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Research Article

EVALUATION OF CYTOTOXIC ACTIVITY OF *CLEOME VISCOSA* L. AND *CLEOME BURMANNI* W. & A. (CLEOMACEAE)

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ABSTRACT

Objective: Cytotoxicity studies are designed for rapid and inexpensive analysis of soluble pharmaceuticals, which gives an idea of the anticancer as well as toxic profile of the studied plant extract. Species of genus *Cleome* have long been used in folklore medicine for the treatment of various ailments. The purpose of the present study is to evaluate the cytotoxic potential of methanol and chloroform extracts of *Cleome viscosa* and *Cleome burmanni.*

Method: The toxic nature of the plant extracts was assessed by a simple and low cost assay such as brine shrimp lethality assay.

Results: The methanol extracts of both plants exhibited significant toxicity against the shrimp nauplii when compared to potassium permanganate (positive control) and thus is possibly a good indicator of toxicity.

Conclusion: The results thus warrant a follow-up through bioassay directed isolation of the active principles and further analysis of cytotoxicity of these active principles using specific cell lines.

Keywords: *Cleome viscosa, Cleome burmanni*, Brine shrimp, Cytotoxic, Potassium permanganate.

INTRODUCTION

Cancer is a public health problem and is the major cause of human mortality all over the world. Lung, bronchus, breast and colorectal cancers in women happen to be the most common fatal cancers with half of the incidence and mortality occurring in Asia [1]. The etiology of cancer is primarily from unhealthy lifestyle and pollution. The implication of free radicals in different steps of carcinogenesis (initiation, promotion and progression) is well documented [2]. Chemotherapeutic drugs are still considered as the most important treatments for cancer, but this kind of treatment triggers enormous side effects. Considering this fact, attention has been focused on the anticancer properties of medicinal plants for quite some time, more so in the recent past. Many plant extracts and phytoconstituents have been tried for their cytotoxic and anticancer potential and most of these plants tend to exert their anticancer properties through antioxidant mechanisms. Herbal drugs are in great demand now more than ever before. There is also a general awareness among the public regarding the safety and efficacy of herbal drugs. Around half of the drugs currently in clinical use as anticancer drugs are of natural product origin, and it has been estimated that about 60% of new chemical entities introduced in the 1981-2002 period in this field were natural products or were derived from a natural lead compound [3]. Therefore, urgent measures should be adopted to screen traditional medicinal plants in order to identify and isolate new cytotoxic compounds for life threatening diseases like cancer. Cytotoxicity usually gives a preliminary idea on the anticancer potential of plant extracts. Such plant extracts may contain bioactive compounds that are toxic to the living body at higher doses and have a pharmacologically beneficial effect at lower doses. Among the available cytotoxicity screening assays, brine shrimp lethality (BSL) bioassay appears to be the most rapid (24 hours), simple (no aseptic techniques are required), easily mastered, and inexpensive method. Moreover, it requires only small amount of test material (2 or 20 mg or less) [4].

Brine shrimp has been used as a "bench top bioassay" for the discovery and purification of bioactive natural products and also for evaluating the anticancer, antimicrobial and pharmacological activities of natural products [5]. Bioactive compounds are almost always toxic at high doses. BSL bioassay was developed by Michael *et al*. [6] and modified by others [7, 8]. This *in vivo* lethality test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays, once the active principles have been isolated. By this method, natural product extracts, fractions as well as the pure compounds can be tested for their biological activity.

Traditional medicines hold great opportunities as sources of easily available, effective healing agents to the people. It is in this context that the people consume several plants or plant derived preparations to cure different diseases. The genus *Cleome* (Cleomaceae) is one such genus reportedly used in traditional systems of medicine [9, 10, 11, 12, 13]. *Cleome viscosa* L., the most commonly occurring species of *Cleome* is an annual herb, which is reported to possess rubefacient, vesicant, expectorant, astringent, antispasmodic, contact insecticidal, repellent, antifeedant, nematicidal, antipyretic, antidiarrhoeal, immunomodulatory, local anesthetic and anthelmintic properties [14, 15, 16, 17, 18, 19]. *Cleome burmanni* W. & A., also a herb, is reported to exhibit anthelmintic, nutritional and antioxidant properties [14, 20, 21].

Literature review indicated that cytotoxic studies of *C. viscosa* and *C. burmanni* have not been undertaken as yet. Considering the above, the present study is aimed at evaluating the cytotoxic potential of the methanol and chloroform extracts of *C. viscosa* and *C. burmanni*.

MATERIALS AND METHODS

The plant samples, *Cleome viscosa* and *C. burmanni* were collected from Kariavattom, Thiruvananthapuram.

Preparation of extract

Methanol and chloroform extracts of *Cleome viscosa* and *C. burmanni* were prepared from shade-dried plant parts. About 20 g of the powdered plant material from each sample was subjected to extraction by soxhlet apparatus using 300 ml each of methanol and chloroform. The extracts were then concentrated under reduced pressure and kept at 4^{o}C until further use.

Brine shrimps

Cytotoxicity assay was done using Brine shrimp (*Artemia salina*), commonly known as 'sea- monkeys'. Brine shrimp is a simple invertebrate organism about one mm in size, brownish red in colour and found in saline aquatic and marine ecosystem. It plays an

important role in the energy flow of the food chain [5]. The freeze-dried cysts were procured from the Department of Aquatic Biology and Fisheries, University of Kerala, Kariavattom. The lethality test involves the culture of brine shrimp larvae, treatment with test-extracts and data-analysis.

Hatching of shrimps

The cysts hatch to nauplii when deposited in saline sea-water. About a spoonful of the cysts (shrimp eggs) were put into the sea water, taken in a glass trough, properly sealed with aluminium foil and maintained at an ambient temperature (37°C). A few holes were made on the aluminium foil covering for the free passage of air into and out of the container. The shrimp hatch and mature as nauplii in two days. The larvae were allowed another 48 h in sea water to ensure survival and maturity before use. These nauplii were taken for the bioassay.

Lethality bioassay

Five concentrations of both the plant extracts (2, 4, 6, 8 and 10 µg/ml) in 5% DMSO were prepared and tested. Each extract concentration to be tested was dispensed in 10 ml volumes and tested in triplicate. After labeling the glass vials properly, ten living shrimps were added to each vial with the help of a Pasteur pipette [7]. About 10 ml of DMSO in sea water and different concentrations of potassium permanganate (as in the sample vials) were taken as negative and positive controls respectively. The vials were kept for 24 hours. Larvae were considered dead if they did not exhibit any internal or external movement during the several seconds of observation. The larvae were not provided with any food. To ensure that the mortality observed in the bioassay could be attributed to bioactive compounds and not due to starvation; the dead larvae in each treatment were compared with the dead larvae in the negative control.

Counting nauplii and analysis of data

After 24 hours, the vials were inspected using a magnifying glass and the number of surviving larvae were counted. The percentage of mortality was calculated at each concentration. The concentration-mortality data were analyzed statistically. The concentration-mortality relationship of the plant product indicates its effectiveness and is usually expressed as a median lethal concentration (LC_{50}). The LC_{50} value was determined using the Probit analysis method [22]. The LC_{50} value represents the concentration of the chemical that produces death in half of the subjects after a certain exposure period.

The percentage of mortality at each test dose and the control was determined using the formula:

% of mortality = (no. of dead nauplii/total number) x 100 …… (a)

In many experiments, it is desirable to correct the mortality in the experimental treatments by the mortality that occurs in the control treatment.

The percentage of mortalities for 0 and 100% was corrected by the following formulas (b & c) proposed by Ghosh [23] before the determination of Probits.

For 0% mortality: 100 x (0.25/n)…….. (b)

For 100% mortality: 100 x (n-0.25/n)……(c)

Where, n = total number of animals in each group.

When there are a small number of treatments, correction of control mortality has traditionally involved the use of Abbot's formula. It is a mathematical formula used to correct mortality in the untreated check. The adjusted value is permissible when mortality in control does not exceed 20% [24].

For the percentage mortality values between 0 and 100, the formula is provided below:

Corrected % mortality = $\{(M_{obs} - M_{control}) / (100 - M_{control})\}$ x 100 …. (d)

Where, M_{obs} = observed % mortality; $M_{control}$ = control % mortality

Table 1: Results of the brine shrimp lethality bioassay in *Cleome viscosa* and *C. burmanni*

Tested material	Conc.(µg/ml)	Total shrimps	No. of shrimps alive	No. of shrimps dead	% mortality	Corrected mortality (%)	Probit
Negative control	2	10	10	0	0	0	0
Methanol extract							
C.viscosa	2	10	8	2	20	20	0.132
	4	10	7	3	30	30	0.331
	6	10	5	5	50	50	0.595
	8	10	2	8	80	80	0.821
	10	10	0	10	100	97.5	0.944
C. burmanni							
	2	10	10	0	0	2.5	0.170
	4	10	9	1	10	10	0.183
	6	10	7	3	30	30	0.260
	8	10	5	5	50	50	0.538
	10	10	2	8	80	80	0.797
Chloroform extract							
C.viscosa	2	10	0	0	0	2.5	0.046
	4	10	8	2	20	20	0.138
	6	10	7	3	30	30	0.310
	8	10	4	6	60	60	0.538
	10	10	3	7	70	70	0.755
C.burmanni	2	10	0	0	0	2.5	0.020
	4	10	9	1	10	10	0.073
	6	10	8	2	20	20	0.193
	8	10	6	4	40	40	0.389
	10	10	4	6	60	60	0.619
Positive control	2	10	7	3	30	30	0.265
	4	10	5	5	50	50	0.509
	6	10	3	7	70	70	0.750
	8	10	1	9	90	90	0.907
	10	10	0	10	100	97.5	0.976

RESULTS0

Following the procedure of Mayer *et al* [7], the lethality of the extracts of *Cleome viscosa* and *C. burmanni* to brine shrimp was determined on *Artemia salina* after 24 hours of exposure of the samples and comparing them relative to the positive control, potassium permanganate. In the brine shrimp lethality (BSL) bioassay, both plant extracts showed lethality against the brine shrimp nauplii comparable to the standard (Table 1). The nauplii showed different rates of mortality at different concentrations in a dose dependent manner.

The LC_{50} values were deduced from Probit analysis which is presented in table 2. Potassium permanganate showed significant toxicity to brine shrimps (LC_{50}<30µg/ml). However, mortality for the negative control was observed to be nearly zero. The BSL assay was considered valid as the negative control showed mortality rate of 'zero' as expected. R^2 values (>0.1) shows that the test was statistically significant (Table 2) and indicates an adequate goodness of fit. R^2 values close to 1 indicate an ideal fit in mathematical and graphical equations.

Table 2: Calculation of LC_{50}, regression equation, confidence limit and Chi square by probit analysis

Tested material	LC_{50} (µg/ml)	95% confidence limit (µg/ml)	Regression equation	R^2
Cleome viscosa (Methanol)	5.285	3.83 - 6.59	y = 10.25x-6	0.976
Cleome burmanni (Methanol)	7.741	6.55 – 9.40	y = 9.75x-24	0.961
Cleome viscosa (Chloroform)	7.671	6.28 – 9.81	y = 8.75x-16	0.973
Cleome burmanni (Chloroform)	8.957	7.49 – 12.46	y = 7.25x- 17	0.957
Potassium permanganate	3.920	1.78 – 5.21	y = 8.75x+15	0.980

DISCUSSION

The cytotoxic activity of the methanol and chloroform extracts of *Cleome viscosa* and *C. burmanni* were tested using brine shrimp lethality assay (BSLA). This assay has been routinely used in the primary screening of the extracts as well as isolated compounds. BSLA assesses the toxicity towards brine shrimp, which could also provide an indication of possible cytotoxic properties of the test materials [25]. As noted previously, brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal and antitumor [26]. Brine shrimp nauplii have been utilized in the analysis of pesticidal residues, mycotoxins, stream pollutants, anesthetics, dinoflagellate toxins, morphine like compounds, carcinogenicity of phorbol esters and toxicants in marine environment [25].

In the present investigation, varying degrees of lethality were observed with exposure to different dose levels of the test samples. The degree of lethality was found to be directly proportional to the concentration of the extracts tested (Table 1). In other words, mortality increased gradually with an increase in concentration of the test samples. The variation in BSLA results observed for *C. viscosa* and *C. burmanii* may be due to the difference in the amounts and types of cytotoxic substances (e.g. Tannins, flavonoids, saponins, anthocyanins or triterpenoids) present in these extracts. Most of these compounds are known free radical scavengers, reactive species quenchers, hydrogen donors, antioxidant enzyme activator, detoxification inducers, normal cell differentiation promoters, tumor production and proliferation cell inhibitors and apoptosis inducer [27, 28]. Plant extracts obtained from the two solvents (methanol and chloroform) was found to show potent activity against brine shrimp nauplii comparable to the positive control potassium permanganate. The methanol extract of both plants were more effective against the brine shrimp when compared to the chloroform extract (Table 1). Compared to other solvents methanol has been known to be more effective in dissolving active compounds within cells, since methanol easily penetrates the cellular membrane to extract the intracellular ingredients from plant materials [29]. The methanol extract of *C. viscosa* appears to be highly effective as it showed an LC_{50} value of 5.285µg/ml which can be considered to be comparable to the standard, potassium permanganate (LC_{50} value of 3.920µg/ml) (Table 2). The chloroform extract of *C. burmanni* was the least effective and showed an LC_{50} value of 8.957µg/ml (Table 2). An extract having LC_{50} below 30µg/ml is generally considered as a potent bioactive extract [25]. Therefore, the positive response obtained in this assay suggests that the extracts of both *C. viscosa* and *C. burmanni* may contain antitumor, antibacterial and pesticidal compounds which may be utilized beneficially. The R^2 values obtained in the present study were almost close to 1 (Table 2). R^2 values determine how closely a certain function fits a particular set of experimental data. R^2 values range from 0 to 1, with 1 representing a perfect fit between the data and the line drawn through them, and 0 representing no statistical correlation between the data and a line.

BSLA is known to have a good correlation with the results obtained for human solid tumor cell lines. The inhibitory effect of the extract might be due to the toxic compounds present in the active fractions that possesses ovicidal and larvicidal properties. The metabolites either affect the embryonic development or slay the eggs [30]. Thus, the results of the present study could be utilized to determine a possible relationship between brine shrimp lethality and other cytotoxicity assays.

The BSLA is used as a preliminary screening assay and therefore the results of the study may be used to focus research on to the particular plant part, plant extract/fraction to prioritize for further fractionation and isolation of bioactive compounds. In order to understand the mechanism of cytotoxicity better, further *in vitro* cytotoxicity assays involving specific carcinoma cell lines should be conducted using the active fractions/ compounds.

CONCLUSION

The results of the present study using BSLA suggest that the methanol and chloroform extracts of *C. viscosa* and *C. burmanni* possesses significant cytotoxic activity. It is mandatory to conduct further experiments to determine the pharmaceutical potential of these plants. It is expected that, in the long run, active principles isolated from *C. viscosa* and *C. burmanni* will be valuable in cancer chemotherapy.

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