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Asian Journal of Plant Science and Research, 2014, 4(4):44-47

ISSN : 2249-7412
CODEN (USA): AJPSKY

Evaluation of cytotoxicity in selected species of *Caralluma* and *Boucerosia*

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ABSTRACT

Caralluma and Boucerosia are xerophytic succulent genus of Asclepiadaceae, with distribution in America, Africa, Asia, South Africa and North Western Europe. The present study was carried out on cytotoxicity of methanolic extracts of four species of Caralluma CAA, CAF, CS, CSL and two species of Boucerosia BL and BU by brine shrimp lethality assay for preliminary screening of toxicity. All the six species have shown variability in their ED_{50} values. ED_{50} means 50 % mortality observed in brine shrimps. ED_{50} value of positive control podophyllotoxin was 4.01. The extract showing the lowest ED_{50} value was BU (39.95 µg/ml) followed by CAF (40.64 µg/ml) and CAA (42.46 µg/ml) were significantly active and had potential for further study. Cytotoxicity screening methods provided important preliminary data to select plant extracts with potential antineoplastic properties.

Keywords: *Caralluma*, *Boucerosia*, Cytotoxicity, Brine shrimp lethality

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INTRODUCTION

The natural compounds derived from medicinal plants may serve as source for development and production of modern chemotherapeutic agents. In order to minimize the poisonous effects and ensure safe utilization, the demand for research on cytotoxic studies of natural products has been increased. The toxic nature of plant body depends upon dose, absorption, detoxification and excretion [1] and their dose depends on the stage of plant growth, the environment, season and parts of plant used.

Brine shrimp lethality is a rapid general bioassay for identifying toxic dose of a bioactive compound. Preliminary screening of bioactive natural products can be conveniently monitored by *in vivo* lethality in a zoological organism namely *Artemia salina* (Leach). The selected plants are screened for their cytotoxic effect on *Artemia salina* and correlate results of toxicity with known ethnopharmacological activities. For the assessment of cytotoxicity, brine shrimp lethality assay is given preference over whole animal bioassays and cell line assays [2]. The advantages of brine shrimp lethality assay include rapid, cost effective, no need of special equipment and animal serum. The assay can be performed with small amount of sample (less than 20 mg), without any objection from animal right advocates to the use of these invertebrates for the experiment [3].

The assay is based on their ability to kill larvae of brine shrimp and became a useful tool for preliminary screening of toxicity [4]. The assay was used to study the toxic effect of fungi [5], plant extracts [6], heavy metals [7], cyanobacteria [8], pesticides [9] and dental material [10]. The ethanolic root extract of *Calotropis gigantea* [11], methanolic extract of *Calotropis procera* [12] and plant extract of *Phyllanthes engleri* exhibited potent cytotoxic potential almost similar to standard anticancer drug cyclophosphamide and they can be used for the development of a new cancer drug. From pharmacological point of view, brine shrimp lethality assay can be used successfully to detect antitumor phytoconstituents in terrestrial plant extracts. Published data on cytotoxicity studies revealed that there is a good correlation between the two assays, brine shrimp lethality assay and bioactivity against tumor cell lines [13] as well as brine shrimp lethality and hepatotoxic activity [14]. Englerin, a selective anticancer drug against kidney cancer cells was isolated from *Phyllanthes engleri*, predicted by brine shrimp test [15].

Based on above literature, the present comparative study was carried out on cytotoxicity of methanolic extracts of four species of *Caralluma* and two species of *Boucerosia* by shrimp lethality assay for preliminary screening of toxicity.

MATERIALS AND METHODS

Caralluma R.Br. such as *Caralluma adscendens* (Roxb.) R. Brown var. *attenuata* (Wight) Grav. & Mayur. (CAA), *Caralluma adscendens* (Roxb.) R. Brown var. *fimbriata* (Wall.) Gravely & Mayur. (CAF), *Caralluma stalagmifera* C.E.C. Fisch. (CS) and *Caralluma stalagmifera* C.E.C. Fisch. var. *longipetala* Karupp. & Pull. (CSL) and as well as two species of *Boucerosia* Wight & Arn. such as *Boucerosia lasiantha* Wight. (BL) and *Boucerosia umbellata* (Haw.) Wight & Arn. (BU) were collected from Gooty, Tadipathri and Penukonda areas of Anantapur district and were taxonomically identified by comparing with Gamble flora [16] and other taxonomical literature, voucher specimens i.e. VM 46, VM 47, VM 48, VM 49, VM 50 and VM 51 were deposited in Montessori Mahila Kalasala, Vijayawada.

Brine shrimp toxicity screening

Cytotoxicity of the methanolic extracts of four species of *Caralluma* and two species of *Boucerosia* were determined by brine shrimp lethality bioassay.

Brine shrimp lethality bioassay was proposed by Michael *et al.*, [17] and modified by [18, 19 & 20] carried out to evaluate the cytotoxicity of medicinal plants of India. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a 1 L capacity conical shaped vessel filled with artificial sea water. The ASW was prepared by using the composition 38 g of sea salt dissolved in 1 L of distilled water and pH was adjusted to 8.5 using 1 N NaOH. Under constant aeration for 48 h and by providing direct light and warmth (24 - 26 ^{o}C) the active nauplii free from egg shells were collected from brighter portion of hatching chamber and used for assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. In each experiment, 0.5 ml of plant extract was added to the vial containing 4.5 ml of brine solution and maintained at room temperature for 24 hours under the light and surviving larvae were counted where as their shells were left in another side. Experiments were conducted along with control (vehicle treated) and different concentrations (1-5000 µg/ml) of the test substances in a set of three tubes per dose.

Lethality concentration determination

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values (ED_{50}) were obtained from the best fit line plotted concentration *vs*. percentage lethality. Podophyllotoxin was used as a positive control in the bioassay.

Statistical analysis

The percentage lethality was calculated from the mean survival larvae of extracts treated tubes and control. ED_{50} values were obtained by best fit line method.

RESULTS AND DISCUSSION

The selected species show minor variations in their morphological features at intervarietal and interspecific levels. Differentiation of methanolic extracts of four species of *Caralluma* and two species of *Boucerosia* such as CAA, CAF, CS, CSL, BL and BU were carried out on the basis of cytotoxicity activity determined by brine shrimp lethality assay. All the six species have shown variability in their ED_{50} values. ED_{50} means 50 % mortality observed in brine shrimps. ED_{50} values of CAA, CAF, CS, CSL, BL and BU were 42.46, 40.64 µg/ml, 189.91 µg/ml, 240.32 µg/ml, 179.40 µg/ml and 39.95 µg/ml respectively. ED_{50} value of positive control podophyllotoxin was 4.01 (Table 1). The extract showing the lowest ED_{50} value was BU (39.95 µg/ml) followed by CAF (40.64 µg/ml) and CAA (42.46 µg/ml) were significantly active and had potential for further study (Fig 1). Cytotoxicity screening methods provided important preliminary data to select plant extracts with potential anti neoplastic properties.

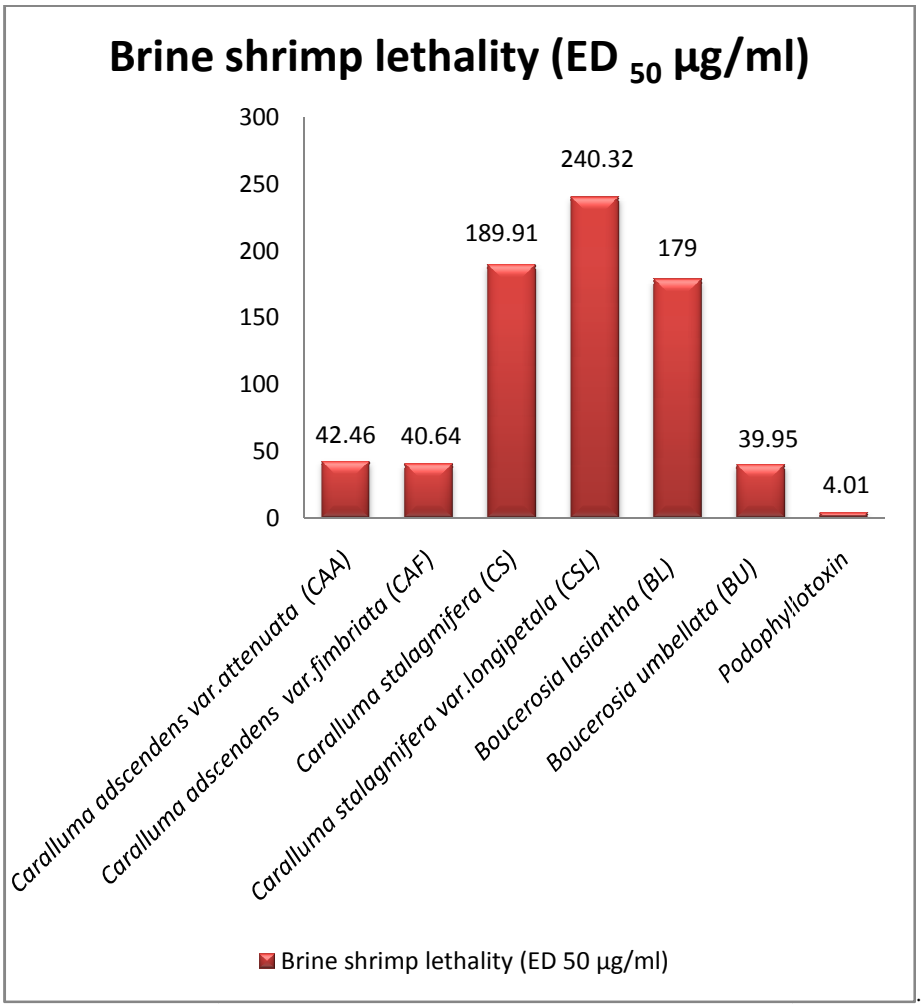
A general bioassay, brine shrimp lethality is an indicative of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions (Mc Laughlin *et al*., 1991). It has been observed that one-tenth ED_{50} values in the brine shrimp test are about ED_{50} values for general cytotoxicities (McLaughlin *et al*., 1991). Literature revealed that brine shrimp lethality assay has been used for the isolation of cytotoxic [21], antimalarial [22], insecticidal [23], and anti feedant [24] compounds from plant extracts. The petroleum ether extract of the of *Allophylus cobbe* L plant exhibited strong cytotoxicity in the brine shrimp lethality bioassay test [25]. All the vegetable extracts from Bangladesh showed considerable general toxicity towards brine shrimps and suggested to be good source of antibacterial and anticancer agents [26].

Table 1: Cytotoxicity activity of methanolic extracts of *Caralluma* and *Boucerosia* species by brine shrimp lethality assay

S.No	*Caralluma* and *Boucerosia* species	Brine shrimp lethality (ED_{50} µg/ml)
1	*Caralluma adscendens* var .*attenuata* (CAA)	42.46±0.63
2	*Caralluma adscendens* var. *fimbriata* (CAF)	40.64±0.75
3	*Caralluma stalagmifera* (CS)	189.91±1.21
4	*Caralluma stalagmifera* var. *longipetala* (CSL)	240.32+1.93
5	*Boucerosia lasiantha* (BL)	179±0.46
6	*Boucerosia umbellata* (BU)	39.95±0.72
7	Podophyllotoxin	4.01±0.01

**All test samples run in triplicates and one way ANOVA test was carried. Values are expressed as mean ± standard deviation (n = 3). The results of ANOVA analysis show significant differences (p<0.05) in the means of total cytotoxicity by means of effective dose value (µg/ml).*

Fig 1. Cytotoxic effect of selected species of *Caralluma* and *Boucerosia* by brine shrimp lethality assay



**ED_{50} indicates effective dose (µg/ml) of test sample required for 50 % lethality of brine shrimp larvae.*

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. The percentages of deaths and survivors of shrimps were calculated. During observation, if no mobility is observed externally and internally then larvae were considered to be dead. The dead larvae in each treatment was compared to that of control in order to confirm that mortality is not due to starvation but due to bioactive compounds in the plant extract. In any case, nauplii can survive up to 48 h without food because they still feed on yolk-sac. However, if control deaths were observed, then percentage mortality can be calculated by subtracting percentage of survivors in the treatment from percentage of survivors in the control.

CONCLUSION

In a reference survey, no more reports about the comparative study of cytotoxic activities of six species using brine shrimp lethality bioassay protocol of CAA, CAF, CS, CSL, BL and BU have been offered so for. The methanolic

extracts of BU, CAF and CAA were shown to possess a comparatively significant cytotoxicity activity in this study over the rest of the species CSL, CS and BU. The significant lethality of BU, CAF and CAA to brine shrimp lethality is an indicative of the presence of potent cytotoxic components which warrants further investigation.

Acknowledgement
Authors are gratefully acknowledged to the Chairman, Laila nutraceuticals, Vijayawada for providing necessary laboratory facilities

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