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MECHANISMS AND ACTIVITY OF BIOPHYTUM SENSITIVUM (L.) AGAINST BIOCIDAL AGENTS AND MOSQUITO LARVAE

[1]JASMIN. S [2]THAMPIRAJ. S

[1]JASMIN. S Research Scholar, (Register No-12314), Department of Botany and Research Center, Scott Christian College, Nagercoil, Kanyakumari District, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamilnadu, India.

[2]THAMPIRAJ. S (Guide), Assistant Professor, Department of Botany and Research Center, Scott Christian College, Nagercoil, Kanyakumari District, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli, Tamilnadu India

*Corresponding Author: **Jasmin. S**, E-mail: jasmins0570@gmail.com

ABSTRACT

Medicinal plants are very interesting, began to focus on discovery of natural products as potential active principles against various diseases. In this study proposes to evaluate the antibacterial and larvicidal activities of selected plant *Biophytum sensitivum* (L.). The antibacterial activities were determined against human pathogens viz *Escherichia 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 best result of larvicidal activity against the mosquito larvae *Aedes aegypti*. This study *Biophytum sensitivum* in the traditional system of to treat various infectious diseases, caused by the microbes as well as in mosquitos.

Key words: antimicrobial activity, *Biophytum sensitivum*, bioactivity, human pathogens.

Introduction

Medicinal plants are largely used by all divisions of the population either directly as folk medications or indirectly in the preparation of recent pharmaceuticals (Pushpangadan *et al*., 1995). 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 represents continuous efforts to act against multidrug resistance organisms (Crag *et al*., 1997). The expanding bacterial resistance to antibiotics has becoming a growing concern worldwide (Gardam, 2000). Use of plants as a source of medicine has been inherited and is an important component of healthcare system in India (Seth *et al*., 2004).

Increasing bacterial resistance is promptering a resurgence in research of the antimicrobial role of herbs against resistance strains (Hemaiswaraya *et al*., 2008; Alviano and Alviano 2009). A vast number of medicinal plants have been recognized as

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valuable resource of natural antimicrobial compounds (Mahady 2005). In India, it is declared that traditional healers use 2500 plant species and 100 species of plants serve as natural principles of medicine. With a view to increasing the wide range of medicinal usage, the present day entails new drugs with more potent and desired activity with less or no side effects against particular disease (Roy *et al.*, 2009).

Mosquitoes are responsible for more diseases than any other group of arthropods (Cepleanu, 1993). The mosquito *Aedes aegypti* acts as a vector for an arbovirus responsible for yellow fever in Central and South America and in West Africa. (Maillard *et al.*, 1993). Indeed, the present recrudescence of these diseases is due to the higher number of breeding places in today's throwaway society and to the increasing resistance of mosquitoes to current commercial insecticides. An alternative for conventional chemical control is the utilization of natural products from plants (Consoli and Oliveira 1994). This has necessitated the need for the search and development of environmentally safer, low cost, indigenous method for vector control. The objective of the study which deals with the effect of different solvent extracts of *Biophytum sensitivum* on human pathogens and mosquito larvae.

MATERIALS AND METHODS

Collection of plant materials

Fresh and healthy plants were collected from various locations of Kanyakumari District. Freshly collected plant leaves were shade dried for ten days and grind. 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 the solvent extracts were kept at room temperature for 10 days with periodic shaking. The extracts obtained from the respective solvents were stored 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. Thus, the disc completely saturated with the extract was 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. An 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 18 hrs, then diluted to 1/10 the concentration to yield a culture density of approximately 10^8 CFU/ML.

Preparation of Nutrient Agar

Solid media of nutrient agar was prepared by dissolving 2.8gm of nutrient agar in 100ml of distilled water. About 25ml of nutrient agar media was poured into a Petri dish allowed to solidify.

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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 and synthetic discs were dried and placed on the agar surface with the help of sterile forceps. Inoculated petri dishes were incubated at 37^0C over night and the inhibition zone was recorded (Bauer *et al.*, 1996). Disc (5mm) without plant extract was used as control. The inhibitory zone around test paper discs indicates the absence of bacterial growth and that was recorded as positive test and the absence of zone as negative test.

Larvicidal Assay by Serial Dilution method

The larvicidal activity was tested on the larvae of the mosquito *Aedes aegypti* in the bioassay laboratory of the IQB/UFAL, based on methodology described by the WHO. After 100 mosquito larvae (*Aedes aegypti*) in the IVth instar stage were collected from freshwater. The susceptibility of the mosquito larvae to the selected concentration of the extracts was studied by this method. Then 10 larvae were prepared for immense into the crude extract. Ten larvae were placed in each bowl containing different concentration of the extract (0.1% to 1%). Another set of 10 larvae were introduced into separate bowls as considered as control. Then the bowl was left undisturbed the activity of the tested extracts was established based on the average percentage of mortality of the larvae after their periodic time.

Results and discussion

The result revealed that ethanolic extract of *Biophytum sensitivum* exhibited significant antimicrobial activity. The zone of inhibition obtained between 0 to 23mm (Table 1). More over the bioactivity exhibited showed a wide variation among the selected pathogens with the plant extracts studied.

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

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	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 were tested against four bacterias *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 20mm) 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)

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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

Results of the present study reveals that the chloramphenicol inhibited the growth of two bacterial strains such as *Staphylococcus aureus* and *Proteus vulgaris* with zone of inhibition above 17mm in *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* showed resistance against chloramphenicol. Tetracycline inhibited the growth of four bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* with the zone of inhibition above (22mm). *Proteus vulgaris* was not sensitive to tetracycline. *Staphylococcus aereus* alone was sensitive to ampicillin, whereas all the other bacterial strains showed resistance against ampicillin. Erythromycine showed antibacterial activity against *Proteus vulgaris*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* with the zone of inhibition above (11mm) *Escherichia coli* showed resistance against erythromycin. Kanamycin inhibited the growth of all the tested bacterial strain with the zone formation above (15mm). Neomycin also showed antibacterial activity against all the tested human pathogens with the zone of inhibition above (20mm). All the tested human pathogens were highly sensitive to ciprofloxacin with the zone of inhibition above (33mm).

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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	+	-	-	+++	+	++	++

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 mean of evaluation of the potential larvicidal effects and highest mortality was observed with the n-butyl alcohol of *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*. The other extracts like Acetone, Benzene, Isopropyl alcohol and Ethanol were showed moderate level of larvicidal activity.

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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 (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	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

Concentration	0.60%				0.70%				0.80%				0.90%				1.00%			
S.No	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	Death Rate (Nos)	Initial Time	Final Time	Time Duration (Min)	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												

CONCLUSION

The antibiotic compounds are synthetic compounds that particularly inhibit individual micro-organisms. They contain a known concentration of a particular compound. However, the natural compounds are synergetic compounds and their susceptibility is low. Hence the antibacterial activity and larvicidal activity is higher than the natural compounds.

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