

## Research Article



## ***In Vitro Analysis of Cytotoxicity and 5-Lipoxygenase Inhibition Activity by Using Callus Extract of *Biophytum sensitivum* (L) DC.***

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### ABSTRACT

Objective of the study is to screen for *in vitro* pharmacological activities of 5-Lipoxygenase and cytotoxicity of using methanolic callus extracts of *Biophytum sensitivum*. *In vitro* Brine shrimp lethality assay was carried out according to the method of Meyer *et al.*, (1982) and the lipoxygenase inhibition activity was assayed using photometric method as described by Reddanna *et al.*, (1985). The callus extract has shown 5-Lipoxygenase potency was found to be low. The Graph pad Prism Version-5 soft ware is used to analyze data in the form of figures. Fenny probed analysis software is used in Brine shrimp lethality assay to obtain ED<sub>50</sub> values. But important finding in this study was that the extract has shown significant and remarkable cytotoxicity. The callus extract has shown 5-Lipoxygenase potency was found to be low. This callus extract deserves further evaluation to screen *in vivo* anti-cancer activity.

**Keywords:** *In vitro* pharmacological activity, *Biophytum sensitivum* callus, Cytotoxicity, 5- Lipoxygenase.

### INTRODUCTION

**M**edicinal plants play a vital role for the development of new drugs. Although very few plant cell processes are operating commercially, the most successful commercial pharmaceuticals produced from undifferentiated cell cultures are anti-biotic compounds.<sup>1</sup> *Biophytum sensitivum* is an important medicinal plant extensively used in traditional oriental herbal medicines. Though the mature wild plant has been screened for various pharmacological activities but *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.<sup>2</sup> Hence the present study was undertaken to scientifically investigate various *in vitro* pharmacological activities like cytotoxicity and 5-lipoxygenase of methanolic callus extracts of *Biophytum sensitivum*. Our study revealed that, it can be explored as good source of cytotoxic and 5-lox inhibition chemicals.

### MATERIALS AND METHODS

#### 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 three times washing with sterile distilled water and the explants were aseptically inoculated on Murashige and Skoog medium supplemented with various

concentrations and combinations of phytohormones for induction of callus.

#### Callus culture

The explants were cultured on MS (Murashige and Skoog, 1962)<sup>3</sup> 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 mg / l is the best concentration for callus induction. After 30 days, old callus was collected and sub cultured on to fresh medium with same growth regulator combinations twice in four week time interval. All the cultures were incubated at 24 ± 2 °C under 16 h photoperiod provided by cool white fluorescent lights.

#### 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 (Image A, B and C). Dried calli was homogenized in to a fine powder and stored in airtight bottles. 25g of shoot 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* analysis.

#### *In vitro* methods

##### **Brine Shrimp Lethality assay**

Brine shrimp lethality assay was carried out according to the method of Meyer *et al.*, (1982).<sup>4</sup> Brine Shrimp (*Artemia salina*) nauplii were hatched in sterile brine solution (sea salt 38 g / l pH 8.5) under constant aeration for 38h. After hatching, 10 nauplii were placed in each



vial and added various concentrations of callus extract in a final volume of 5 ml, maintained at 37° C for 24 h under the light of incandescent lamps and surviving larvae were counted.<sup>5</sup> Each experiment was conducted along with control at various concentrations of the test substances. Percentage lethality was determined by comparing the mean surviving larvae of test and control tubes. The result of the test compound was compared with the positive control Podophyllotoxin.

#### 5-Lipoxygenase inhibition assay

The lipoxygenase inhibition activity was assayed using photometric method as described by Reddanna *et al.*, (1985).<sup>6</sup> The assay mixture contained 2.97 ml of 50 mM phosphate buffer (pH 6.3), 5µl of 80 mM Linoleic acid and sufficient amount of potato 5-Lipoxygenase enzyme. The enzyme solution was stored in ice and controls were measured at intervals throughout the experimental period to ensure that enzyme activity was constant. The reaction was started by the addition of substrate (Linoleic acid) and the increase in UV absorption at 234 nm was followed at 25° C. The activities were measured at every 2 minutes. The reaction was linear during this time period. In the inhibition studies, the activities were measured in the presence of various concentrations of extract.<sup>7</sup> All the assays were performed in duplicate or triplicate. The percent inhibition of 5-Lipoxygenase activity was calculated as follows:

5-Lipoxygenase was calculated using following formula.

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

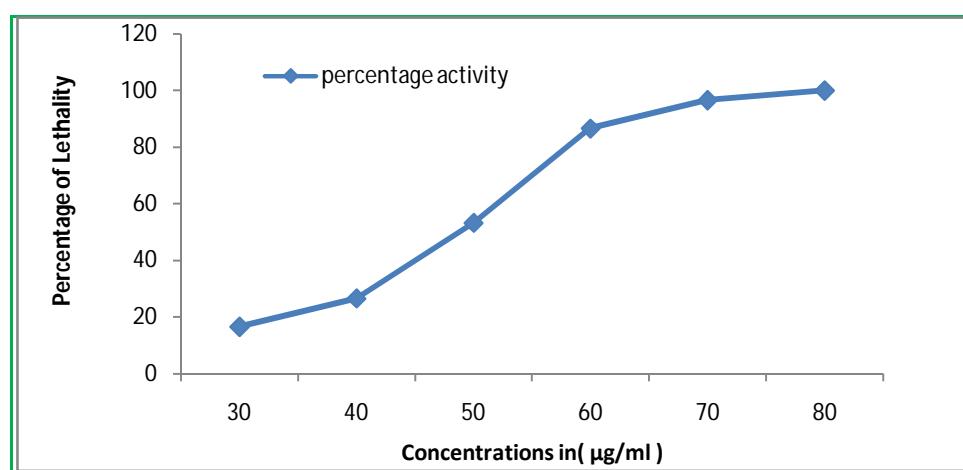
#### RESULTS AND DISCUSSION

##### Brine Shrimp Lethality Assay

Following the procedure of Meyer *et al.*, (1982)<sup>4</sup> the lethality of crude methanol extract of *Biophytum sensitivum* to Brine Shrimp was determined on *Artemia salina* after 24 hours of exposure of the samples and the positive control was Podophyllotoxin. The test samples (extract) were prepared by dissolving in water to attain concentrations like 30, 40, 50, 60, 70, 80 µg / ml (Table – 1). Then matured shrimps were applied to each of all experimental and control vials. ED<sub>50</sub> values were obtained using Fenny probed analysis software with increase in the concentration of extracts the percentage of lethality also increased (Figure 1) and the ED<sub>50</sub> value was 44.85 µg / ml (Table – 1) for experiments where as the control podophyllotoxin showed ED<sub>50</sub> value at 3.77 µg / ml (Table – 1). This indicated the presence of cytotoxic principles of this extract.

**Table 1:** Brine shrimp lethality bioassay of methanol callus extract of *Biophytum sensitivum*

Test substance	Solubility	ED <sub>50</sub> (µg/ml)	Degrees of freedom	UCL	LCL
Methanol callus extract of <i>Biophytum sensitivum</i>	water	44.85	5.61	48.01	41.42
Podophyllotoxin (Standard)	DMSO	3.77	3.07	4.54	2.99

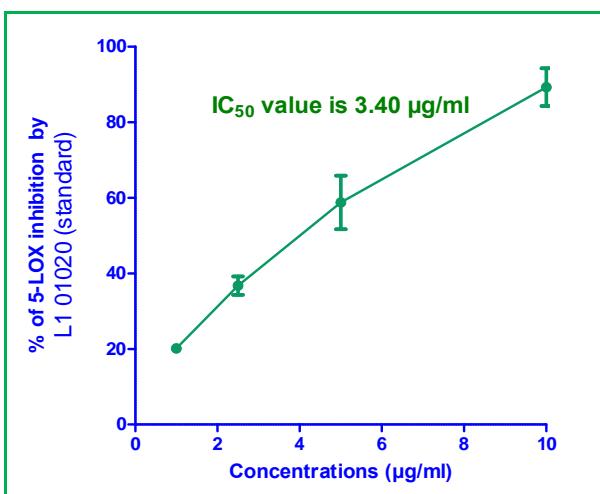


**Figure 1:** Brine shrimp lethality assay of methanol extract of *Biophytum sensitivum*.

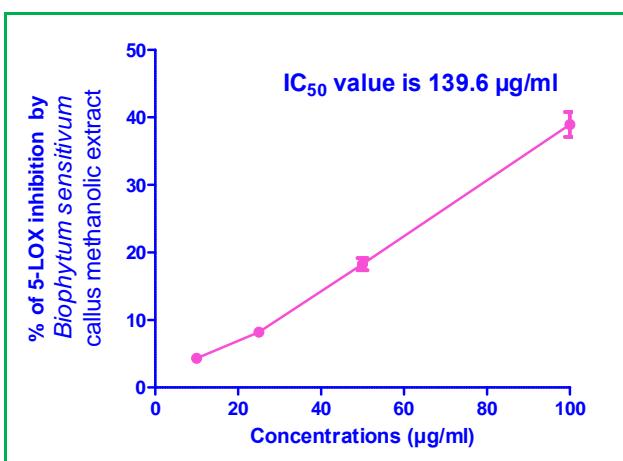
#### Lipoxygenase inhibition assay

The 5-Lipoxygenase inhibition assay of methanol crude extract at concentrations of 10, 25, 50, 100 µg / ml was investigated and compared to that of Zileuton, standard (Figure 2). The crude extract possessing 5-Lipoxygenase inhibitory activity with IC<sub>50</sub> value less than 139.6 µg / ml

while IC<sub>50</sub> value of standard drug, Zileuton is 3.40 µg / ml. The results elucidated that the inhibitory potential of *Biophytum sensitivum* against 5-Lipoxygenase inhibition was comparatively lower (Figure 3). For the first time, we report the 5-Lipoxygenase inhibition of methanol callus crude extract of *Biophytum sensitivum*.



**Figure 2:** 5-LOX inhibition by inhibition of *B.sensitivum* callus extract.



**Figure 3:** 5-LOX inhibition by inhibition of standard

Plant cell culture extracts have also been used widely in the form of fractions and isolated compounds as potential bioactive molecules.<sup>8</sup> The 5-Lipoxygenase inhibition assay previously reported in *Celastrus paniculatus* methanolic extract.<sup>9</sup> In the present study, methanolic callus extract of *Biophytum sensitivum* significantly inhibits 5-LOX *in vitro*. This provides evidence for the possible anti-inflammatory properties of the same. Our result is also in support with the Guruvayoorappan & Kuttan, (2008)<sup>10</sup> studies which demonstrated the methanolic extract of *Biophytum sensitivum* altered the cytokine profile and inhibits iNOS and COX-2 expression in LPS/ConA stimulated macrophages. Though anti-inflammatory activity of extracts of *Biophytum sensitivum* in carrageenan induced paw edema is well reported by Jachak, (1994)<sup>11</sup> anti-inflammatory properties in terms of 5-LOX inhibition may probably be useful in the treatment of respiratory disease states like asthma and chronic obstructive pulmonary disease (COPD). Active principles present in the methanolic callus extract of *Biophytum sensitivum* might be screened for asthma and COPD. Previously reported the *in vitro* pharmacological screening for methanol callus extracts revealed that lack of potent antioxidant,  $\alpha$ -glucosidase, acetylcholesterase and tyrosinase activities

of *Biophytum sensitivum*.<sup>12</sup> Though callus extract has shown *in vitro* inhibition of enzyme activities like, 5-lipoxygenase activity.

The brine shrimp lethality test is normally used to predict the presence of toxic bioactive compounds.<sup>13</sup> Hence this, analysis will lead to the discovery of new cytotoxic compounds. Our results further support the previous findings<sup>14</sup> that *Biophytum sensitivum* is a traditional oriental herbal medicine that is known for its immunostimulatory and antitumor effects.<sup>15</sup> Tumor metastasis is the most important cause of cancer death. In our study, methanolic *Biophytum sensitivum* callus extract has shown significant cytotoxic activity indicating that it can be selected for further cell line assay, because many scientists have shown a correlation between cytotoxicity against the brine shrimp nauplii by using extracts.<sup>16</sup> Although *Biophytum sensitivum* was shown to inhibit metastasis, the mechanism underlying this action is not well understood. In addition, it was demonstrated that *Biophytum sensitivum* inhibits tumor cell invasion and metastasis in lung tissue.<sup>17</sup> All the evidences support the strong anti-cancer potentiality of this plant extract.

Important finding in this study is the methanol extracts of *Biophytum sensitivum* has shown significant and remarkable cytotoxic activity. *Biophytum sensitivum* has shown to contain biflavones and flavonoids.<sup>18</sup> At present we do not know whether these compounds are responsible for the cytotoxicity produced by this extract. Further studies using isolated compounds are in progress.

## CONCLUSION

For the first time, we are reporting *in vitro* pharmacological screening of methanolic callus extracts of *Biophytum sensitivum*. In our study, methanolic callus extract has shown significant cytotoxic activity indicating that is can be selected for further cell line assay or *in vivo* anti cancer activity. Basing on the results, it is concluded that the *Biophytum* plants can explored as potent source of cytotoxic chemicals.

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