) and *Chrysanthemum indicum (Asteraceae)* contains terpenoid, flavonoids, oxygenated terpens, sesqueterpenes 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 (Dadsena *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).

Sphagneticola trilobata (L.) Pruski. (Previously accepted name, *Wedelia trilobata* L.) is a member of *Asteraceae*, (Meena, *et*

al., 2011), its common name is "Wedelia" or trailing daisy. It is a creeping, perennial herb, stem rooting at the nodes, leaves shortly petiolate, opposite-decussate, ovate, lobed, irregularly toothed, capitula heterogamous, receptacle convex, ray florates are golden yellow in colour (Hossain and Hossan, 2005). *Sphagneticola 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 Hossan, 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 amnenorrhaea 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 *Sphagneticola trilobata was* reported by many earlier workers, Taddi and Rosas Romero (1999), Utrakoon *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) (Bitsori, 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 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. floccosum is an* another anthropophilic dermatophyte. Its infection usually occur on the skin and nails. It is not known to invade hair. *E. floccosum* 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 inguinal 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 (Traczyk and Dutckiewicz, 2000). It has been claimed to be involved in a wide range of human infections including invasive aspergillosis (Ribeiro *et al.,* 2005), aspergilloma, otomycosis (Yasin *et al.,* 1978), brain granuloma and onychomycosis (Cornere and Eastman, 1975; Piraccini, 2002).

The pathogenic feature of considered bacteria viz. *Pseudomonas aurigunosa*, *Salmonella typhi, Staphylococcus aureus and Mycobacterium tuberculosis* and fungus viz. *Microsporum canis, Trichophyton rubrum, E. floccossum* 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 resistancy 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 *Sphagneticola trilobata* and constantly screened for their possible pharmacological value.

MATERIALS AND METHODS

Collection and identification of plants

The plant *Sphagneticola 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 8537km^2. The area which was selected for the collection of the plant materials for the present study was 50km^2. Around the district headquarter. The identification and authentication of the plants was carried out

Table 1: Showing zone of inhibition (in mm) by *Sphagneticola trilobata* against four Bacteria at 400µL conc. in two solvents

S.N.	Name of organism	*Sphagneticola trilobata*							
		Stem		Root		Leaf		Flower	
		M	A	M	A	M	A	M	A
1	*Staphylococcus aureus*	8.66±0.43	-	9.19±0.34	-	8.99±0.46	-	23.79±0.27	-
2	*Salmonella typhi*	-	-	-	-	16.92±0.58	-	19.66±0.94	-
3	*Pseudomonas aeruginosa*	-	-	-	-	12.93±0.28	-	23.60±0.92	-
4	*Mycobacterium tuberculosis*	-	-	-	-	-	-	-	-

M-Methanol extract, A-Aqueous extract

Table 2: Showing zone of inhibition (in mm) by *Sphagneticola trilobata* against four fungal organisms at 400µL conc. in two solvents

S.N.	Name of organism	*Sphagneti cola trilobata*							
		Stem		Leaf		Root		Flower	
		M	A	M	A	M	A	M	A
1	*Microsporum canis*	-	-	-	13.73±0.49	-	-	-	-
2	*Epidermophyton floccossum*	-	-	17.73±0.46	15.66±0.63	16.19±0.33	-	-	-
3	*Trichophyton rubrum*	-	-	17.33±0.34	-	-	-	-	-
4	*Aspergillus flavus*	-	-	-	-	-	-	-	-

M-Methanol extract, A-Aqueous extract

at Department of Botany, Govt.V.Y.T.PG. Autonomous College, Durg, C.G. India and latter also confirmed by Botanical Survey of India, Kolkata.

Preparation of plant extract

The root, stem, leaves and flowers of *Sphagneticola 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 ratio 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 bacteria 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), *Epidermophyton floccossum* (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

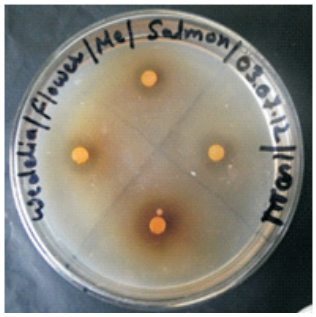
The antimicrobial activity was evaluated by agar disk diffusion method accepted by NCCLS which is a modification described by Bauer *et al.,* 1966. The disk of 6.00mm of Whatman filter paper no. 1 was saturated with plant extracts and allowed to dry. The impregnated disks were then placed on to the surface 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 *Epidermophyton floccossum,* Czapack 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 37ºC and plates containing *Mycobacterium tuberculosis* was incubated for three weeks at 37ºC. Fungal seeded plates were incubated for 72h at 25ºC, except *Trichophyton rubrum* which is incubated at 30ºC 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

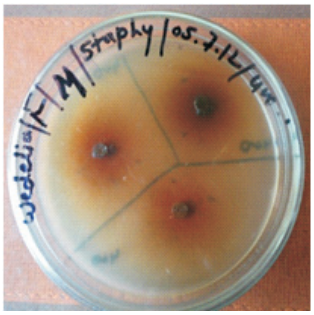
The antibacterial activity of *Sphagneticola trilobata* was found significant against three bacteria, *Pseudomonas aeruginosa, Staphylococcus aureus* and *Salmonella typhi*. The methanolic extract of leaf showed 8.99±0.46mm, 16.92±0.58mm, 12.93±0.28mm and flower extract of methanol showed 23.79±0.27mm, 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 *Sphagneticola trilobata* was found significant against three fungal organisms *Microsporum canis, Trichophyton rubrum* and *Epidermophyton floccossum* 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 *Epidermophyton floccossum*. The methanolic extract of leaf showed 17.33±0.34mm zone of inhibition against *Trichophyton rubrum* and aqueous extract of leaf, showed 13.73±0.49 mm zone of inhibition against *Microsporum canis,* all extracts were not effective against *Aspergillus candidus*. (Table 2, Fig. 2).

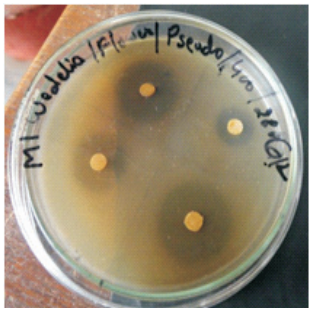
Some previous literature related to antibacterial activity of n-hexane extract of *Sphagneticola trilobata* are available against *Bacillus subtilis, Mycobacterium smegmatis, S. aureus, S.epidermidis, E.coli, Proteus vulgaris, P. aeruginosa, Salmonella paratyphi* and *Shigella sonnei*. The aqueous extract was inactive against the tested bacteria (Taddi *et al.,* 1999).



S.*trilobata* flower against *S.thyphi*

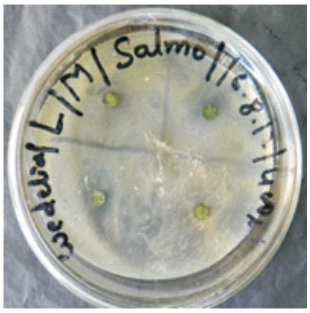


S.trilobata* flower against *S.aureus

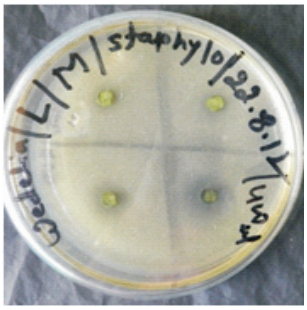


S.trilobata* flower against *P.aeruginosa

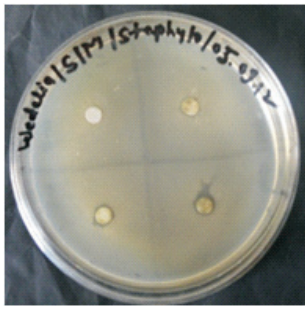
S.trilobata* leaf against *P.aeruginosa



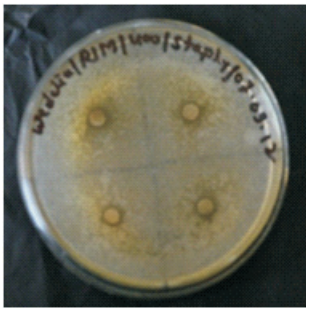
S.trilobata* leaf against *S.thyphi



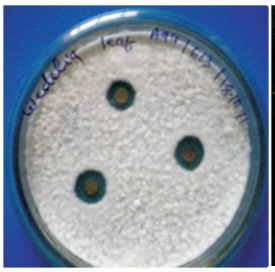
S.trilobata* leaf against *S.aureus



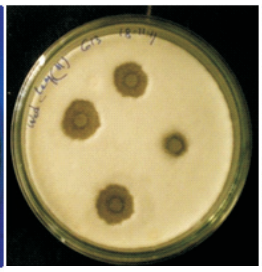
S. trilobata* stem against *S.aureus



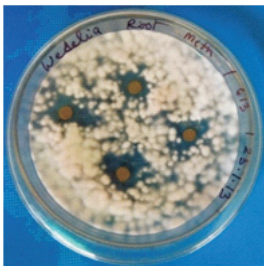
S.trilobata* stem against *S.aureus



S.trilobata* leaf(A)against *E. floccosum

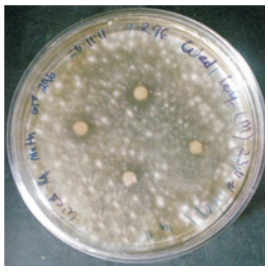


S. trilobata* leaf (M) against *E. floccossum



S.trilobata* root(M) against *E.floccossum

S.trilobata* leaf (A) against *M. canis



S.trilobata* leaf (M) against *T.rubrum

Figure 1: Above figure showing zone of inhibition by different extracts of *S.trilobata* for various bacterial strains

The antibacterial activity of ethanol extract of leaf and stem of *Sphagneticola trilobata* against *E. coli, S.typhi, P. aeruginosa, S.aureus* and *K. pneumoniae, Xanthomonas oryzae* and *X.axanopodis* was reported by Govindappa *et al.* (2011), but we found antibacterial property of methanolic extract of leaf of *Sphagneticola trilobata* against *Staphylococcus aureus, Salmonella typhi and Pseudomonas aeruginosa* and methanoloic extracts of stem and root of *Sphagneticola trilobata* against *Staphylococcus aureus* only. Methanolic extract of flower of *Sphagneticola trilobata* was reported against *Staphylococcus aureus* only by Chethan *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 *Sphagneticola trilobata* exhibited less activity on the species of *Fusarium* and *Aspergillus,* but in this study, first time we are reporting significant antifungal property of *Sphagneticola trilobata* against *Epidermophyton floccossum* in methanolic and aqueous extract of leaf and in methanolic extract of root. Both the leaf and root part were found effective against *Epidermophyton floccossum*. 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 *Sphagneticola 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. Utrakoon *et al.* (2009) reported the efficacy of essential oil extracted of *Sphagneticola trilobata* leaves on the growth of *Aspergillus flavus* but we found antifungal activity in aqueous and methanolic extract of leaf against *Epidermophyton floccossum,* 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 *Sphagneticola trilobata* against particular microorganism for pharmaceutical application.

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