Original Article

In vitro pharmacological investigations of *Biophytum sensitivum* callus extract: Lack of potent activities

Sirigiri Chandrakala, Kokkanti Mallikarjuna, Challa Siva Reddy[1]

Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur, [1]Department of Pharmacology, KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, India

Abstract

Objective: *Biophytum sensitivum* is an important medicinal plant extensively used in traditional oriental herbal medicines. Though medicinal use of this plant is known, the active principles responsible such property is not known. Pharmacological screening of this plant may lead to discovery of new activity with new mode of treatment. Hence, screening for *in-vitro* pharmacological activities of methanolic callus extracts of *Biophytum sensitivum* has been carried out. Experiments were designed according to the standard methods and processes. **Materials and Methods:** Leaf cutting derived callus on MS medium supplemented with BA (Benzyl adenine) 1.0 mg/l + NAA (1-naphthaleneacetic acid) 1.0 mg/l is used as a source, and compounds were extracted from dried callus using methanol solvent with Soxhlet apparatus. **Results:** The callus extract has shown antioxidant activity, *in-vitro* inhibition of enzyme activities like α-glycosidase, acetyl cholesterase, and tyrosinase, but potency was found to be low. The Graph pad Prism Version-5 software is used to analyze data in the form of Figures. **Conclusion:** For the first time, we are reporting *in-vitro* pharmacological screening of methanolic callus extracts of *Biophytum sensitivum*.

Key words: Acetyl cholinesterase, anti-oxidant, *Biophytum sensitivum* callus extracts, Ttyrosinase, α-glucosidase

INTRODUCTION

Biophytum sensitivum (Linn.) DC. (Syn.*Oxalis sensitiva* Linn.) is a small annual herb belonging to the family Oxalidaceae. It grows in shady places in dry parts of India during the rainy season. It is extensively used in traditional oriental herbal medicines.[1] *Biophytum sensitivum* flower is used in athapoo, a special floral formation, that adores courtyards and public places during Onam festival in Kerala. Since the plant is reputed for its folkloric uses in various diseases,[2] it draws our attention for it's *in-vitro* pharmacological screening.

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Plant tissue culture technology has resulted in the production of many pharmaceutical substances for new therapeutics. Successful attempts to produce some of these valuable pharmaceuticals in relatively large quantities by cell cultures are illustrated. The pharmacological drugs are either too expensive or have undesirable side effects contraindications.[3] Pharmacologically, the *Biophytum* plant has been investigated for its hypoglycemic,[4] anti-inflammatory,[5] hypocholesterolemic,[6] and anti-cancer effect.[7] It is a known traditional remedy for the treatment of diabetes[8] and anti-tumor activities.[9] It possess a wide spectrum of medicinal properties for asthma, snakebites, stomachalgia and phthisis, positive effects in inflammatory diseases and antioxidant activity,[10] and leaf extracts with anti-bacterial activity.[11]

Address for correspondence:
Dr. K. Mallikarjuna, Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, India.
E-mail: mallikarjunaanu@gmail.com

Biophytum sensitivum extracts lowered blood sugar on streptozotocin and nicotinamide-induced diabetes in rats.[12]

Though the mature wild plant has been screened for various pharmacological activities, *in-vitro* screening for various pharmacological activities using callus is lacking. As the *in-vitro* callus represent good source of secondary metabolites, screening of such callus may give insight into the presence of unknown compounds with new activity.[13] Hence, the present study was undertaken to scientifically investigate various *in-vitro* pharmacological activities like antioxidant and inhibition assays of α-glucosidase, acetyl cholinesterase and tyrosinase of methanolic callus extracts of *Biophytum sensitivum*. Our study revealed that, indeed it shows antioxidant and inhibition activities of various enzymes indicating that it can be explored as antioxidant chemicals.

MATERIALS AND METHODS

Preparation of plant material

The fresh matured plants (100 no.) of the *Biophytum sensitivum* collected from A.N.U Campus, Guntur District were used as a source of explants. The leaf explants were excised into 1 cm long segments and were washed with liquid detergent (5% Teepol, Qualigens, India) followed by Bavistin (1% w/v) for 3 min, after that continued washing with mercuric chloride (0.1% w/v) for 1 min. Finally, the explants were washed with 70% ethanol followed by 3 times washing with sterile distilled water, and the explants were aseptically inoculated on Murashige and Skoogs[14] medium supplemented with various concentrations and combinations of phytohormones for induction of callus.

Callus culture

The explants were cultured on MS (Murashige and Skoog)[15] basal medium supplemented with various concentrations of BA (Benzyl Adenine) (0.5-5.0 mg/l) + NAA (1-naphthaleneacetic acid) (0.5-5.0 mg/l) for callus induction. BA 1.0 mg/l + NAA 1.0 g/l is the best concentration for callus induction [Figure 1]. After 30 days, old callus was collected and sub-cultured on to fresh medium with same growth regulator combinations twice in 4-week time interval. All the cultures were incubated at 24 ± 2° C under 16 h photoperiod provided by cool white florescent lights.

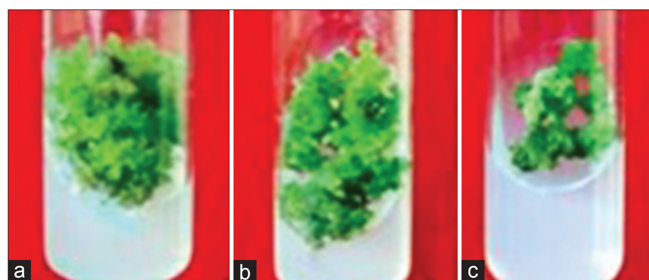

Figure 1: (a, b) *In-vitro* regenerated callus of *Biophytum sensitivum* after eight weeks of culture with BA 1.0 mg/l + NAA 1.0 mg/l. (c) Callus after six weeks of culture with BA 5.0 mg/l + NAA 5.0 g/l

Extraction from callus cultures

About 6-8 week-old calli derived from the leaf cuttings were collected and dried in an oven at 40 ± 1°C for 5 hours [Figure 1a, b and c]. Dried calli was homogenized to a fine powder and stored in airtight bottles. 25 g of leaf calli powder were extracted with 150 ml of solvent methanol for 24 h by using Soxhlet apparatus (Borosil, India). The extract was dried in a flash evaporator for 30 min, and the left over powder was considered 100%. 100 mg/ml were prepared by re-dissolving the extracted powder in the same solvent, which was used in the extraction. This crude callus extract is used for pharmacological *in-vitro* analyzes.

In-vitro methods

DPPH free radical scavenging activity

DPPH (1, 1-diphenyl-2picryl-hydrazyl) free radical scavenging of test compounds was determined by the method of Lamaison *et al*., (1991),[16] which depends on scavenging of colored free radical (DPPH) in methanol solution by the test drugs. The reaction mixture was prepared using DPPH and test drugs in final concentrations of 3 ml. Absorption of DPPH at its adsorption maximum 516 nm is inversely proportional to the concentrations of the scavenger (Test drug). The activity was expressed as inhibitory concentrations 50 (IC50) i.e., the concentration of the test solution showed 50% reduction in absorbance of the test solution as compared to that of blank solution.

$$IC50 = [(\text{OD of control} - (\text{OD of test} - \text{OD test blank})) \div \text{OD of control}] \times 100$$

DPPH Free radical scavenging activity inhibition was calculated using following formula.

$$\%\ \text{inhibition} = \left[\frac{\text{Control O.D.} - \text{Test O.D.}}{\text{Control O.D.}}\right] \times 100$$

α- glucosidase inhibition assay

α-glucosidase inhibitory activity was determined according to method of Padmanabha Rao and Jamil (2011).[17] In a microplate well, 50 μl of enzyme (0.4 U/ml) was taken, to this, 90 μl of 100 mM

phosphate buffer pH 7.0 and 10 µl test substances was added and mixed well. The reaction mixtures was incubated at room temperature for 5 min and 50 µl of p-Nitro phenyl α-D- glucosidase (20 mM) as substrate was added, mixed well, and incubated for 15 min at room temperature. The reaction was stopped by the addition of 30 µl of sodium carbonate solution (200 mM). The absorbance was measured at 405 nm using microplate reader. Control and test blank OD's were obtained by replacing enzyme with buffer.

α- glucosidase inhibition was calculated using following formula.

$$\% \text{ Inhibition} = \left[\frac{\text{Control O.D.} - \text{Test O.D.}}{\text{Control O.D.}}\right] \times 100$$

Estimation of acetylcholinesterase assay

The acetylcholinesterase activity was determined using photometric method as described by Ellman *et al*., (1961).[18] Acetylthiocholine substrate is hydrolyzed by AchE in the sample and forms thiocholine, which will react rapidly and irreversibly with 56-thio-bis-nitro benzoic acid (DTNB) producing a yellow anion of 5-thio-2 nitro benzoic acid. The increase in color intensity was measured specrophotometrically at 412 nm. Twenty micro liters of 0.075 M acetylcholine iodide was added to the reaction mixture in the cuvette and mixed well and the absorbance was for 5 min at an interval of 15 seconds each, the change in absorbance per minute (ΔA) was calculated (Lowry *et al*. 1951).[19]

AChE activity was calculated using following formula.

$$5.74 \times 10^{-4} \times \left[\frac{\Delta A}{\text{Protein content of the sample}}\right]$$

Tyrosinase inhibition assay

Tyrosinase inhibition assay was carried out according to the method of Ohguchi and Tanaka (2003).[20] The assay mixture contained 250 µl of enzyme, 50 µl of drug carrier, and 1250 µl of 8 mM M L - Dopa. The reaction was started by addition of substrate. Then, incubated for 1 min, the activities were measured at 475 nm. In the inhibition studies, the activities were measured in the presence of various concentrations of test substances. All the assays were performed in duplicate or triplicate.

Tyrosinase inhibition was calculated using following formula

$$\% \text{ Inhibition} = \left[\frac{\text{Control O.D.} - \text{Test O.D.}}{\text{Control O.D.}}\right] \times 100$$

RESULTS

DPPH – inhibition assay

For determining the antioxidant potential (DPPH radical scavenging assays) of *B. sensitivum*, the stock solution (100 µg/ml) of methanol callus extract was prepared. From this stock solution, different dilutions (25, 50,100, 200 µg/ml) of extracts were prepared in methanol and water and were taken in 4 different test tubes, compared with standard vitamin – C (1, 2.5, 5, 10 µg/ml). Experiments on antioxidant potential of *B. sensitivum* revealed that the percentage of DPPH inhibition increased with increase in the concentration, highest percentage of inhibition was observed at 27.13 µg/ml [Figure 2]. The IC50 value of *B. sensitivum* was 736.5 µg/ml, indicating the less degree of *in-vitro* antioxidant potential of *B. sensitivum* when compared with control vitamin C [Figure 3]. Vitamin-C showed highest percentage of inhibition of DPPH at 160, even at 10 µg/ml.

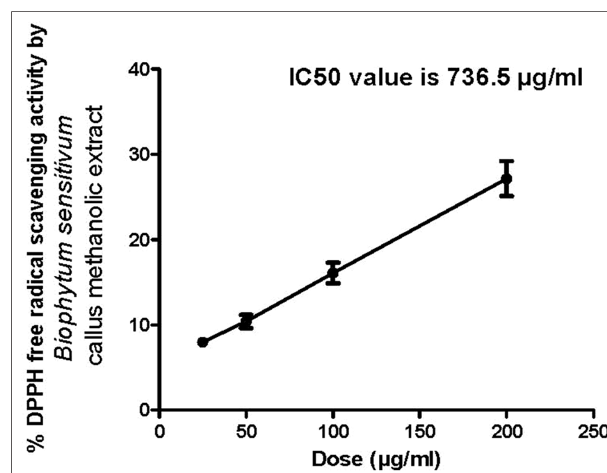

Figure 2: DPPH scavenging activity of *B. sensitivum* callus methanol extract

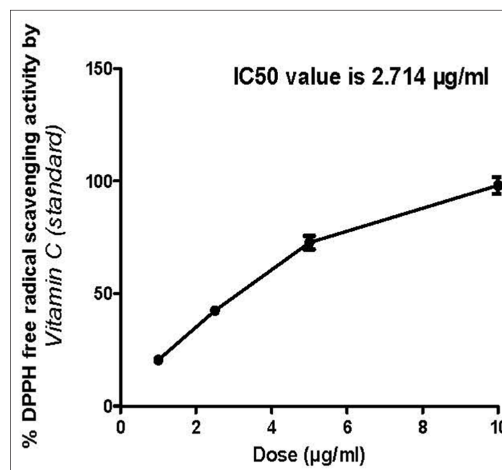

Figure 3: DPPH scavenging activity by vitamin C (standard)

α- Glucosidase inhibition assay

The present study was to investigate *in-vitro* α-glucosidase inhibition at different concentrations: 37.5, 75, 150, 300 μg/ml of extract percentage of inhibition were 0.47, 4.25, 12.99, 30.02 in comparison to Acarbose (DMSO) at 10 ng/ml of using standard. The extract is found to be active at a concentration (300 μg/ml) and exhibited 30.02% inhibition. The crude extract has α- glucosidase with IC 50 value is 504.8 μg/ml [Figures 4 and 5].

Estimation of acetylcholinesterase

The acetylcholinesterase activity carried out according to the method of Ellman *et al*. 1961. The AChE inhibitory activity of methanol crude extract at concentrations of 100, 250, 500, 1000 ng/ml was investigated and compared to the Neostigmine (Standard) [Figure 6]. This extract possessing AChE inhibitory activity with IC 50 value is 1587 ng/ml [Figure 7] while IC50 value of standard drug is 38.93 ng/ml.

Tyrosinase inhibition assay

Plant extracts were screened for tyrosinase inhibition assay, was carried out at 4 different concentrations of 25, 50, 100, 200 μg/ml, then the percentage of inhibition were observed respectively as 8.62, 14.39, 23.32, and 42.13% with an IC 50 values 280.5 μg/ml [Figure 8]. In comparison, the IC 50 for Resveratrol, which is used as a positive control for tyrosinase inhibition, was 8.52 μg/ml [Figure 9]. For the first time, we report tyrosinase inhibitory activity of *Biophytum sensitivum*.

DISCUSSION

It is well-known that, *in-vitro* cultures are able to produce secondary metabolites in quantities more than that of original parts of the plants.[21] In the past few decades, secondary metabolite production from plant tissue culture has been identified as a tremendous resource for new drug development and clinical research in the fields of pharmacology and medicine.

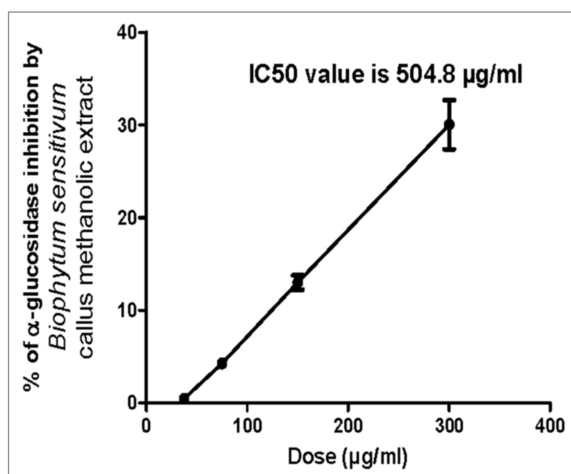

Figure 4: α-glucosidase inhibition of *B. sensitivum* callus methanol extract

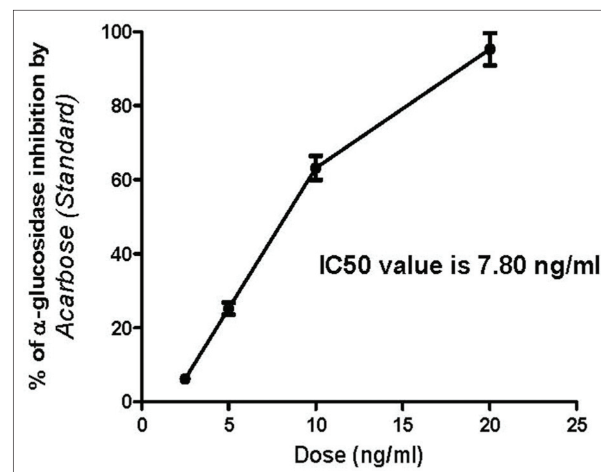

Figure 5: α-glucosidase inhibition by acarbose standard

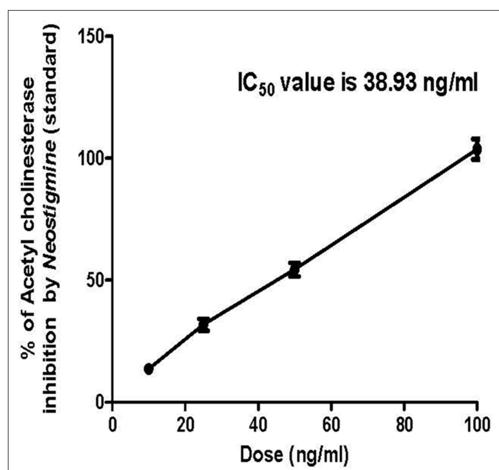

Figure 6: Acetyl cholinesterase inhibition of Neostigmine

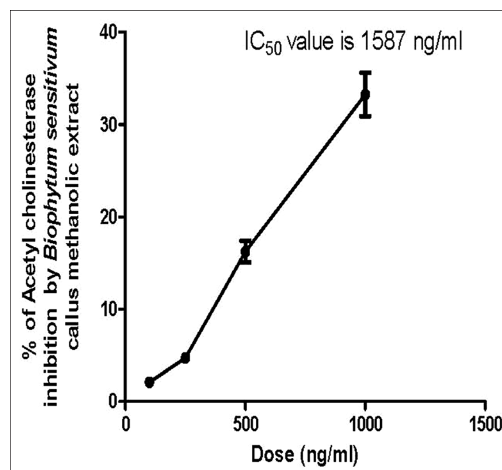

Figure 7: Acetyl cholinesterase inhibition of *B. sensitivum* callus extract

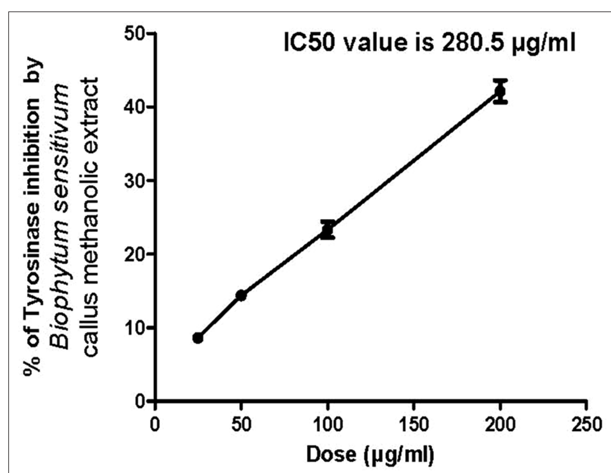

Figure 8: Tyrosinase inhibition of *B. sensitivum* callus extract

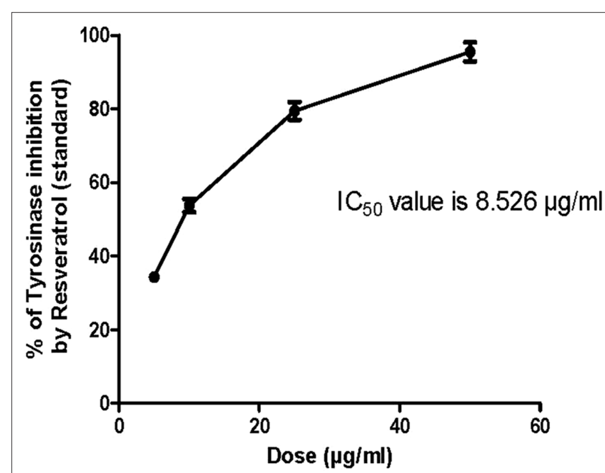

Figure 9: Tyrosinase inhibition of resveratrol

Callus culture is one of the main biotechnological approaches in the production of medicinal compounds from plants.[22] Plant cell culture extracts have also been used widely in the form of fractions and isolated compounds as potential bioactive molecules. *In-vitro* studies are highly instrumental in selecting a drug for a particular disease and also in getting the preliminary evidence to proceed for further *in-vivo* pharmacological research. Hence, the study is designed to screen and identify the therapeutic suitability of this plant extract for the treatment of a particular disease.

Therefore, in this study, antioxidant, α-glucosidase, acetylcholesterase, tyrosinase activity evaluated to determine the medicinal values. Leaf extract of *Biophytum sensitivum* used in traditional Nepalese folk medicine for the treatment of hyperglycemic patients.[4] Our *in-vitro* enzymes studies revealed that *Biophytum sensitivum* callus extract inhibits the –α-glucosidase, but the potency of this callus extract less, when compared to the standard drug, acarbose. The present study clearly indicates that the active principles in this plant extract may be helpful in preventing the glucose absorption in the gastrointestinal tract of diabetic patients. This could be an additional evidence to validate the use of this plant extract in addition to the previous findings[14] suggest that the hypoglycemic response of *B. sensitivum* may be mediated through stimulating the synthesis/release of insulin from the beta cells of Langerhans. Plant extracts were screened for tyrosinase inhibitory activity.[23]

Furthermore, flavonoids and other phenolic compounds have been shown to possess anti-cholinesterase properties[24] as observed in the case of *Biophytum sensitivum* callus extracts (un published data). The results elucidated that the inhibitory potential of *Biophytum sensitivum* against AchE was comparatively lower. This is because crude extracts contain non-active components along with active ones. Therefore, to isolate the active compounds from this plant, callus will help to identify the potent natural inhibitors of AchE. The presences of flavonoids possess anti-allergic, anti-inflammatory, anti-viral, and anti-oxidant activities.[25]

For the first time, we report in-vitro pharmacological screening for methanol extract of callus *Biophytum sensitivum*. Though callus extract has shown *in-vitro* inhibition of enzyme activities like α-glucosidase, acetyl cholinesterase, and tyrosinase, the potency (IC_{50}) was found to be low including with antioxidant activity. The occurrence of antioxidant activity may be due to the presence of phenolic compounds in the methanol callus extract. The reason for low antioxidant activity of *B.sensitivum* callus extract could be due to presence of phenol compounds in low concentration. This type of results previously reported that media with NAA concentration from 0.5 to 1.0 mg/ml had adverse effect where antioxidant activity was to be low (10-15%), as reported in the case of *Ipomea aquatica* leaf callus antioxidant activity.[26]

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