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Biocidal activity of *Biophytum sensitivum* (L.) against human pathogens and mosquito larvae

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Abstract
Medicinal plants are very interesting, began to focus on discovering natural products as potential drugs against various ailments. In this study proposes to evaluate the antibacterial and larvicidal activities of selected plant *Biophytum sensitivum* (L.). Antibacterial activity has been tested against human pathogenic E. coli, *Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus* and *Proteus vulgaris*. The highest value observed from ethanol extracts of *Biophytum sensitivum* (L.) showed antibacterial activity against *Escherichia coli*. All the tested human pathogens were highly sensitive to ciprofloxacin. The N-butyl alcohol extracts of *Biophytum sensiivum* showed the best results of larvicidal activity against Aedes aegypti mosquito larvae. This study *Biophytum sensitivum* in the traditional system of to treat various infectious diseases, caused by the microbes as well as in mosquitos.

Keywords: antimicrobial activity, *Biophytum sensitivum*, bioactivity, human pathogens

Introduction

Medicinal plants are widely used by all parts of the population, either directly as traditional medicine or indirectly in the preparation of recent pharmaceutical products. (Pushpangadan *et al*., 1995) [9]. According to WHO the increase of resistant to antibiotics by bacterial pathogens is a growing problem in both developed and developing countries. The systematic screening of antibacterial plant extracts represent continuous efforts to act against multidrug resistance organisms (Crag *et al*., 1997) [2]. The expanding bacterial resistance to antibiotics has becoming a growing concern worldwide (Gardam, 2000) [5]. The use of the plant as a raw material for medicine has been inherited and is an important part of the Indian healthcare system. (Seth *et al*., 2004) [11].

The rise in antimicrobial resistance is driving a resurgence in research into the antibacterial role of herbs against drug-resistant strains. (Hemaiswaraya *et al.,* 2008; Alviano and Alviano 2009) [6, 1]. Numerous medicinal plants are recognized as valuable sources of natural antimicrobial compounds (Mahady 2005) [7]. In India, 2500 plant species are used by traditional healers and 100 plants are said to serve as principles of natural medicine. With the aim of expanding their wide range of medical applications, the modern era has brought new drugs with more potent and desirable activities with fewer or no side effects for certain diseases. (Roy *et al.*, 2009) [10].

Mosquitoes cause more diseases than any other arthropod group (Cepleanu, 1993) [3]. The mosquito *Aedes aegypti* serves as a vector for the arbovirus that causes yellow fever in Central and South America and West Africa. (Maillard *et al.*, 1993) [8]. In fact, the current resurgence of these diseases is due to an increase in the number of breeding sites in today's throwaway society and increased resistance of mosquitoes to common over-the-counter insecticides. An alternative to conventional chemical control is to use natural products of plant origin (Consoli and Oliveira 1994) [4]. This requires research and development of a native vector control method that is safer for the environment and at a lower cost. The objective of the study was concerned with the effects of different solvent extracts of *Biophytum sensitivum* on human pathogens and mosquito larvae.

Materials and Methods

Collection of plant materials

Fresh and healthy plants are harvested from various places in the Kanyakumari district. The leaves of the fresh plant after being harvested are dried in the shade for 10 days and then crushed. One gram of grinded powder was soaked in 10 ml of different solvents such as ethanol, n-butyl alcohol, isopropyl alcohol, benzene and acetone. All solvent extracts were stored at room temperature for 10 days and shaken periodically. Extracts obtained from each solvent were saved for further use.

Preparation of natural disc and Synthetic disc

Sterile discs were obtained and stored at 4°C. Discs were handled using a pair of pre-sterilized forceps. The extract was loaded on to the disc carefully using capillary tube, without spreading out. The disc is completely saturated with the extract for testing anti-bacterial activity. The synthetic disc used were chloramphenicol, tetra-cycline, ampicillin, ciprofloxacin, erythromycin and neomycin. A total of five human pathogens were used in the study. They are *Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi*. All selected human pathogens were obtained from clinical laboratories. Inoculum of each pathogen was suspended in 3ml of nutrient broth. The bacteria species were cultured in the nutrient broth. All cultures were cultured in the nutrient broth and were incubated at 37°C for 18hrs, then diluted to 1/10 the concentration to yield a culture density of approximately 108 CFU/ML.

Preparation of Nutrient Agar

A nutrient agar solid medium was prepared by dissolving 2.8 g of nutrient agar in 100 ml of distilled water. Approximately 25 mL of nutrient agar was poured into a Petri dish and allowed to set.

Antibacterial activity

Inoculate an agar plate with size 106 inoculum and dip a sterile swab into the diluted culture inoculum. Sterile discs and synthetic discs containing plant extracts were dried and placed on the agar surface using sterile forceps. Inoculated petri dishes were incubated overnight at 37° C. and zones of inhibition were recorded (Bauer *et al*., 1996). Discs (5 mm) without plant extract were used as controls. A zone of inhibition around the test strip disc indicates no bacterial growth. This is recorded as a positive test and the absence of zones is recorded as a negative test.

Larvicidal Assay by Serial Dilution method

The larvicidal activity was tested on *Aedes aegypti* mosquito larvae at the IQB/UFAL Bioassay Laboratory based on the methodology described by WHO. Subsequently, 100 of his 4th instar mosquito larvae (*Aedes aegypti*) were collected from freshwater. In this method, we investigated the susceptibility of mosquito larvae to selected concentrations of the extract. Ten larvae were then dissected into crude extracts. He placed 10 larvae in each dish containing different concentrations of the extract (0.1% to 1%). Another set of 10 larvae was introduced into another dish as a control. The shell was then left undisturbed. The activity of the tested extracts was determined based on the average percentage of post-period larval mortality.

Results and discussion

The results showed that *Biophytum sensitivum* ethanol extract possessed significant antibacterial activity. The resulting inhibition zones range from 0 to 23 mm (Table 1). Moreover, the biological activities showed great variation among the pathogens selected in the tested plant extracts.

Table 1: Antibacterial activity of different solvent extracts of *Biophytum sensitivum*

Human Pathogens	Solvents	Inhibition zone
Escherichia coli	Ethanol	23mm
	Acetone	-
	Benzene	15mm
	n-butyl alcohol	11mm
	Iso propyl alcohol	9mm
Pseudomonas aeruginosa	Ethanol	16mm
	Acetone	-
	Benzene	-
	n-butyl alcohol	11mm
	Iso propyl alcohol	-
Salmonella typhi	Ethanol	9mm
	Acetone	14mm
	Benzene	11mm
	n-butyl alcohol	9mm
	Iso propyl alcohol	13mm
Staphylococcus aureus	Ethanol	20mm
	Acetone	-
	Benzene	12mm
	n-butyl alcohol	18mm
	Iso propyl alcohol	22mm
Proteus vulgaris	Ethanol	11mm
	Acetone	-
	Benzene	20mm
	n-butyl alcohol	9mm
	Iso propyl alcohol	12mm

Ethanolic extract of *Biophytum sensitivum* (L.) showed maximum antibacterial activity (inhibition zone 23mm) against *Escherichia coli* and minimum activity (9mm) against *Salmonella typhi*. (Natarajan *et al*., 2012) have reported that the antibacterial activity of ethanol extracts tested against four bacteria. The bacteria are *Bascillus subtilis, Staphylo coccus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* Acetone extract of *Biophytum sensitivum* (L.) showed maximum antibacterial activity (inhibition zone - 14mm) against *Salmonalle typhi* and had no activity against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus vulgaris*. In a report antibacterial activity of *Biophytum sensitivum* (L.) all the extracts were inhibited growth of almost all the selected bacteria in the range of (inhibition zone 7.25mm) (Natarajan *et al*., 2010). Benzene extract of *Biophytum sensitivum* (L.) showed maximum antibacterial activity (inhibition zone 20 mm) against *Proteus vulgaris* and had no activity against *Pseudomonas aeruginosa*. The screening of secondary metabolites had shown that higher plants represent a potential source of new anti-infective agents (Kelmanson *et al*., 2001). N-butyl alcoholic extracts of *Biophytum sensitivum* (L.) showed maximum antibacterial activity (inhibition zone 18mm) against *Staphylococcus aureus* and minimum activity (9mm) against *Salmonella typhi* and *Proteus vulgaris*. Iso propyl alcoholic extracts of *Biophytum sensitivum* showed maximum antibacterial activity (inhibition zone 22mm) against *Staphylococcus aureus* and had no activity against *Pseudomonas aeruginosa*. (Natarajan *et al.,* 2010) reported the use of herbal extracts and demonstrate that folk medicine can be

used as effective modern medicine to compact pathogenic microorganisms.

Seven antibiotics are chloramphenicol, tetracycline, ampicillin, ciprofloxacin, erythromycin, kanamycin and neomycin were tested against five bacterial strains *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *salmonella typhi* and *Pseudomonas aeruginosa* to determine the sensitivity towards antibiotics. (Table 2)

Table 2: Drug sensitivity of human pathogens against antibiotics

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

Table 3: Antibacterial activity caused by synthetic compounds through disc diffusion method

Human Pathogens	Antibiotics						
	CH	**T**	**AM**	**CI**	**ER**	**KA**	**N**
Escherichia coli	-	++	-	+++	-	++	++
Klebsiella Pneumoniae	-	++	-	+++++	++	+	+++
Salmonella typhi	-	+	-	++++	+	++	++
Staphylococcus aureus	+	++	+	+++	++	++	++
Proteus vulgarius	+	-	-	+++	+	++	++

The results of this study showed that chloramphenicol showed resistance to chloramphenicol in *Escherichia coli, Pseudomonas aeruginosa, and Salmonella typhi*, with zones of inhibition greater than 17 mm, and in 2 strains such as *Staphylococcus aureus* and *Proteus vulgaris*. It has been shown to inhibit the growth of two bacterial strains. Tetracycline inhibited the growth of her four bacterial strains, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi*, and *Staphylococcus* aureus, in the upper (22 mm) zone of inhibition. *Proteus vulgaris* was insensitive to tetracycline. Only *Staphylococcus aereus* was sensitive to ampicillin, whereas all other bacterial strains showed resistance to ampicillin. Erythromycin showed antibacterial activity against *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi* with a zone of inhibition greater than 11 mm. E. coli showed resistance to erythromycin. Kanamycin inhibited the growth of all bacterial strains tested in the above zoning (15 mm). Neomycin also showed antibacterial activity against all tested human pathogens in the above zone of inhibition (20 mm). All tested human pathogens were highly sensitive to ciprofloxacin, with zones of inhibition greater than 33 mm.

Results reveals that the n-butyl alcohol extract of *Biophytum sensitivum* showed varying mortality rate against the IVth instar larvae of *Aedes aegypti* (Table 3). In our study (1) mortality was observed in 0.1% concentration on n-butyl alcohol extract of *Biophytum sensitivum* while 100% mortality was observed in 1% concentration within 10 minutes. Preliminary screening is a good tool to assess potential larvicidal efficacy, and the highest mortality was observed with n-butyl alcohol for *Biophytum sensitivum*. Acetone extract showed mortality rate (1) in 0.1% concentration within 19 minutes and 100% mortality was observed 1% concentration within 50 minutes. Benzene extract of *Biophytum sensitivum* showed minimum mortality (1) in 0.1% concentration within 53 minutes and 90% mortality rate observed in 1% concentration within 19 minutes.

Isopropyl alcohol extract showed minimum mortality (2) in 0.1% concentration within 58 minutes and 100% mortality was observed in 1% concentration within 61 minutes. Benzene extract showed minimum mortality (6) in 0.1% concentration within 109 minutes and 90% mortality was observed in 1% concentration within 19 minutes. The most powerful larvicidal activity of *Biophytum sensitivum* were shown by N-butyl alcohol extracts, against the mosquito larvae *Aedes aegypti*. Other extracts such as Acetone, Benzene, Isopropyl alcohol and Ethanol showed moderate levels of larvicidal activity.

Table 4: Bio-larvicidal activity on n-Butyl extract of *Biophytum sensitivum* in 0.1% to 1.0% concentration

Concentration	0.1%				0.20%				0.30%				0.40%				0.50%			
S.No	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)
1	11.47	1.05	78	1	11.47	12.50.	64	1	11.45	11.55	10	1	11.33	11.38	7	1	11.34	11.35	5	1
2					11.76	1.05	79	2	11.45	12.20	35	2	11.33	11.42	11	2	11.34	11.36	6	2
3									11.45	12.50	64	3	11.33	11.44	13	3	11.34	11.40.	11	3
4													11.33	11.49	18	4	11.34	11.46	16	4
5													11.33	11.51	20	5	11.34	11.50.	20	5
6													11.33	11.53	22	6	11.34	11.52	22	6
7													11.33	11.54	23	7	11.34	11.53	23	7
8													11.33	11.56	25	8	11.34	11.55	25	8
9													11.33	12.20.	49	9	11.34	11.59	27	9
10													11.33	12.31	60	10	11.34	12.01	30	10

Table 5

Concentration	0.60%				0.70%				0.80%				0.90%				1.00%			
S.No	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Mits)	Death Rate (Nos)
1	11.29	11.33	5	2	11.26	11.30	4	1	11.26	11.30	4	1	11.25	11.27	2	1	11.24	11.26	2	2
2	11.29	11.36	8	4	11.26	11.31	5	3	11.26	11.32	6	3	11.25	11.29	4	2	11.24	11.28	4	3
3	11.29	11.40	12	6	11.26	11.33	7	4	11.26	11.33	7	4	11.25	11.30	5	3	11.24	11.29	5	4
4	11.29	11.44	15	8	11.26	11.34	8	5	11.26	11.34	8	6	11.25	11.32	7	5	11.24	11.30	6	6
5	11.29	11.50	22	9	11.26	11.35	9	6	11.26	11.35	9	7	11.25	11.34	9	7	11.24	11.31	7	8
6	11.29	11.55	27	10	11.26	11.36	10	8	11.26	11.39	13	8	11.25	11.35	10	8	11.24	11.34	10	10
7					11.26	11.40	15	9	11.26	11.40	14	10	11.25	11.36	11	10				
8					11.26	11.41	18	10												

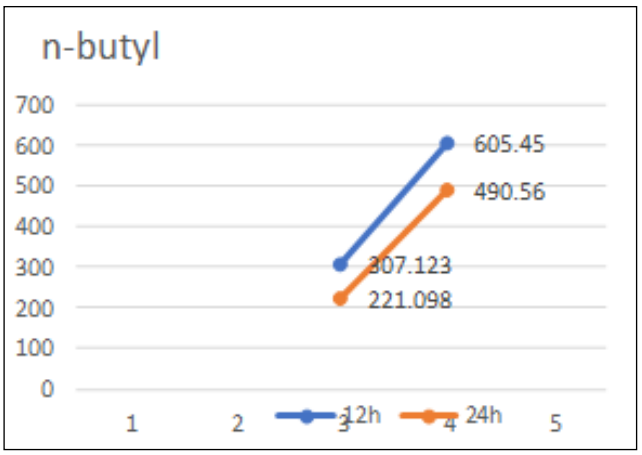

Fig 1

n- butyl alcohol extract shows greatest larvicidal activity than others. LC_{50} and LC_{90} value shows after 12h is 307 and 605 ppm. After 24h it shows 221 ppm and 490 ppm. Isopropyl alcohol shows moderate level of activity. Acetone, ethanol, benzene extract shows low level of larvicidal activity.

Conclusion

Antibiotic compounds are synthetic compounds that inhibit certain microorganisms. They contain specific compounds in known concentrations. However, natural products are synergistic compounds and have low sensitivity. The antibacterial and insecticidal activity is therefore higher than that of the natural compounds.

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