International Journal of Biology Research
ISSN: 2455-6548, Impact Factor: RJIF 5.22
www.biologyjournal.in
Volume 2; Issue 3; July 2017; Page No. 16-19

Anti bacterial activity of root extract of *Biophytum sensitivum* (L.) DC

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Abstract
Medicinal plants are very interesting have the ability to produce remarkable chemical structures with diverse biological activities. *Biophytum sensitivum* L. DC (*Oxalidaceae*) is used as traditional medicine to cure various disease and also is an ethnomedicinal plant used in folk lore medicine. The main aim of this study demonstrates the root extracts of *Biophytum sensitivum* (Acetone, Benzene, Ethanol, Iso-prophyl alcohol and N- butyl alcohol) was evaluated for its antibacterial activity against several human pathogenic bacterial strains (*Escherichia coli, Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus* and *Proteus vulgaris*) by disc agar diffusion method. All the extracts showed various levels of activity on different test organisms and their activity is quite comparable with the standard antibiotics. The n-butyl alcohol extracts of *Biophytum sensitivum* root showed remarkable anti bacterial activity against *Staphylococcus aureus*. The results from these investigations encourage that the plant extracts may be used as anti-infective agents.

Keywords: *biophytum sensitivum*, antibacterial activity, disc agar diffusion method

Introduction
Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials need to occur. Human infections particularly those involving microorganisms ie.bacteria, fungi, viruses they cause many diseases in human beings. The frequency of life threatening infections, caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune compromised patients in developing countries (Al-bari *et al*., 2006) [2]. Many infectious microorganisms have developed resistance against many synthetic antibiotics due to the indiscriminate use of antimicrobial drugs (Ahmed *et al.*, 1998) [1] and some times they associated with side effects (Cunha, 2001) [4]. There is an urgent need to discover an alternative new, more active, broad spectrum and safer antimicrobial agents (Frontling and Rathway, 1987) [6]. The pharmacological investigations of plants were carried out to find noval drugs or templates for the development of new therapeutic agents (Iwu *et al.*, 1999) [12]. Phytomedicines have been used to treat or prevent various disorders (Steenkamp, 2003) [20]. Innumerable drugs from plants found worldwide have been documented in literatures yet there continues to be untapped knowledge on the curative potential of several plants. One of the most popular medicinal herb, *Biophytum sensitivum* (L.) DC (*Oxaliaceae*) is a small annual plant, growing throughout the tropical regions of south Asia, Africa and Madagascar. It possess a wide spectrum of medicinal properties namely antiseptic properties, Asthma and phthisis (Pullaiah, 2002) [16], including positive effects in inflammatory diseases, and diabetes (Kirtikar; 1984, [13] Mitra and Ambasta 1988; [15] Puri *etal*., 1998) [18]. The biological activity of the plant shows hypoglycaemic (Puri and Baral., 1998) [17], immunomodulatory, (Guruvayoorappan and kuttan, 2007), [8] Chemoprotective Guruvayoorappan and Kuttan) 2007 [11] hypocholesterolemic (Dinesh puri, 2003) [5], apoptotic, cell – mediated immune response (Guruvayoorappan and kuttan, 2007) [10] antitumor (Bhaskar and Rajalakshmi, 2010) [3]. The phytochemistry of *Biophytum sensitivum* showed a wide range of chemical compounds including two biflavones, cupressuflavone and amentoflavones three flavonoids, luteolin7 -methyl ether, isoorientin and 3' – Methyoxyluteolin7-o-glusoside; two acids, 4-coffeoylquinic acid and 5-coffeoylquinic acid which were isolated from the aerial parts of *Biophytum sensitivum* (Lin and Wang, 2003) [14].

Material and Methods
Plant material
The mature and healthy plant`s root of *Biophytum sensitivum* was collected from Kanyakumari District, Tamil Nadu. The taxonomic identification was done by referring Herbarium voucher specimens and flora of presidency of Madras (Gamble, 1928) [7]. After the collection of plant parts (root) were washed with tap water, shade dried and weighed. The root was then ground using blender to get powder. The ground dried powder of *Biophytum sensitivum* root (10gram) was sequentially extracted with acetone, benzene, ethanol, iso-propyl alcohol, n-butyl alcohol using soxhlet apparatus for 20 days for aqueous extracts the dried powder was boiled with water for 24 hours continuously. The supernatants were filtered with whatman No.1 filter paper then it was stored in airtight bottles for bacterial study.

Preparation of natural disc
Sterile discs were obtained and stored at 4^0c discs were handled using a pair presterilized forceps. The extracts was loaded on the disc carefully using capillary tube, without spreading out. Thus the discs were completely saturated with the extract was used for testing antibacterial activity

Synthetic discs

The synthetics disc used were Cholramphenicol, Tetracycline, Ampicillin, Ciprofloxacin, Erythromycin, Kanamycin and Neomycin. Human pathogens used in the study of *Staphyolocoous aureus*, *Escherichia coli*, *Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typi*. All human pathogens were obtained from clinical laboratories Kanyakumari District, Tamil Nadu.

Antibacterial Activity

The agar plates were inoculated with inoculums of 10^6 size sterile swab is dipped into diluted culture inoculums. Sterile disc with plant extracts synthetic discs were dried and placed on the agar surface with the help of sterile forceps. Inoculated petridishes were incubated at 36^0C overnight and the inhibition zone was recorded (Bauer *et al.*, 1996). Discs (5mm) without plant extract was used as control. The inhibitory zone around test paper discs indicate the absence of bacterial growth and that was recorded as positive test and absence of zone as negative test.

Result and Discussion

The antibacterial activity of *Biophytum sensitivum* root were assessed using agar disc diffusion method by measuring the diameter of inhibition zones. (Table1: Plate 1.) In the present investigation *Biophytum sensitivum* root extracts possess antibacterial activity against tested gram positive (*Staphylococcus aureus)* and gram negative strains (*Pseudomonas aeruginosa, Proteus vulgaris, Salmonella typhi* and *Escheria coli*). The extracts obtained with different solvents like ethanol, acetone, benzene, n-butyalcohol and iso-propyl alcohol showed varying activity. Among the five extracts of *Biophytum sensitivum* root ethanol and n-butyl alcohol extracts were moderately susceptible to all tested human pathogens (Table 1.) The n-butyl alcohol extracts of *Biophytum sensitivum* root obtained the great activity among all other solvents. The least activity of n-butyl alcohol extracts of *Biophytum sensitivum* root. Against *Proteus vulgaris* (14mm). Higher activity of n-butyl alcohol extract of *Biophytum sensitivum* root was against the *Staphylolcoccus aureus* 30mm) than other solvents.

Table 1: Antibacterial activity of Different solvent extracts of *Biophytum sensitivum* root

Human pathogens	Solvents	Plant extracts of *Biophytum sensitivum* root
Escherichia coli	Ethanol	23mm
	Acetone	-
	Benzene	15mm
	n-butyl alcohol	28mm
	Iso-propyl alcohol	9mm
Pseudomonas aeruginosa	Ethanol	16mm
	Acetone	-
	Benzene	-
	n-butyl alcohol	23mm
	Iso-propyl alcohol	-
Salmonella typhi	Ethanol	9mm
	Acetone	14mm
	Benzene	11mm
	n-butyl alcohol	23mm
	Iso-propyl alcohol	13mm
Staphylococcus aureus	Ethanol	20mm
	Acetone	-
	Benzene	12mm
	n-butyl alcohol	30mm
	Iso-propyl alcohol	22mm
Proteus vulgaris	Ethanol	11mm
	Acetone	-
	Benzene	20mm
	n-butyl alcohol	14mm
	Iso-propyl alcohol	12mm

Table 2: Antibacetrial activity caused by plant extracts of (*Biophytum sensitivum* root) through disc agar diffusion method

Human pathogens	Solvents used				
	Ethanol	Acetone	Benzene	n-butyl alcohol	Iso-prophyl alcohol
Escherichia coli	++	-	+	+	+
Pseudomonas aeriginosa	+	-	-	+	-
Salmonella typhi	+	+	+	+	+
Staphylococcus aureus	++	-	+	+	++
Proteus vulgaris	+	-	++	+	+

(-) Negative activity
(+) Active
(++) moderate activity

Table 3: Drug sensitivity of human pathogens against Antibiotics

Human pathogens	Antibiotics zone formation (mm)						
	CH	**T**	**AM**	**CI**	**ER**	**KA**	**N**
Escherichia coli	R	27mm	R	33mm	R	21mm	25mm
Pseudomonas aeruginosa	R	23mm	R	52mm	23mm	15mm	33mm
Salmonella typhi	R	22mm	R	40m	18mm	23mm	20mm
Staphylococcus aureus	17mm	29mm	12mm	34mm	28mm	23mm	22mm
Proteus vulgaris	18mm	R	R	33mm	11mm	21mm	21mm

Table 4: Antibacterial activity caused by Synthetic compounds through disc diffusion method

Human pathogens	Antibiotics						
	CH	T	AM	CI	ER	KA	N
Escherichia coli	-	++	-	+++	-	++	++
Pseudomonas aeruginosa	-	++	-	+++++	++	+	+++
Salmonella typhi	-	+	-	++++	+	++	++
Staphylococcus aureus	+	++	+	+++	++	++	++
Proteus vulgaris	+	-	-	+++	+	++	++

R-Resistant (+++)-Highly activity T-Tetracycline
(-)-Negative activity ER-Erythromycin KA-Kanacmycin
(++)-Medium activity CH-Cholramphenical N-Neomycin
(+)-Active AM-Ampicillin CI-Ciprofloxacin

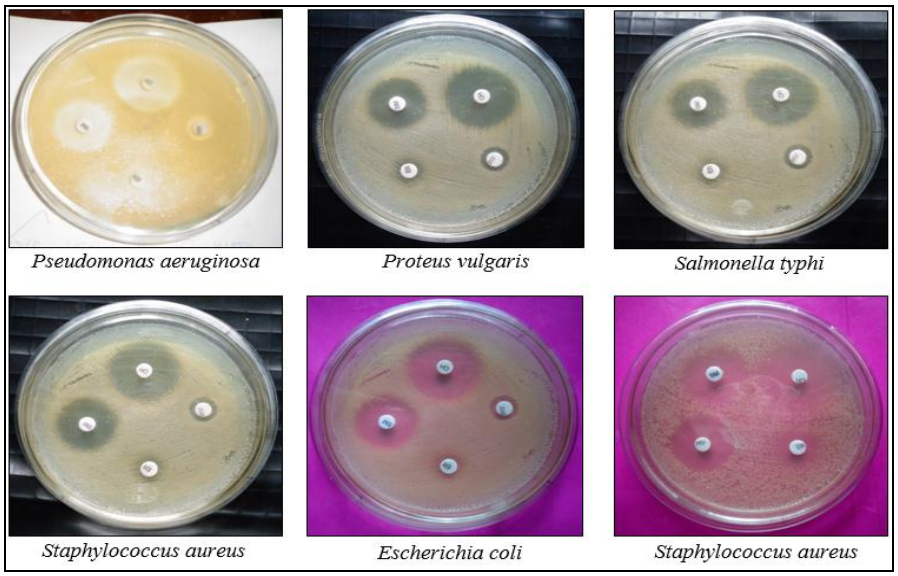

Fig 1

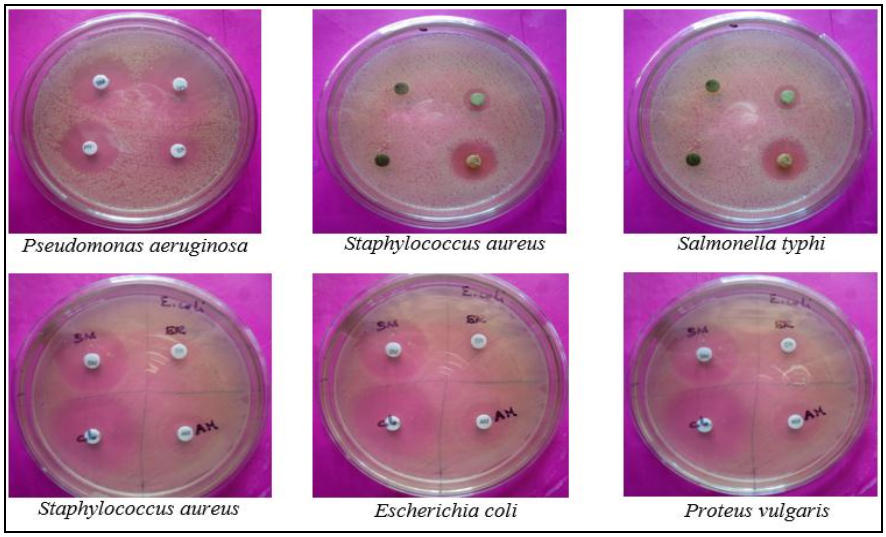

Fig 2

Five different human pathogens were tested against seven different antibiotics such as Chloramphenicol, Tetracycline, Ampicillin, Ciprofloxacin Erythromycin, Kanamycin and Neomycin (Table 3, 4 and plate II) *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Proteus vulgaris* were inhibited by ciprofloxacin with the zone formation above 30mm.Maximum antibacterial activity was observed against *Pseudomonas aeruginosa* with the zone formation of 52mm *Pseudomonas aeruginosa* and *Salmonella typhi* was resistant to Chloramphenicol. In the study exclusive inhibition of S*taphylococcus aures* by Amphicillin was obvious. All the other tested human pathogens *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and P*roteus vulgaris* were resistant to ampicillin. (Table 3, 4 plate II). There are several investigators have proved the antimicrobial potentiality of some *Oxalidacease* members. Valasaraj *et al.,* 1997 [21] reported that the different concentrations (25, 12.5, 6.25and 1.56 mg/ml) of 80% ethanol extracts of *Oxalis corniculata* were tested against four bacteria viz. *Bacillus subtillis, Staphylococcus aureus. Escherchia coli* and *Pseudomonas aeruginosa* by the agar dilution method. Raghvendra *et al.,* 2006 [19] have reported that the antibacterial activity of various extracts of *Oxalis corniculata* plant and showed significant anti bacterial activity of methanolic and ethanolic extracts of the same against Xanthomonas and fourteen human pathogenic bacteria.

Now a days researchers have attracted the attention a lot on medicinal plants globally. In the present study the antibacterial activity of *Biophytum sensitivium* root exhibited different degree of antimicrobial activity against the human pathogens.

The result of present study on *Biophytum sensitivum* root showed broad spectrum of antibacterial activity against the tested bacteria. All the extracts of *Biophytum sensitivum* root inhibition growth of almost all the selected bacteria in the range from 9 to 30mm Among them n-butyl alcohol extract of *Biophytum sensitivum* root showed great activity against *Staphylococcus aureus* (30mm) and *Escherichia coli* (28mm). Result of present investigation highlights the antibacterial potential of *Biophytum senstitivum* root extracts. The study encourages that folk medicine can be used as effective modern medicine to combat pathogenic microorganisms.

Conclusion

The present investigations suggest that different solvent extracts of *Biophytum sensitivum* root showed a good result of antibacterial activity and can be developed as new natural antibacterial drugs.

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