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Biopotency of *Biophytum sensitivum* DC

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The present study was intended to examine the antioxidant, cytotoxicity, larvicidal potentials of *B. sensitivum* DC. The total phenolic content was determined by the standard method described by Siddhuraju and Becker (2003)**. The antioxidant activity of different extracts were determined using the stable radical DPPH, phosphomolybdenum and scavenging of hydrogen peroxide assays according to the standard method. In addition, the larvicidal and cytotoxic properties of the various extract of *B. sensitivum* were examined. Maximum amount of extractable total phenolics was observed in methanolic extracts of *B. sensitivum* (1399.84 ± 215.79mg GAE/g). The best free radical (DPPH) scavenging activity of *B. sensitivum* was observed in acetone extracts with IC_{50} value 30.12µg/ml. The methanolic extracts of *B. sensitivum* displayed the strongest phosphomolybdenum reduction (202.24 ± 11.44 g AA/100 g) compared to other tested extracts. The methanolic extracts of *B. sensitivum* showed highest larval mortality in terms of lethal concentrations for 50% mortality against *Culex quinquefasciatus* with LC_{50}= 215.34 mg/ml. The methanolic extract of *B. sensitivum* displayed most effective at 90% mortality (LC_{90}) of brine shrimp nauplii occurred at 66.34 mg/ml Conclusion: The present investigations suggest that methanolic and acetone extracts showed a good result of antioxidant, larvicidal and cytotoxic activity. It was found that the high rates of phenolic substances widely distributed in *B. sensitivum*.**

Keywords: antioxidant; larvicidal; cytotoxic; *B. sensitivum.*

Introduction

Biophytum sensitivum DC (Oxalidaceae) is a small, sensitive annual herb, growing throughout the tropical regions of South Asia, Africa and Madagascar. More number of pharmacological activities has been reported on *Biophytum sensitivum* including antibacterial 2006). *Biophytum sensitivum* has been studied for its antioxidant both *in vitro* and *in vivo* (Kalita *et al.* 2013), immunomodulatory (Guruvayoorappan and Kuttan, 2007a), anti tumour (Bhaskar and Rajalakshmi, 2010), anti-inflammatory (Jachak *et al.* 1994), antidiabetic (Ananda prabhu *et al.* 2012), chemoprotective properties (Guruvayoorappan and Kuttan, 2007) and wound healing potential. Plant secondary metabolites have proved to be an excellent reservoir of new medical compounds. Antioxidants play an important role to protect cells and tissues against damage by reactive oxygen species (Rajakannu *et al.* 2012). Number of *in vivo* and *in vitro* studies confirmed that the plant derived metabolites are the good source of anti- cancer agents (Krishnaveni and Ragunathan, 2012; Sushma *et al.* 2012; Sumitra and Krunal, 2013). The *in vivo* lethality in a

simple zoological organism (*Artemia salina*) can be used as a suitable technique for screening, detection and observation of bioactive natural products (Guruvayoorappan and Kuttan, 2007). Brine shrimp assay has advantages of being hasty (24 hours), economical, simple and it is an unsurpassed for screening of antitumor compounds. Chandra Kala and Mallikarjuna (2014) illustrated the presence of cytotoxic principles of methanolic callus extract of *Biophytum sensitivum*. Mosquito serve as crucial vectors for a number of arboviruses. The mosquito *Culex quinquefasciatus, Aedes aegypti* acts as a vector for various endemic, disabling and disfiguring diseases (Kalu *et al.* 2010). The discovery and development of synthetic organic chemicals with persistent residual action and also become the major weapon for mosquito control. But the extensive use of synthetic organic insecticides has resulted in environmental hazards and also in the developmental of physiological resistance in vector species. With this knowledge, the present study was intended to examine the antioxidant, cytotoxicity, larvicidal potentials of *B. sensitivum* DC.

Materials and Methods

The mature and healthy plants of *Biophytum sensitivum* DC (Oxalidaceae) were collected from Cheranmahadevi, Tirunelveli district, Tamil Nadu, India. The collected plant was identified by Dr. M. Johnson, Assistant Professor, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai and the specimen was deposited in the Xavier's College Herbarium (XCH). After collection, the whole plant was dried in shade for two weeks and weighed. It was then ground using blender to get powder. The ground-dried powder of *B. sensitivum* (10g) was sequentially extracted with petroleum ether, chloroform and methanol using soxhlet apparatus for 8 h. For aqueous extracts the dried powder were boiled with water for 24 h continuously. The supernatants were filtered and make up to known volume for bio-efficacy studies.

Determination of total phenolics

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003).

DPPH radical scavenging activity

The antioxidant activityof different extracts were determined in terms of hydrogen donating of radical scavenging ability using the stable radical DPPH, according to the method of Brand-Williams *et al*. (1995).

Phosphomolybdenum assay

The total antioxidant activity was evaluated by the green phosphomolybdenum complex formation according to the method described by Prieto *et al.* (1999). The results are mean values expressed as g of ascorbic acid (AA) equivalents/ 100 g extract.

Scavenging of hydrogen peroxide

The ability of extracts to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*. (1989). The percentage inhibition activity was calculated using the formula

$$\% \text{ scavenging activity} = [(\text{Control OD} - \text{sample OD}) / \text{Control OD}] * 100$$

Larvicidal activity

Culex quinquefasciatus (IV instar larvae) were collected from in and around our college sewage tanks, Palayamkottai, Tirunelveli district, Tamil Nadu, India. These larvae were brought to the laboratory and transferred to 18 × 13 × 4 cm size enamel trays containing 500 ml of water maintained in the laboratory. ***Culex quinquefasciatus*** was maintained at 27 ± 2°C, 75–85% RH and 14L: 10D photoperiod cycles. Fourth instar larvae of ***Culex quinquefasciatus*** were transferred in 250 ml glass beaker containing desired plant extracts concentration such as 50, 100, 150, 200 and 250 mg/l. The plant extracts were disolved with acetone for the larvicidal activity. Each and every experiment was performed with five replicates and repeated thrice. 1% acetone was used as negative control. The standard larvicide Temephos (Abate®) was used as positive control. The control mortality was corrected by Abbott's formula and LC50, LC90 regression equation and 95% confidence limit of lower (LCL) and upper confidence limits (UCL) were calculated by using probit analysis.

Cytotoxic activity

In a samll tank, the artificial sea water (38 g NaCl/1000 ml tap water) was taken and *Artemia salina* (shrimp) eggs were added to one side of the tank. The shrimps were allowed for 48 hrs to hatch and mature as nauplii. The hatched shrimps were taken for bioassay. *Biophytum sensitivum* extracts were taken in different concentrations (100, 200, 300, 400 and 500 mg/10 ml) to the sample tubes. The extracts were dissolved with DMSO for the cytotoxic activity. With the help of a Pasteur pipette 10 living shrimps were dropped into each test tube. Control group was added in cytotoxic activity to validate the test method and result obtained due to the cytotoxic activity of the test agent. After 24 hours, the tubes were inspected using a magnifying glass and the number of survived nauplii in each vial was counted and observations were recorded for each vial. Each and every experiment was performed with five replicates and repeated thrice. Using the recorded observations LC_{50}, 95% confidence limit, LC_{90}, and chi square values were calculated. The standard plumbagin was used as positive control.

Results

Total Phenolics

The occurence of total phenolic content of *Biophytum sensitivum* as follows methanol (1399.84 ± 215.79 mg GAE/g) > acetone (743.97 ± 52.11 mg GAE/g) > aqueous (310.79 ± 27.26 mg GAE/g) > chloroform (213.0 ± 38.94 mg GAE/g) > petroleum ether (66.83 ± 6.33 mg GAE/g).

DPPH radical scavenging activity

The results on free radical scavenging activity of the different extracts of *B. sensitivum* were illustrated in table 1. The absorbance decreases as a result of a color change from purple to yellow as radical is scavenged by antioxidants through donation of hydrogen to form the stable DPPH molecule. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was determined. Therefore, lower value of IC_{50} indicates a higher antioxidant activity. The best free radical scavenging activity of *B. sensitivum* was observed in acetone extracts with IC_{50} value 30.12µg/ml (Table 1). The free radical scavenging activity of *B. sensitivum* was exerted as follows acetone > methanol> chloroform > petroleum ether extracts.

Phosphomolybdenum assay

The results of phosphomolybdenum assay of various extracts of *B. sensitivum* were demonstrated in table 1. The methanolic extracts of *B. sensitivum* displayed the strongest phosphomolybdenum reduction (202.24 ± 11.44 g AA/100 g) compared to other tested extracts. The phosphomolybdenum reduction of *B. sensitivum* was exerted as follows: methanolic extracts> chloroform > acetone > petroleum ether>aqueous extracts.

Scavenging of Hydrogen Peroxide

The concentrations of *B. sensitivum* extracts were directly proportional to the H_2O_2 scavenging activity of the *B. sensitivum* extracts and the result of H_2O_2 scavenging activity of the *B. sensitivum* was tabulated in table 1. Aqueous extracts of *B. sensitivum,* showed maximum inhibition (85.44%) followed by methanolic extracts with 69.19% of inhibition. Petroleum ether and chloroform extracts does not show the Hydrogen peroxide activity (Table 1).

Table 1: DPPH radical scavenging activity, Phosphomolybdenum assay and Hydrogen peroxide assay of various extracts of *B. sensitivum*

Extracts	DPPH IC_{50} (µg/ml)	Phosphomolybdenum assay (g AA/100 g extract)	% of H_2O_2 scavenging activity
Pet. Ether	**54.11**	**217.22** ± 14.24	-
Chloroform	**49.36**	**204.74** ± 11.72	-
Acetone	**30.12**	**209.73** ± 12.28	61.84 ± 2.88
Methanol	**30.23**	**202.24** ± 11.44	69.19 ± 2.37
Aqueous	**-**	**380.52** ± 15.34	85.44 ± 2.57

Larvicidal activity

The tested extracts of *B. sensitivum* showed moderate level of larvicidal effect after 24 hrs (Table 2). However the methanolic extracts of *B. sensitivum* showed highest larval mortality in terms of lethal concentrations for 50% mortality against ***Culex quinquefasciatus*** with LC_{50}=215.34 mg/ml followed by petroleum ether extracts with LC_{50}=267.31mg/ml (Table 2).

Table 2: Larvicidal activity of *B. sensitivum* against *Culex quinquefasciatus*

Extracts	LC_{50} (mg/ml)	LCL	UCL	χ2 (df)
Pet. Ether	267.31			6.138
Chloroform	279.95	237.07	391.39	4.546
Acetone	285.47	234.64	434.91	4.297
Methanol	215.34	150.18	1450.8	7.186
Aqueous	660.75			1.378

Brine shrimp bioassay

The increased mortality rate of brine shrimp was observed proportionally with the increasing concentration of the extract. The inhibitory effect of the extract might be due to the toxic compounds present in the crude extracts. The methanolic extract of *B. sensitivum* displayed most effective and 90% mortality (LC_{90}) of brine shrimp nauplii occurred at 66.34 mg/ml followed by petroleum ether with LC_{90}=240.39 mg/ml. The cytotoxic effect of *B. sensitivum* against the brine shrimp nauplii was showed as follows methanol > petroleum ether> acetone >aqueous extracts.

Discussion

Antioxidant based drugs have an pharmacological activity for the prevention and treatment of complex diseases like artery- clogging atherosclerosis, chronic diseases, Alzheimer's disease and cancer. Therefore, the researcher provides special attention to the plant derived antioxidants. The anti- cancer and anti- oxidant properties of the medicinal plants are depends on the presence of phenolic constituent's viz., phenolic acids, flavonoids, tannins, coumarins, lignins, quinnones, stilbenes and curcuminoids (Cai *et al.* 2004).

Phenols and phenolic compounds are greatly used in skin infections, wound healing, inflammation, antioxidant, immune enhancers, anti-clotting and hormone modulators (Hussain *et al.* 2011). The result of the present study showed that the methanolic extracts of *B. sensitivum* have the maximum amount of extractable total phenolics (1399.84 ± 215.79mg GAE/g). The results of the present study coincided with previous observations and supported the pharmacological evidences. It is further investigated through DPPH, phosphomolybdenum assay, hydrogen peroxide assay to validate the antioxidant and free radical scavenging potentials of *B. sensitivum*. Kalita *et al*. (2013) observed maximum frequency (43.96%) of inhibition of DPPH radical activity at a concentration of 110.46 µg/ml of *B. sensitivum* methanolic extract collected from Medinapur. In the present study we observed the best free radical scavenging activity with IC_{50} 30.120µg/ml of *B. sensitivum* acetone extracts than Kalita *et al*. observations. In addition, the aqueous extracts demonstrated efficient H_2O_2 scavenging activity with 85.44%. The methanolic extracts had the strongest phosphomolybdenum reduction (202.24 ± 11.44 g AA/100 g) compared to other tested extracts. The observed antioxidant activity may be due to the presence of higher effective chemical constituents such as steroids, phenolic groups, cardiac glycosides, flavonoids, saponins, tannins and sterols. The results of the present study suggest that *B. sensitivum* collected from Cheranmahadevi possess effective free radical inhibitor than Medinapur populations. Hence the results indicated that the Cheranmahadevi accession of *B. sensitivum* proved as superior species and *B. sensitivum* can act as a good scavenger of such harmful radicals.

The chemical constituents, present in the medicinal plants are toxic against pathogens. Such phytochemicals act as general toxicants like larvicides, oviposition attractants/deterrents, insect growth regulators, repellents and adulticides (Murthy and Rani, 2009; Kasturi Vadeyar *et al.* 2010). The results of the present study suggest that the methanolic extracts of *B. sensitivum* against ***Culex quinquefasciatus*** having the highest larval mortality (LC_{50}=215.34 mg/ml) in terms of lethal concentrations for 50% mortality. It could be helpful to be applied in integrated control strategies to gain maximum impact on vector (mosquitoes) control.

Cytotoxic is the ability of a product to kill cells, which is an important property of anti-cancer therapeutics that works by killing specific tumour cells. Guruvayoorappan and Kuttan (2007) significantly enhanced antibody dependent cellular cytotoxicity (ADCC) and antibody dependent complement mediated cytotoxicity (ACC) in both *Biophytum sensitivum* treated normal as well as tumor bearing animals. Chandra Kala and Mallikarjuna (2014) reported significant cytotoxic activity (ED50 value- 44.85 µg/ml) of methanolic *B. sensitivum* callus extract. Similar to Chandra Kala and Mallikarjuna the methanolic extract of *B. sensitivum* collected from cheranmahadevi displayed most effective and 90% mortality (LC_{90}) of brine shrimp nauplii occurred at 66.34 mg/l. The results suggest that the *B. sensitivum* collected from cheranmahadevi possess more amounts of metabolites.

Conclusion

The present investigations suggest that methanolic and aqueous extracts showed a good result of antioxidant activity. It was found that the high rates of phenolic substances widely distributed in *B. sensitivum* collected from cheranmahadevi. From the results, it is concluded that the *B. sensitivum* can explored as potent source of anti-cancer agent.

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