



Received on 24 July 2018; received in revised form, 29 November 2018; accepted, 20 January 2019; published 01 April 2019

## ANTI-MITOTIC ACTIVITY OF *GANODERMA APPLANATUM*

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### Keywords:

*Ganoderma applanatum*, Anti-mitotic activity, Legume seeds, Germination

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**ABSTRACT:** Traditionally, mushrooms have 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. The current work is carried out to screen antimitotic activity of *Ganoderma applanatum* on legume seeds. Three legume seeds viz., Bengal gram, green gram, and fenugreek were selected for the study and the ability of the methanol extract to inhibit the germination was calculated. The results indicated that the extract is having a significant inhibition in the growth of the radicle of the seeds. The antimitotic 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 metabolite which is responsible for the reported activity.

**INTRODUCTION:** Mushrooms are macrofungus, having a fruiting body which can be seen with naked eye. Mushrooms either wild or cultivated are becoming an important part in our diet due to their nutritional value. Mushrooms are having immunomodulatory and health-promoting activity. So, they are also classified under nutraceuticals; they are the foods which give both energy and health<sup>1</sup>. Mushrooms are rich with carbohydrates, proteins, vitamins<sup>2</sup>. *Ganoderma* is one of the famous genus in mushrooms belongs to Ganodermataceae<sup>3</sup>. 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 have double walled basidiospores which give uniqueness to the genus<sup>4</sup>. The previous reports on *Ganoderma* genus revealed that it is having anti-cancer<sup>5</sup>, antiviral<sup>6</sup>, hepatoprotective<sup>7</sup>, antidiabetic<sup>8</sup>, anti-inflammatory<sup>9</sup>, antihyperlipidemic activity<sup>10</sup> and anti-oxidant<sup>11,12</sup>. The *Ganoderma* species possess mainly steroids, triterpenoids and polyphenols<sup>13</sup>.

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 are reported to have apoptotic activity<sup>14,15</sup>. Since, *Ganoderma applanatum* possess various phytochemicals including polyphenols, the present study is to

	<p style="text-align: center;">DOI: 10.13040/IJPSR.0975-8232.10(4).1912-15</p>
	<p style="text-align: center;">The article can be accessed online on <a href="http://www.ijpsr.com">www.ijpsr.com</a></p>
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screen anti-mitotic activity by using different legume seeds. The inhibition of sprouting in Bengal gram (*Cicer arietinum*), Green gram (*Vigna radiata*) and Fenugreek (*Trigonella foenum-graecum*) indicate that the extract is involving in the M phase of the cell division and causing mitotic arrest.

## 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 (VN. 174) 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<sup>16</sup>. The results were depicted in **Table 1**.

**Preparation of Legume Seeds:** High-quality Bengal grams, green grams, and fenugreek were procured from the local market. Seeds with equal weight were taken and soaked overnight with water to accelerate the germination<sup>17</sup>.

**Preparation of Extracts:** The three extracts were prepared in different concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml). Gum acacia was used wherever it is necessary. One positive control (Doxorubicin) and one negative control (Distilled water) were also maintained.

**Determination of Antimitotic Activity:** Bengal gram, green gram, and fenugreek seeds are having good quality were taken and soaked for overnight. After 24 h, the seeds with good size and texture were selected. All the seeds were divided into groups having 10 seeds, and an average weight of 10 seeds was recorded. The seed samples were added to the Petri dish containing moist filter paper and allowed for germination for two days with continuous monitoring. Moisture is maintained in Petri dishes throughout the experiment. The length of the radicles of the three seeds was measured

after 2 days and calculated the percentage of inhibition<sup>18</sup>. The results are illustrated in **Table 2-4**.

**Statistical Data:** All the values are expressed as Mean  $\pm$  SD. One-way ANOVA and Dunnett's test was used for comparison.

## 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 shown in **Table 1**. Since, the methanol extract is rich in phytochemicals; it is selected for the screening of antimitotic activity<sup>18</sup>.

**TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF GANODERMA APPLANATUM**

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

+ Present, - Absent

**Anti-mitotic Activity:** The antimitotic activity of *Ganoderma applanatum* methanol extract shows that the mushroom has a significant antimitotic activity in Bengal gram, green gram and fenugreek seeds **Table 2-4** in dose-dependent manner by taking Doxorubicin as standard. The percentage inhibition in the imbibitions of the seed germination indicates that the methanol extract is inhibiting the seed germination by interfering in the cell division. The extract, even at a dose of 0.5 mg/ml, is capable of inhibiting the germination of Bengal gram by 50%, green gram by 39.62% and fenugreek by 26.08% which shows its potent anti-mitotic activity.

From the results, it is also clear that the extract is completely inhibiting the germination at a dose of more than 5 mg/ml. The results also indicate that the extract is sensitive to the Bengal gram.

**TABLE 2: ANTI-MITOTIC ACTIVITY OF GANODERMA APPLANATUM BY INHIBITING SPROUTING IN BENGAL GRAM SEEDS**

Concentration	Bengal gram	% inhibition
Negative control (Distilled water)	2.74	
Positive control (Doxorubicin)	0	100
0.5 mg/ml	1.36	50.36
1mg/ml	0.76*	72.26
5mg/ml	0.08*	97.08
10mg/ml	0*	100

P-values are expressed in comparison with the control, \*P<0.05 are considered as statistically significant.

**TABLE 3: ANTIMITOTIC ACTIVITY OF GANODERMA APPLANATUM BY INHIBITING SPROUTING IN GREEN GRAM SEEDS**

Concentration	Green gram	% inhibition
Negative control (Distilled water)	2.12	
Positive control (Doxorubicin)	0	100
0.5 mg/ml	1.28*	39.62
1mg/ml	0.96*	54.71
5mg/ml	0.2*	90.56
10mg/ml	0*	100

P-values are expressed in comparison with the control, \*P<0.05 are considered as statistically significant.

**TABLE 4: ANTIMITOTIC ACTIVITY OF GANODERMA APPLANATUM BY INHIBITING SPROUTING IN FENUGREEK SEEDS**

Concentration	Fenugreek	% inhibition
Negative control (Distilled water)	0.92	
Positive control (Doxorubicin)	0	100
0.5 mg/ml	0.68	26.08
1mg/ml	0.12	86.95
5mg/ml	0.02*	97.82
10mg/ml	0*	100

P-values are expressed in comparison with the control, \*P<0.05 are considered as statistically significant.

**CONCLUSION:** In conclusion, the experimental results revealed that the methanol extract of *Ganoderma applanatum* has various phytochemicals. It is also can be concluded that it also exhibits antimitotic activity against the germination of the legume seeds. The probable inhibition of cell division in the legume seeds indicates that *Ganoderma applanatum* methanol extract is capable of producing lead molecules for the treatment of deadly diseases like cancer. Further investigation is needed in isolation and characterization of the bioactive molecules from *Ganoderma applanatum* which are having the potency to treat cancer.

**ACKNOWLEDGEMENT:** Authors are thankful to the management of Vijaya College of Pharmacy for providing necessary facilities. We are also thankful to Dr. K. Sunil Kumar for statistical data.

**CONFLICT OF INTEREST:** Authors do not have any conflict of interest.

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**How to cite this article:**

Dokuparthy SK, Prabhakar L, Srikanth D, Narender K, Divya C and Maddala Y: Anti-mitotic activity of *Ganoderma applanatum*. *Int J Pharm Sci & Res* 2019; 10(4): 1912-15. doi: 10.13040/IJPSR.0975-8232.10(4).1912-15.

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