



BRINE SHRIMP LETHALITY BIOASSAY OF *GANODERMA APPLANATUM*

*Sudheer K Dokuparthi., Prabhakar L., Srikanth D., Narender K., Suresh Kandagatla and Yesubabu Maddala

Vijaya College of Pharmacy, Department of Pharmacognosy, Hyderabad

ARTICLE INFO

Article History:

Received 5th January, 2018

Received in revised form 20th

February, 2018 Accepted 8th March, 2018

Published online 28th April, 2018

Key words:

Ganoderma applanatum, brine shrimp, cytotoxicity, mortality.

ABSTRACT

Traditionally, mushrooms are having an importance in the diet. Since they possess both nutritional and medicinal value, research on mushrooms is the new area for developing lead molecules to treat cancer and other ailments. *Ganoderma* is a famous genus in the medicinally important mushrooms. Cancer is a fatal disease in which uncontrolled proliferation is the major crunch point to be considered. In our current study, various extracts of *Ganoderma applanatum* has been investigated for the evaluation of the cytotoxic activity. All the extracts were screened for their cytotoxicity by using brine shrimp nauplii (*Artemia salina*) lethality bioassay. The cytotoxicity was assessed in terms of LC₅₀ (lethality concentration), 10 nauplii were taken into three replicates of each concentration of the extract. After 24 hrs, brine shrimps were checked for the mortality, surviving brine shrimps were counted and LC₅₀ was evaluated. The results showed that all the extracts were showing potent toxicity to the nauplii. The LC₅₀ values were compared to the standard potassium dichromate. It indicates that the extracts are toxic even at low doses. Further investigation is needed to study the acute and sub acute toxicity of the extracts for its safe application to the humans. The anti mitotic activity can be attributed to the chemical constituents present in the extract. The phytochemical screening showed that the species is rich in various phytochemicals like alkaloids, glycosides, terpenoids, saponins, steroids, tannins, carbohydrates and proteins. These bioactive compounds, especially the tannins, terpenoids, and steroids might be responsible for the said activity. Further research is in the process to identify and isolate the secondary metabolites which are having potent cytotoxic property.

Copyright©2018 Sudheer K Dokuparthi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Mushrooms are macrofungus, having a fruiting body which can be seen with naked eye. Mushrooms either wild or cultivated are become an important part of our diet due to their nutritional value. Mushrooms are having immuno-modulatory and health-promoting activity. So, they are also classified under nutraceuticals, they are the foods which give both energy and health (Barros et al., 2007). Mushrooms are rich in carbohydrates, proteins, vitamins (Okwulehie et al., 2004). *Ganoderma* is one of the famous genera of mushrooms belongs to Ganodermataceae (Ainsworth, 2005). The *Ganoderma* is generally grown on dead stumps of plants and synthesizes various components which are not essential for their normal physiological functions. But, they will play a vital role in their defense mechanism, which makes it unique in its survival, protection from the other microorganisms and herbivorous animals. These mushrooms are having double walled basidiospores which give uniqueness to the genus (Smith & Sivasithamparam, 2003).

The previous reports on *Ganoderma* genus revealed that it is having anti-cancer, anti viral, anti tumor, anti proliferative, cytotoxic, hepatoprotective, anti diabetic, anti inflammatory and anti hyperlipidemic activity (Ajith & Janardhanan, 2007; Acharya et al., 2005). The *Ganoderma* species possess mainly steroids, triterpenoids, and polyphenols (Smania et al., 2003; Abdul W. Kidukuli et al., 2010).

Cancer is a deadly disease, which involves the abnormal proliferation of cells along with malignancy and metastatic behavior. The current drugs in cancer chemotherapy mainly target the highly dividing cells. These drugs are cytotoxic and cause mitotic arrest. Some drugs may lead to a programmed cell death called apoptosis. The polyphenols like tannins, flavonoids are reported to have apoptotic activity. Since *Ganoderma applanatum* posses various phytochemicals including polyphenols and flavonoids.

The brine shrimp lethality bioassay is a simple and rapid method to evaluate the cytotoxic activity. It inexpensive and requires small amounts of test materials (Ghisalberti EL, 1993). The bioassay has a good correlation with pesticidal activity and with cytotoxic activity in solid tumors. This invivo

*Corresponding author: Sudheer K Dokuparthi

Vijaya College of Pharmacy, Department of Pharmacognosy, Hyderabad

lethality assay can be successively applied as a primary screening that can be backed up by more sophisticated and specific evaluation bioassays (McLaughlin JL *et al.*, 1998). Our, present study is to investigate the toxicity of n-hexane, ethyl acetate and methanolic extracts of *Ganoderma applanatum* by brine shrimp lethality bioassay. All the extracts were prepared by Soxhlet method and these extracts were used for further studies.

MATERIALS AND METHODS

Collection and extraction

The mushrooms were collected from the local forest field of Hayathnagar, Hyderabad. The species authenticated and a voucher specimen was preserved in the college herbarium for future reference. The mushroom was washed to remove earthy matter and shade dried for few days. After complete drying, they are subjected to soxhlet extraction by using various solvents starting from non-polar to polar. N-Hexane, Ethyl acetate and Methanol were used for the exhaustive extraction which yielded the respective extracts.

Preliminary phytochemical screening

The three extracts were screened for phytochemicals by using standard procedures(Harborne JB, 1973). The results were depicted in Table 1.

Table 1 Preliminary phytochemical screening of *Ganoderma applanatum*

Phytochemicals	n-Hexane extract	Ethylacetate extract	Methanol extract
Alkaloids	+	-	+
Glycosides	-	+	+
Terpenoids	+	+	+
Steroids	+	+	-
Saponins	-	+	+
Flavonoids	-	+	+
Tannins	-	+	+
Carbohydrates	-	-	+
Lipids	+	-	-
Proteins	-	-	-

+ present, - Absent

Hatching of brine shrimp

The eggs of brine shrimp were procured from the local market and hatched in a glass compartment with sea water. The glass compartment is having two partitions, one is illuminated. After 24 hrs of hatching at room temperature, the eggs hatch into larvae (nauplii), which will swim towards the illumination chamber by leaving their shells in the dark chamber. The nauplii were collected carefully by pipette for the bioassay.

Preparation of test samples

32 mg of each of the test samples were taken and dissolved in 200 µl of pure dimethyl sulphoxide (DMSO). The final volume was made to 20ml with sea water. The concentration of the stock solution is 1600 µg/ml. Eight samples of volume 5ml having different concentration 1600, 1000, 800, 500,250,100, 50, 25 µg/ml were prepared by diluting the stock solution with sea water.

Control group

This group is used to validate the test method and ensure that the cytotoxicity is only due to the activity of the test compounds. Two types of control groups were used in this

experiment- Potassium dichromate (200µg/ml) as the positive control and 50 µg/ml of DMSO in 4.95ml of sea water as the negative control.

Brine shrimp assay

To the pre-marked vials containing above-prepared concentrations, 10 nauplii were added. After 24hrs, the nauplii in each vial were counted with the aid of a 3x magnifying glass for the surviving brine shrimp. The mortality of the brine shrimp larva is identified by their movement. The endpoint of this bioassay is the absence of controlled forward motion during the 30s of observation. These results were depicted in Table 2.

Table 2 Mean percentage of mortality of *Artemia salina* brine shrimp after 24 hrs at different concentrations of the *Ganoderma applanatum* extracts.

Extract	Mean percentage(%) of mortality at concentration ppm (µg/ml)								
	Negative control	25	50	100	250	500	1000	1600	Positive control
n-Hexane	0	27	43	70	80	90	100	100	100
Ethyl acetate	0	32	53	75	84	91	100	100	100
Methanol	0	37	58	74	87	93	100	100	100

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening

The preliminary phytochemical screening of the methanol extract of *Ganoderma applanatum* revealed the presence of various phytochemicals like alkaloids, glycosides, terpenoids, steroids, saponins, flavonoids, tannins, carbohydrates and proteins. The results were showed in Table 1. Since the methanol extract is rich in phytochemicals, it is selected for the screening of anti mitotic activity.

Brine shrimp lethality assay

Brine shrimp nauplii are simple zoological organisms which can be used to evaluate the lethality (Meyer BN *et al.* 1982). It is a simple and very useful tool to screen diverse compounds. This bioassay is also used to screen plant extracts. It is a safe, economical method for determination of the cytotoxicity of synthetic and plant products (Almeida PA *et al.*, 2002). The bioassay can be correlated significantly to the inhibition of human solid tumor cell lines. It is the primary tool to detect the anti-tumor properties (Anderson JE *et al.*, 1991). The toxicity of extracts can be evaluated by their LC₅₀ values. If LC₅₀ values are lower than 1000 µg/ml are considered as cytotoxic (Meyer BN *et al.*, 1982). This bioassay can also be used for the evaluation of antifungal, teratogenic effect, pesticidal effect and environmental toxicity (Vanhaecke P *et al.*, 1980b). The brine shrimp bioassay results illustrated in Table 2. In this study, it was observed that n-Hexane, Ethyl acetate, and methanolic extracts were toxic to the brine shrimps nauplii. They exhibit potent toxicity, compared to the standard potassium dichromate. For n-Hexane, Ethyl acetate and methanolic extracts respectively the brine shrimp lethality was found to be directly proportional to the concentrations of the extracts ranging from lowest concentration (25 µg/ml) to highest concentration (1600 µg/ml). This dose-dependent increment of the toxicity may be due to the presence of cytotoxic principles present in the extracts of *Ganoderma applanatum*. The presence of steroids, alkaloids, saponins, and tannins are already reported to have the cytotoxic property.

CONCLUSION

From the above study, it is observed that all the extracts of *Ganoderma applanatum* exhibited cytotoxicity against the brine shrimp nauplii. The LC₅₀ values are found to be less than 1000mg/ml. It may be due to the presence of bioactive components. This bioassay can only give information that it is having very potent biologically active components. But, this assay is inadequate to understand the mechanism of action of the phytochemicals present in it. Further investigations are needed in isolation, structure elucidations of the phytochemicals present in *Ganoderma applanatum* and toxicity towards the cell lines will help us to understand its molecular mechanism of their bioactivity.

Acknowledgement

Authors are thankful to the management of Vijaya College of Pharmacy, for providing necessary facilities.

Conflict of interest:

Authors do not have any conflict of interest.

References

- Abdul W. Kidukuli1, *et al.*, In vivo antiviral activity of selected Tanzanian wild edible mushrooms. *J. Appl. Biosci.* 2010, 31, 1887-94.
- Acharya, K. *et al.*, Antioxidant and nitric oxide synthase activation properties of *Ganoderma applanatum*. *Indian Journal of Experimental Biology*, 2005, 43(10), pp.926–929.
- Ainsworth, A.M., Tanzanian mushrooms. Edible, harmful and other fungi. *Mycologist*, 2005, 19(3), p.134.
- Ajith, T.A. & Janardhanan, K.K., Serial Review Indian Medicinal Mushrooms as a Source of Antioxidant and Antitumor Agents. *J. Clin. Biochem. Nutr*, 2007, 40(May), pp.157-162.
- Almeida PA, Silva TMS, Echevarria A 2002. Mesoionic 5-alkyl-1,3-dithiolium-4-thiolates: Synthesis and brine shrimp toxicity. *Heterocycl Comm* 8: 593-600.
- Anderson JE, Goetz CC, McLaughlin JL, Sufness M 1991. A blind comparison of simple bench-top bioassays and human tumor cell cytotoxicities as antitumor prescreens. *Phytochem Analysis* 2: 107-111.
- Barros, L. *et al.*, Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, 2007, 55(12), pp.4781-4788.
- Ghisalberti EL. Detection and isolation of bioactive natural products. In: Colegate SM, Molyneux RJ, editors. Bioactive natural products: Detection, isolation and structure elucidation. New York: CRC Press; 1993. p. 15-8.
- Harborne JB. Phytochemical Methods. Chapman and hall Ltd., London: U.K., 1973, 49-188.
- McLaughlin JL, Rogers LL, Anderson JE. The use of biological assays to evaluate botanicals. *Drug Inform J* 1998;32:513-24.
- Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica* 1982; 45:31.
- Okwulehie, I.C., Odunze, E.I., Evaluation of the nutritional value of some tropical edible mushrooms. *Journal of Sustainable Agriculture and the Environment*, 2004, 157-162.
- Smania, E.F.A. *et al.*, Antifungal activity of sterols and triterpenes isolated from *Ganoderma annulare*. *Fitoterapia*, 2003, 74(4), pp.375-377.
- Smith, B.J. & Sivasithamparam, K., Morphological studies of *Ganoderma* (Ganodermataceae) from the Australasian and Pacific regions. *Australian Systematic Botany*, 2003, 16(4), pp.487–503.
- Vanhaecke, P. & Sorgeloos, P., 1980b. International Study on *Artemia*. 14. Growth and survival of *Artemia* larvae of different geographical origin in a standard culture test. *Mar. Ecol. Prog. Ser.* 3: 303-307.

How to cite this article:

Sudheer K Dokuparthi *et al* (2018) 'Brine Shrimp Lethality Bioassay of *Ganoderma Applanatum*', *International Journal of Current Advanced Research*, 07(4), pp. 11815-11817. DOI: <http://dx.doi.org/10.24327/ijcar.2018.11817.2058>
