ISSN: 2226-7522
Science, Technology & Arts Research
Jan-March 2012, 1(1): 57-59

Short Communication

Cytotoxic Activity of *Croton gibsonianus* Nimm. Grah

Prashith Kekuda T.R[1*], Raghavendra H.L[2], Vinayaka K.S[3]

[1] Department of Microbiology, SRNMN College of Applied Sciences, NES Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India

[2] Faculty of Medical Sciences, Wollega University, Post Box No: 395, Nekemte, Ethiopia.

[3] Department of PG Studies and Research in Botany, Kuvempu University, Jnanasahyadri, Shankaraghatta-577451, Karnataka, India.

Abstract

The present study was designed to investigate the cytotoxic activity of methanol extract of *Croton gibsonianus* Nimm. Grah (Euphorbiaceae) leaves by brine shrimp lethality bioassay. The powdered leaf material was extracted with methanol. The cytotoxicity activity of methanol extract was tested using brine shrimp (*Artemia nauplii*) lethality assay. The mortality of shrimps were determined and LC_{50} was calculated. Cytotoxic activity, in terms of mortality of brine shrimps, was dependent on concentration of the extract. Highest mortality (87%) was observed at extract concentration 1000µg/ml. The LC_{50} of methanol extract was found to be 109.48µg/ml. The result of the study indicated that the extract possesses cytotoxic property. Further studies on isolation of active constituents possessing cytotoxic activity their cytotoxicity on various cancer and tumor cell lines are under investigation.

Article Information

Article History:

Received : 15-01-2012
Revised : 10-02-2012
Accepted : 16-02-2012

Keywords:

Croton gibsonianus
LC_{50}
Brine shrimp lethality

***Corresponding Author:**

Prashith Kekuda T.R
E-mail: p.kekuda@gmail.com
Phone: +91 97398 64365

INTRODUCTION

Croton gibsonianus Nimm. Grah is a shrub belonging to the family Euphorbiaceae. It is growing in under story of evergreen forests of Western Ghats. Branchlets are stellately pubescent; leaves opposite, elliptic oblong, glandular-serrate, apex shortly acuminate, base rounded with a pair of glands. Inflorescence is terminal racemes. Flowers are unisexual, male flowers are above and female at the base. Ovary is globose, densely hairy, fruit is tricarpellary and is berry. Flowering and fruiting occur in October to December (Ramaswamy *et al*., 2001). The methanol extract of leaves was shown to exhibit antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*; antifungal activity against *Aspergillus niger*, *Trichophyton rubrum*, *Candida albicans* and *Chrysophyllum indicum*; and scavenging activity against DPPH free radical with IC_{50} of 43.78µg/ml (Vinayaka *et al*., 2010). Swathi *et al* (2011) showed that the methanol extract of leaves possess antibacterial activity against isolates of *Streptococcus mutans* recovered from dental caries subjects.

The brine shrimp lethality test is considered to be very useful in determining various biological activities such as cytotoxic, phototoxic, pesticidal, trypanocidal, enzyme inhibition, and ion regulation activities (Jerry *et al*., 1998; Anderson *et al*., 1991; Fatope *et al*., 1991; Solis *et al*., 1993; Zani *et al*., 1995; Ozala *et al*., 1999). The assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. The brine shrimp lethality assay is based on the ability of the extract to show lethality in laboratory

cultured *Artemia nauplii* brine shrimp. It is considered as useful tool for preliminary assessment of toxicity. It has also been suggested for screening pharmacological activities of plant extracts (McLaughlin, 1991; Luis *et al*., 2002). The literature review revealed that cytotoxic activity in terms of brine shrimp lethality of *C. gibsonianus* remains unexplored. Hence, in continuation of our previous work, the present study was undertaken to investigate cytotoxic activity of methanol extract of *C. gibsonianus* leaves.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plants were collected in Hulikal Ghat region (alt-2115, lat-13º 44' N to 75º 01' E) of Karnataka (Western Ghats region of Southern India) during May 2009 and authenticated by Prof. KG Bhat, Udupi, Karnataka, India. Voucher specimen (KU/AB/KSV/2032) was deposited in the University herbaria at P.G Department of Studies and Research in Botany, Shankaraghatta, Karnataka for future reference.

Extraction

The leaves were washed thoroughly, shade dried, powdered and used for extraction. A known amount of powdered leaf material (500gm) was subjected to soxhlation and exhaustively extracted with methanol for about 48 hours. The extract was filtered and concentrated in vacuum under reduced pressure using rotary-flash evaporator and dried in the desiccator (Manjunatha *et al*., 2006).

Cytotoxic Activity of Methanol Extract

The brine shrimp lethality test was conducted according to the method of Raghavendra *et al* (2010) to determine cytotoxic nature of methanol extract. Brine shrimp *Artemia nauplii* eggs (Nihon Animal Pharmaceutical Inc., Tokyo, Japan) were hatched in a container filled with air-bubbled artificial sea water which was prepared with 10g of a commercial salt mixture (GEX Inc., Osaka, Japan) and 500 ml of distilled water. After 36-48 hours, the shrimps were collected by pipette for bioassay. The different concentrations of methanol extract (10-1000µg/ml) were tested in vials containing 5ml of brine and 25 shrimp in each of three replicates. The vials were incubated at 25°C and surviving shrimps were counted after 24 hours. LC_{50} values greater than 1000µg/ml for plant extracts were considered inactive (non-toxic).

RESULTS

The yield of extract was 4.5%. The result of cytotoxic activity of methanol extract in terms of mortality of brine shrimps is presented in Table 1. The degree of lethality of extract was directly proportional to the concentration of the extract. Highest lethal effect was observed at 1000µg/ml extract concentration at which the mortality was found to be 87%. The LC_{50} was found to be 109.48µg/ml and hence the extract is toxic.

Table 1: Cytotoxic activity of methanol extract of *C. gibsonianus.*

Concentration (µg/ml)	Mortality (%)	LC_{50} (µg/ml)
0	0.0	
10	13.0	
100	46.0	109.48
1000	87.0	

DISCUSSION

The brine shrimp lethality bioassay is a rapid method utilizing only 24 hours, inexpensive and needs no special equipment. It is so simple that no aseptic technique is required. It utilizes a large number of organisms for validation and a relatively small amount of sample. It does not require animal serum as needed for other methods of cytotoxicity testing (Hossain *et al*., 2009). Several studies have been carried on brine shrimp lethality of extracts from natural sources. In a study, Raghavendra *et al*. (2010) showed cytotoxic activity of methanol Extract of *Putranjiva roxburghii* Wall (Euphorbiaceae) Seeds. The cytotoxicity of methanol extract of leaves of *Abrus pulchellus* Wall (Fabaceae) using brine shrimp lethality bioassay revealed dose dependent activity with LC_{50} of 281.70µg/ml (Kekuda *et al*., 2010).

CONCLUSION

The brine shrimp lethality assay has proven to be a convenient system for monitoring biological activities which in most cases correlates reasonably well with cytotoxic and anti-tumor properties. The lethal nature of extract could be attributed to the presence of phytochemicals. In suitable form, the extract could be used to prevent and treat cancer. Isolation of active components from crude extract and their cytotoxic activity is under study.

REFERENCES

Anderson, J.E., Goetz, C.M., McLaughlin, J.L., Suffness, M. (1991). A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. *Phytochemical Anal*ysis 2: 107-111.

Fatope, M.O., Ibrahim, H., Takeda, Y. (1991). Screening of higher plants reputed as pesticides using the brine shrimp lethality assay. *International Journal of Pharmacognosy* 31: 250-254.

Hossain, A.M., Ferdous, T., Salehuddin, S.M and Das, A.K. (2009). *In vitro* cytotoxicity (LC_{50}) of extracts obtained from the seeds of Zea mays. *Asian Journal of Food and Agro-Industry* 2(3): 336-34.

Jerry, L., McLaughlin., Lingling Rogers, L. (1998). The use of biological assays to evaluate botanicals. *Drug Information Journal* 32: 513-524.

Manjunatha, B.K., Patil, H.S.R., Vidya, S.M., Kekuda, T.R.P., Mukunda, S and Divakar, R. (2006). Studies on the antibacterial activity of *Mucuna monosperma* DC. *Indian Drugs*. 43: 150-152.

McLaughlin, J.L. (1991). Methods in plant biochemistry. In: Hostettmann, K., (Eds.) Assay for bioactivity. Academic press. London.1-32.

Ozala, T., Vuorela, P., Kivranta, J., Vuorela, H., Hiltunen, R. (1999). A bioassay using *Artemia salina* for detecting phototoxicity of plant coumarins. *Planta Medica* 65: 715-718.

Ramaswamy, S.N., Rao, R.M., Govindappa, D.A. (2001). Flora of Shimoga district, Karnataka. Prasaranga, Manasagangothri, Mysore University, Mysore, Karnataka. Pp 531.

Solis, P.N., Wright, C.W., Anderson, M.M., Gupta, M.P., Phillipson, J.D. (1993). A microwell cytotoxicity assay using Artemia salina (brine shrimp). *Planta Medica* 59: 250-252.

Swathi, D., Suchitha, Y., Venugopal, T.M., Kekuda, P.T.R, Mallikarjun, N. (2011). Inhibitory Efficacy of *Croton gibsonianus* Nimm. Grah (Euphorbiaceae) Against Oral Isolates of Streptococcus mutans. *International Journal of Pharmaceutical & Biological Archives* 2(3): 896-899.

Vinayaka, K.S., Swathi, D., Kekuda, T.R.P., Bhagath, K and Mallikarjun, N. (2010). Antibacterial, antifungal and free radical scavenging activity of *Croton gibsonianus* Nimm. Grah. (Euphorbiaceae). *Journal of Natural Pharmaceuticals* 1(1): 46-50.

Zani, C.L., Chaves, P.P.G., Queiroz, R., Oliveira, A.B., de Cardoso, J.E., Anjos, A.M.G., Grandi, T.S.M. (1995). Brine shrimp lethality assay as a prescreening system for anti-*Trypanosoma cruzi* activity. *Phytomedicine* 2: 47-50.