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Brine Shrimp Lethality Bioassay of *Careya Arborea* Leaves and Aerial Parts of *Cleome Viscosa*.

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ABSTRACT

The aim of present study was to evaluate in vivo brine shrimp lethality assay of the various fractions of Careya arborea leaves and Cleome viscosa aerial parts. Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). 10-15 larvae of brine shrimp were placed into three replicates of each concentration of various fractions of the selected plants and lethality was evaluated after 24 hrs. Results showed that none of fractions of C. arborea were found to be toxic upto a dose level of 1071 µg/ml. However, hexane and chloroform fraction of C. viscosa exhibited significant toxicity against brine shrimp larvae with LC_{50} values 155.54 µg/ml and 222.25 µg/ml respectively, while aqueous fraction exhibited weak cytotoxicity with LC_{50} values 516.36 µg/ml and ethyl acetate fraction was found to be non toxic with LC_{50} 1018.07 µg/ml. In the assay the positive control showed LC_{50} values less than 100 µg/ml. Thus, the results support the medicinal uses of these plants in traditional system.

Keywords: Brine shrimp, lethality assay, cytotoxicity, LC_{50}

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INTRODUCTION

Cleome viscosa Linn. (Capparidaceae), commonly known as wild mustard, is an annual, sticky herb found as common weed all over the plains of India and throughout the tropics of the world. In Ayurvedic system of medicine, the plant is considered to be diuretic, stomachic, laxative, anthelmentic and to cure earache, and ulcers [1, 2]. It is also reported to be useful in treatment of fever, skin diseases, leprosy, blood diseases and uterine complaints [3]. Ethnobotanically, the leaves are useful in healing the wounds, ulcers and seeds are useful in fever, diarrhoea, and convulsion [4, 5]. Cleome viscosa is highly effective in a wide spectrum of diseases and reported to possess analgesic, antidiarrheal, antipyretic, psychopharmacological, immunomodulatory and gastroprotective activity [6, 7]. The plant contains a number of medicinally active compounds including naringenin, β-amyrin, glucocapparin, glucocleomin and lupeol. A series of coumarino-lignans (cleomiscosins) have been isolated from the seeds [8].

Careya arborea commonly known as Wild Guava and Kumbhi in Ayurveda, is a medium sized deciduous tree, widely available in India, Sri Lanka, Malay, and Peninsula [9]. Ethnobotanically, the leaves are used to treat ulcer and its pulp used as poultice rapidly heal wound, ulcers and root is used for the treatment of tuberculosis and skeletal fractures [10, 11]. Leaves and flowers are used in the form of paste to cure several skin diseases. Traditionally, the bark has been reported to be used in the treatment of tumors, bronchitis, epileptic fits, abscesses and antidote to snake-venom [12, 13]. C. arborea is reported to possess analgesic, antidiarrhoeal, hepatoprotective, antitumor, CNS depressant, anticoagulant, in vitro cytotoxic and gastroprotective activity [14, 15]. Phytochemical investigation revealed the presence of careaborin-I, careyagenolide, triterpanoid sapogenols, β-amyrin, β-sitosterol and taraxerol in C. arborea leaf [13].

Due to wide application of these medicinal plants in traditional system of medicine, the current efforts therefore evaluate the safety and efficacy of different fractions of Careya arborea leaves and Cleome viscosa aerial parts against brine shrimp nauplii. The brine shrimp lethality assay has been used routinely in the primary screening of the crude extracts as well as isolated compounds to access the toxicity towards brine shrimp. Brine shrimp lethality assay consists of exposing larvae of Artemia salina to test sample in saline solution and lethality is evaluated after 24 hrs. The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs makes the assay a very useful bench top method [16].

MATERIALS AND METHODS

Plant material

Candidate plants were collected from their natural habitat. Leaves of Careya arborea plant was collected from Bauli jungal, Rewa district, India in the month of March 2010 and aerial parts of Cleome viscosa plant was collected from near around Kanpur, India, in the month of August 2010.

The plants materials were identified, authenticated taxonomically by Dr. Tariq Husain, Taxonomist, National Botanical Research Institute (NBRI), Lucknow, India and a voucher specimen of the plants were deposited in the herbarium section of departmental museum. The freshly collected plant materials to be used were further dried in tray drier under controlled conditions and finely powered with mechanical grinder avoiding elevation of temperature above 40°C.

Extraction and Fractionation

Dried and powered leaf of Careya arborea and Cleome viscosa aerial parts were exhaustively extracted with 70% alcohol for 24 hr (3 times) by maceration. The extracts thus obtained were filtered, concentrated using rotavapour and the concentrate was subjected to partitioning with hexane, chloroform and ethyl acetate. All the fractions were subjected to activity studies.

Hatching Brine shrimps

The Brine shrimp eggs were gifted by Aquatic Enterprise Co. Malaysia. The Artemia salina eggs were hatched in artificial seawater prepared by dissolving 40 g sodium chloride in 1lt. of distilled water. Two unequal compartments of plastic chamber with several holes on the divider were used for hatching [17]. After

36 h incubation at room temperature (22-29°C) under light source, the active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay.

Brine shrimp assay

Biosafety of all the fractions of C. arborea and C. viscosa extracts was measured by brine shrimp lethality test. All the fractions (20 mg of each) were dissolved in 10 ml of pure dimethyl sulfoxide (DMSO) to get stock solutions of 2 mg/ml. Each of the stock solution (250, 500, 750, 1000 µl) were transferred into plates and 5 ml of prepared sea water was added to each plates. The corresponding concentrations were 100, 200, 300, 400 µg/ml respectively [18]. Dimethyl sulfoxide was used as negative control and thymol [19] used as positive control. After hatching and maturation of A. salina, 10-15 larvae were placed in each plate using glass capillary and incubated at 25-27°C for 24 h under illumination. After 6 h and 24 h, the numbers of dead naupli in each plate were counted. The experiment was done in triplicate.

Lethality concentration determination and statistical analysis

The percentage lethality was determined by comparison of mean surviving larvae of the test and control plates. The percentage of mortality in each concentration was determined and LC_{50} values were obtained from best fit line plotted concentration verses percentage lethality using Microsoft excel 2007. The statistical analysis was analysed using GraphPad Prism software version 4.01.

RESULTS

Brine shrimp lethality test after 24 hours of exposure to all the test samples, show that percentage mortality increases with the increase in concentration of test samples (Table 1, Figure 1a, 1b & 2a, 2b). The positive control compared to the negative control (DMSO + Sea water) was highly lethal, giving significant lethality to the shrimps. The lethal concentration (LC_{50}) of the test samples was obtained by plot of percentage of mortality versus the sample concentration and the best fit was obtained from the curve data by means of regression analysis and with software GraphPad Prism version 4.01. The criterion of toxicity for fractions was established by following the findings established as LC_{50} values >1000 µg/ml (non toxic), ≥ 500 ≤ 1000 µg/ml (weakly toxic) and < 500 µg/ml (toxic) [20]. In the brine shrimp assay, among the evaluated fractions of C. arborea, hexane (LC_{50} 1071 µg/ml), chloroform (LC_{50} 1327 µg/ml), ethyl acetate (LC_{50} 1078 µg/ml) and aqueous (LC_{50} 1118 µg/ml) fractions were found to be non toxic. On the other hand hexane and chloroform fraction of C. viscosa was toxic to brine shrimp with LC_{50} value 155.54 µg/ml and 222.25 µg/ml respectively, while the aqueous fraction was weakly toxic with LC_{50} 516.36 µg/ml and ethyl acetate fraction was found to be non toxic with LC_{50} 1018.07 µg/ml. In the assay positive control showed the LC_{50} less than 100 µg/ml and negative control showed LC_{50} 1060.3.

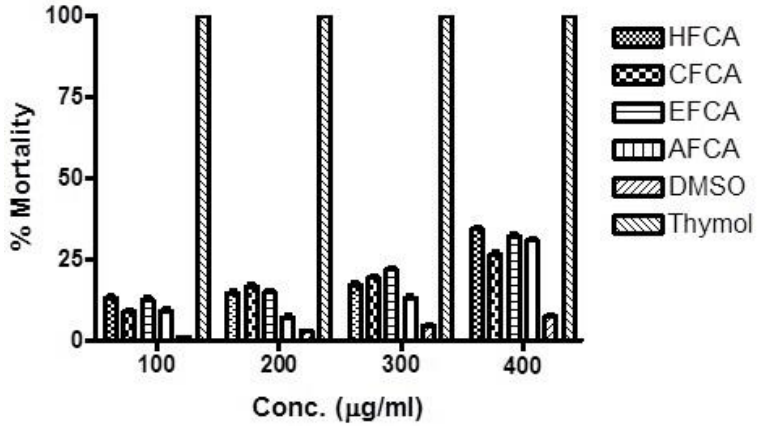

Figure 1a: Concentration vs percentage mortality of various fractions of *C. arborea* leaf extract, negative control (DMSO) and positive control (Thymol)

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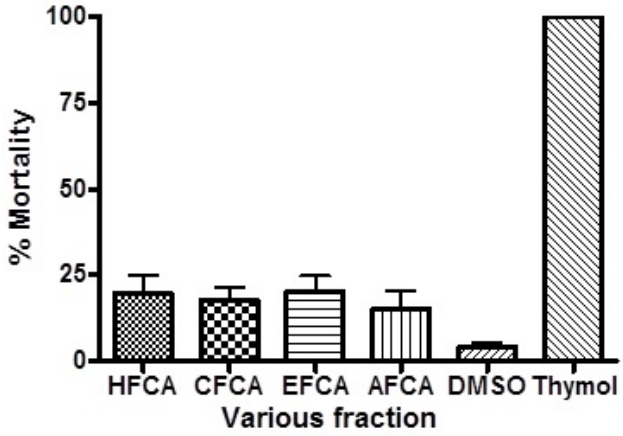

Figure 1b: Percentage mortality of various fractions of *C. arborea* leaf extract compared with positive control thymol.

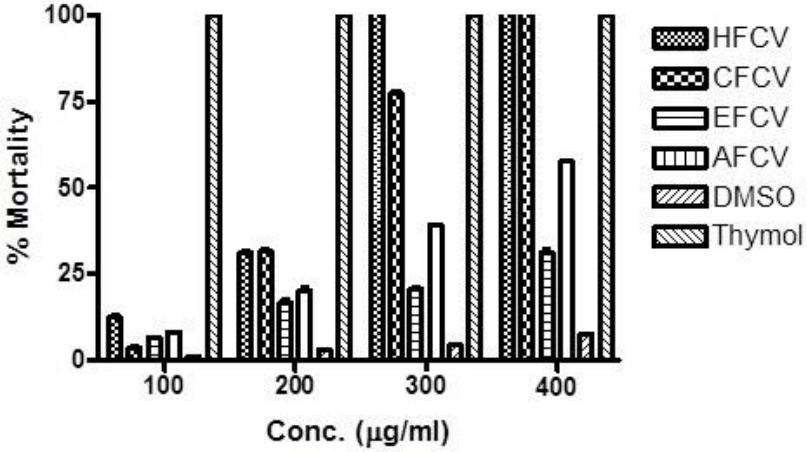

Figure 2a: Concentration vs percentage mortality of various fractions of *C. viscosa* aerial parts extract, negative control (DMSO) and positive control (Thymol)

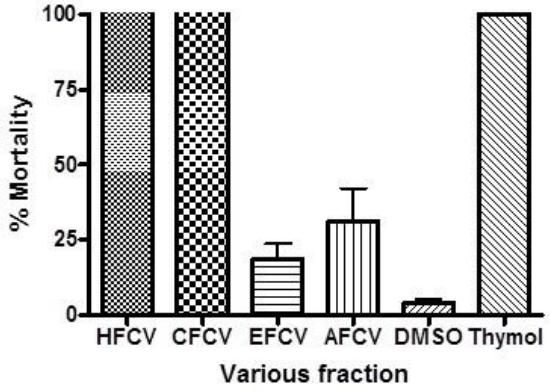

Figure 2b: Percentage mortality of various fractions of *C. viscosa* aerial parts extract compared with positive control thymol.

Table 1: Brine shrimp lethality bioassay results of plant extracts

Test sample	LC 50 (µg/ml) after 24 hr
Careya arborea	
Heaxane (HFCA)	1071
Cloroform (CFCA)	1327
Ethyl acetate (EFCA)	1078
Aqueous (AFCA)	1118
Cleome viscosa	
Heaxane (HFCV)	155.54
Cloroform (CFCV)	222.25
Ethyl acetate (EFCV)	1018.07
Aqueous (AFCV)	516.36
DMSO (- Control)	1060.3
Thymol (+ Control)	<100

DISCUSSION

The brine shrimp lethality bioassay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic property. Brine shrimp lethality assay is used as a screening tool for the determination of bioactivity of different extracts, fractions and pure compounds. Various studies have shown that brine shrimp assay has been an excellent method for preliminary investigations of toxicity, to screen medicinal plants popularly used for several purposes and for monitoring the isolation a great variety of biologically active compounds [21, 22].

The brine shrimp toxicity assays have been carried out for all the fraction C. arborea leaf and C. viscosa aerial parts extract. The brine shrimp test results indicate that all the fraction of C. arborea extract tested had LC_{50} values above 1000 µg/ml which suggests that they are practically non toxic while hexane and chloroform fraction of C. viscosa extract showed toxicity and ethyl acetate fraction exhibited no toxicity. The results obtained suggest the selected plant can be used safely as per the traditional claims and can be tested further for acute toxicity on animal model to correlate the two methods of toxicity evaluation.

CONCLUSION

The ethno-pharmacological activities of these selected plants are due to their different bioactive compounds present in the plants. Further work is needed to carry out bioassay guided separation of pure compound form promising fraction and lethality bioassay should be carried out to determine potent cytotoxic ones.

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