ISSN: 0974 - 0376

The Ecoscan : Special issue, Vol. IX: 837-843: 2016
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES
www.theecoscan.com

ANTIBACTERIAL ACTIVITY OF WILD MEDICINAL MUSHROOM: *GANODERMA APPLANATUM* (PERS.) PAT.

Sukumar Dandapat *et al.,*

KEYWORDS

Antibacterial
Fungi
Disease
Phytochemical
Nutrition

Proceedings of National Conference on
Harmony with Nature in Context of
Resource Conservation and Climate Change
(HARMONY - 2016)
October 22 - 24, 2016, Hazaribag,
organized by
Department of Zoology, Botany, Biotechnology & Geology
Vinoba Bhave University,
Hazaribag (Jharkhand) 825301
in association with
National Environmentalists Association, India
www.neaindia.org

The Ecoscan
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

SUKUMAR DANDAPATÁ, M .P. SINHAÁ AND T. C. SARMA[3]
áDepartment of Zoology, Ranchi University, Ranchi- 834 008, Jharkhand, INDIA.
[2]Department of Botany, Gauhati University, Guwahati - 781 014, Assam. INDIA.
e-mail: scholar.sukumar27@gmail.com

ABSTRACT

In the present study antibacterial activity of medicinal mushrooms *G. applanatum* was carried out by agar well diffusin and broth dilution methods and MIC was calculated. *G. applanatum* extract contains phytochemicals alkaloids, phytophenols, tannins, saponins, etc.) which possess therapeutic efficacy. Saponin content of aqueous extra (0.06 ± 0.05mg/100g) and methanolic extract (0.04 ± 0.005 mg/100g) significantly ($p < 0.001$) low among all the studied phytochemicals and phenolic (20.81± 0.14 mg/100g) and flavonoid content (23.89 ± 0.38mg/100g) of methanolic extract is significantly ($p < 0.001$) higher among the studied phytochemicals. Methanolic extract of *G. applanatum* was much effective in agar disc diffusion method (showed 3.21 ± 0.06 mm ZOI and 3.02 ±0.03 ZOI against *Salmonella typhi* and *Proteus mirabilis* respectively). Broth dilution method showed 100% inhibition against both the studied pathogenic bacteria. As a potent antimicrobial, nutritional potentiality of *G. applanatum* is high (222.08Kcal/100g) and among the nutritional components the fungi contain carbohydrate (42.72%) significantly ($p < 0.001$) higher and crude fat (0.48%) significantly ($p < 0.001$) lower quantity. Nutritionally *G. applanatum* is good having high calorific value (222.08 ± 2.26; K Cal/ 100g) and contains highly nutritional components (11.72 ±0.25% crude protein, 0.48 ±0.03% crude fat and 42.72 ±0.54% carbohydrate). Therefore, *G. applanatum* can be used in pathogenic disease caused by *S. typhi* and *P. mirabilis*.

***Corresponding author**

INTRODUCTION

Nowadays, infectious diseases are one of the major causes of morbidity in developing countries of the world and major agent of pathogenesis is pathogenic bacteria (Solanki, 2010; Kumar *et al.,* 2013). Two most common pathogenic bacteria *Salmonella typhi and Proteus mirabilis* have been studied by several workers. *P. mirabilis* is causative agent of diseases such as, urethitis, cystitis, prostatitis and pneumonia etc. (Crump *et al.,* 2004) and Typhoid is predominantly caused by *S. typhi* via contaminated food, water and other vectors (Arumo, 1998; Nagshetty *et al.,* 2010; Dandapat *et al.,* 2013).

Synthetic antimicrobial drug and antibiotic chemotherapy has been used as most important weapons against pathogenic bacterial infections since their introduction in the field of medicine. However, synthetic drugs are sometimes associated with adverse effects on the host, including hypersensitivity, immune laboratory suppression and allergic reactions and initiation of oxidative stress and metabolic imbalance (Mizuno, 1995; Wasser and Weis, 1999; Kumar *et al.,* 2013; Dandapat *et al*., 2014).

Ethnomycologically used macrofungi *G. applanatum* belonging to the phylum basidiomycota and with approximately 700 species of this phylum have been reported for their significant pharmacological activity (Taylor and Webster, 2011.Karaman *et al.,* 2012; Dandapat and Sinha, 2015). Macrofungi (mushroom) contain various bioactive primary metabolites which provide nutrition and secondary metabolites which possess therapeutic efficacy against diseases and disorders (Sharma *et al*., 2013). *G. applanatum* belonging to family ganodermataceae has been used in typhoid, tuberculosis, pneumonia, hepatitis, renal infection and other diseases (Kumar *et al*., 2013; Kumar *et al*., 2013)

Therefore, present study was undertaken to estimate the phytochemical composition of *G. applanatum* to investigate nutritional potentiality and antipathogenic activity against two major pathogens *P. mirabilis* and *S. typhi*.

MATERIALS AND METHODS

Collection of Macro fungi

Fresh fruiting bodies of *G. applanatum* were collected from different adjoining sites of two National Parks (Orang National Park, Kaziranga National Park) and Manas Biosphere Reserve (Ultapani) of Assam and were identified in laboratory of Department of Botany, Gauhati University, Guwahati, Assam and brought to Department of Zoology, Ranchi University, Ranchi to evaluate antibacterial efficacy.

Extract preparation

The fresh fruiting bodies were washed and disinfected by treating with $HgCl_2$ and washed again. The basediocarps were dried in shade under room temperature for six to seven days, powered and sieved (Sofowara, 2008). 50 g of the powder was subjected to extraction chambers of Soxhlet using methanol, ethanol, chloroform and distilled water for extraction. The extract obtained was filtered, concentrated and dried in rotary flash evaporator maintained at 45ºC. Percentage yield of each

extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies (Dandapat *et al.*, 2013).

Mycochemical screening

Estimation of total phenol

Estimation of total phenol was done by phenolic -catechol method according to the procedure described by Malick and Singh (2. Dilute aqueous extract (0.5 mL of 1:10 g/L) was pipetted out in series of test tubes and volume was made up to 3 ml with distilled water. Folin-Ciocalteau reagent (0.5mL) was added to each tube and incubated for 3 min. at room temperature and then sodium carbonate (20%; 2ml) solution was added, mixed thoroughly and the tubes were incubated for 1 min. in boling water bath and total phenols was determined by colorimetry at 650nm against a reagent blank (distilled water). The standard curve was plotted using 10, 50, 100 ìg/mL solution of standard phenolic -catechol was prepared. From the standard curve, concentration of phenols in the test samples was determined and expressed as mg of catechol equivalent.

Estimation of total flavonoid

10ìg/mL, 50ìg/mL and 100ìg/mL of samples were pipette out in series of test tubes and volume was made up to 0.5 mL with distilled water. Sodium nitrite (5%; 0.03mL) was added to each tube and incubated for 5 minutes at room temperature. Aluminium chloride solution (10%; 0.06mL) solution was added and incubated for 5 minutes at room temperature. Sodium Hydroxide solution (1M; 0.2mL) solution was added and total volume was made up to 1mL with distilled water. Absorbance was measured at 510nm against a reagent blank (Distilled water). Standard curve using 10ìg/mL, 50ìg/mL and 100ìg/mL concentrations of rutin was prepared and from the standard curve, concentration of flavonoids in the test samples was determined and expressed as mg of rutin equivalent (Helmaj *et al.*, 2007).

Estimation of total alkaloids

1 g of the dry extract was taken in a 250 mL beaker and 40 mL of 10% acetic acid was added in 60 mL ethanol covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed (Harborn, 1973).

Estimation of tannin

50 mL of the aqueous extract evaporated to dryness in an oven at 105ºC for 4 hours and dried residue was weighed (T_1). 80 mL of the extract was mixed 6.0g of hide powder and shake for 60 minutes and then filtered and evaporated 50.0 mL of the clear filtrate to dryness in an oven at 105ºC and the residue was weighed (T_2). 6 g of hide powder was shaken with 80 ml of water, filtered, evaporated and residue was dried at 105ºC and weighed (T0). The quantity of tannins was calculated as percentage using the formula suggested in the Quality control methods (WHO, 1998):

$$\text{Tannin}(\%) = \frac{[T1 - (T2 - T0)]X\ 500}{W}$$

Where,

W = the weight of the extract in grams.

Nutritional potentiality

Nutritional potentiality of *G. applanatum* was estimated on the basis of composition of carbohydrate, protein, fat, fiber and ash content of the fruiting body by using the formula proposed by Nile and Kobhragade (Nile and Khobragade, 2009).

Antibacterial assay

Antibacterial activity of *G. applanatum* extract was estimated by agar well diffusion and broth dilution method. The minimum inhibitory concentration (MIC) and zone of inhibition was estimated by agar well diffusion method and by broth dilution method %inhibition and complete inhibition represents the MIC was estimated.

Bacteria

Antibacterial activity of fungal extracts were carried out against *Salmonella typhi* (MTCC 3216) and *Proteus mirabilis* (MTCC 1429) was procured from Institute of Microbial Technology, Chandigarh, India and the culture of the bacteria was revived in nutrient broth media at 37ºC for 18 hours for agar well diffusion and broth dilution method.

Agar well diffusion method

The plates having nutrient agar media (Peptone-10 g, NaCl-10g and Yeast extract- 5g, Agar- 20g in 1000 ml of distilled water) were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100ì L of 10^{-4} cfu) and spread evenly on the plate. After 20 min, the wells were filled with 25 μg/mL to 800 μg/mL of extract. The control wells with gentamycin were also prepared to compare the antimicrobial efficacy of the extract. All the plates were incubated at 37ºC for 24 h and the diameter of inhibition zones and MICs (lowest concentration of the extract that showed inhibition) were noted (Threfall *et al.*, 1999; Kumar *et al.* 2013).

Broth dilution method

The test tubes containing nutrient broth media (Peptone-10 g, NaCl-10g and Yeast extract- 5g, in 1000 mL of distilled water) were prepared, autoclaved and different concentrations of the extract were added. Each tube was inoculated with 18 h old cultures (100ì L of 10^{-4} cfu). A control tube with inoculums and without any sample was prepared along with a sterile media tube as blank. All the tubes were incubated at 37ºC on a shaker with 140 rpm for 24 h and the growth was measured at 660 nm. The % of inhibition was calculated by using the formula below (Walker, 2000; Kumar *et al.*, 2013):

$$\%\,\text{Inhibition} = 100 - \left[\frac{\text{OD of culture with sample (test)}}{\text{OD of culture without sample (control)}}\right]X100$$

Statistical analysis

All results were expressed as mean ± standard deviation (SD). Data were analyzed using Student's t-test, $p < 0.05$ considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

Results of phytochemical screening of methanolic, ethanolic, chloroform and aqueous extract of *G. applanatum* are presented in Table 1. The results reveal that saponin content of aqueous extract (0.06 ± 0.05mg/100g) and methanolic extract (0.04 ± 0.005 mg/100g) is significantly ($p<0.001$) lower among all the studied phytochemicals. However, phenolic content (20.81± 0.14 mg/100g) of methanolic extract and flavonoid content (23.89 ± 0.38mg/100g) is significantly ($p<0.001$) higher among the studied phytochemicals quantity presented in Table 1.

Udu-Ibiam *et al*. (2014) reported 64.12 ± 1.2 mg/g phenols, 0.016 ± 0.001 mg/g flavanoid, 0.28 ± 0.04mg/g saponins, 0.1 ± 0.04% alkaloids and 0.014 ± 0.003 % tannins in *Tricholoma nudum* and 6.012 ± 0.91 mg/g phenols, 0.031 ± 0.02 mg/g flavanoid, 0.27 ± 0.008mg/g saponins, 2.0 ± 0.01% alkaloids and 0.014 ± 0.001 % tannins in *Psalliotaca mpestris*. Secondary metabolites such as phenols, tannins, saponins, alkaloids, flavonoids are responsible for inhibition of growth of pathogenic bacteria (Kumar *et al.,* 2013; Udu-Ibiam *et al.,* 2014; Kumar *et al.,* 2014). In the present study phytochemical compositions of *G. applanatum* extracts are higher than the above studied edible mushrooms. Therefore, *G. applanatum* may possess good antipathogenic activity.

Antipathogenicactivity

Antipathogenic efficacy of methanolic, ethanolic, chloroform and aqueous extract of *G. applanatum* were quantitatively assessed on the basis of zone of inhibition (ZOI) following the agar well diffusion method and minimum inhibitory concentration by broth dilution method. The test organisms were inoculated with standard antibiotic: gentamycin to compare the efficacy of fungal extract for their antimicrobial properties. The results reveal that synthetic antibiotic, Gentamycin show significantly ($p<0.001$) higher ZOI than extracts (Table 2 and Fig. 1) of *G. applanatum*. However, methanolic extract of *G. applanatum* showed significantly ($p<0.001$) higher ZOI than aqueous extract against *S. typhi* and *P. mirabilis* (Table 2 and Fig. 1).

The MICs of Gentamycin is higher than extracts of *G. applanatum* against both the tested pathogenic bacteria but the extracts also possess good MICs against tested pathogens. The MICs of methanolic, ethanolic and chloroform extract against *P. mirabilis* was same as compared with the MICs of methanolic and ethanolic extracts against *S. typhi* (Table 4).

In broth dilution method, the MICs of Gentamycin against *P. mirabilis* and *S. typhi* significantly ($p<0.001$) higher than the extracts of *G. applanatum* but the MICs of the extracts are also good against the tested pathogenic bacteria (Table 5). However, aqueous extract possess high MICs than methanolic extract against the studied pathogenic bacteria (Table 6).

Oyetayo and Ariyo (2013) studied the antibacterial activity of ethanolic extract of *Pleurotus ostreatus* and reported the MICs 15 mg/mL and 6.25 mg/mL against *S. typhi* and *P. mirabilis* respectively. Nagaraj *et al*. (2013) studied the antibacterial activity of *G. applanatum* against *K. pneumonia* and *P. aeruginosa* and reported 20mg/mL and 35mg/mL MICs of methanolic extract of *G. applanatum* against *K. pneumonia* and *P. aeruginosa* respectively. In the present study

Table 1 : Phytochemical composition of different extracts *G. applanatum* (M ± SD; n = 6).

Phytochemicals (mg/100g)	Different extracts of *G. applanatum*			
	Methanol	Ethanol	Chloroform	Aqueous
Alkaloid	0.83 ± 0.04*	0.8 ± 0.01**	0.53 ± 0.01*	0.75± 0.01*
Flavonoid	23.89 ± 0.38*	21.30 ± 0.63**	15.65 ± 0.53*	18.56± 0.28*
Tannins	2.31± 0.09*	1.35 ± 0.1**	0.76 ± 0.03*	1.28± 0.06**
Phenol	14.63± 0.21*	15.60 ± 0.61***	12.65 ± 0.17*	20.81± 0.14*

* = ($p<0.001$); ** = ($p<0.005$); *** = ($p<0.05$); ns = non significant

Table 2.: ZOI (in mm) and MIC (μg/mL) of *G. applanatum* extracts against *S. typhi* and *P. mirabilis* (M ± SD; n = 6).

Concentration (μg/mL)	Zone of Inhibition (mm) against *S. typhi*			
	Methanolic	Ethanolic	Chloroform	Aqueous
25	0	0	0	0
50	0	0	0	0
100	0	0	0	0
200	0	0	0	0
400	0	0	0	0
800	3.21 ± 0.06*	2.65± 0.01*	0	0
MIC (μg/mL)	800	800	800	0
Concentration (μg/mL)	Zone of Inhibition (mm) against *P. mirabilis*			
	Methanolic	Ethanolic	Chloroform	Aqueous
25	0	0	0	0
50	0	0	0	0
100	0	0	0	0
200	0	0	0	0
400	0	0	0	0
800	3.02 ±0.03*	2.31± 0.02*	1.98± 0.01*	0
MIC (μg/mL)	800	800	800	0

* = ($p<0.001$).

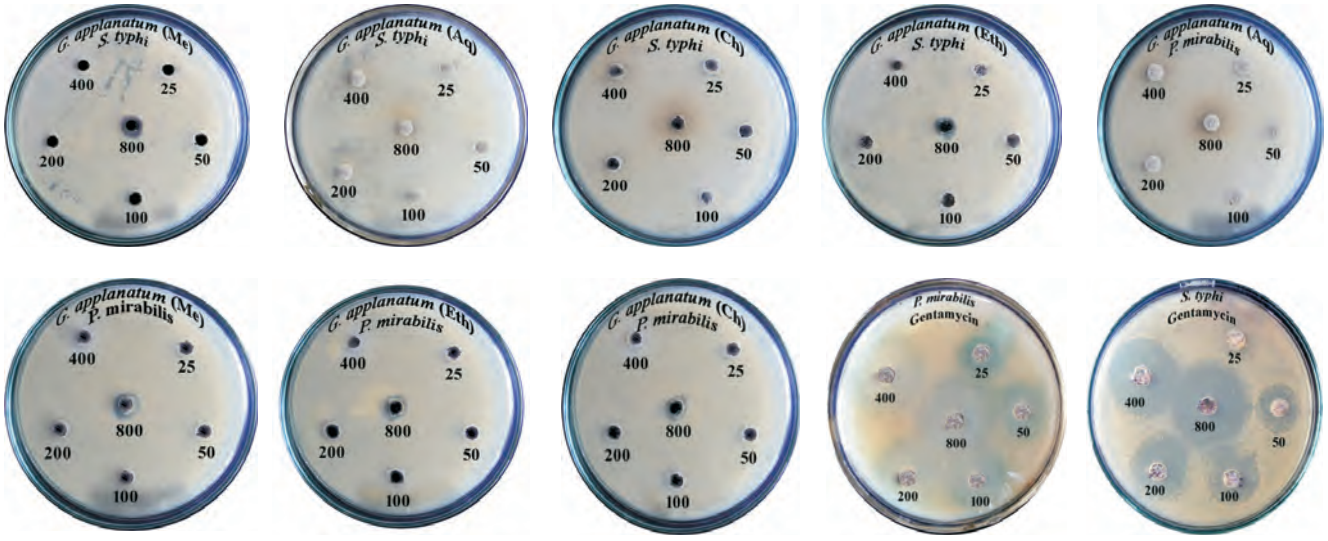

Figure 1: a, b, c and ZOI of methanol, aqueous, chloroform and ethanol extract of *G. applanatum* against *S. typhi*; e, f, g and h ZOI of aqueous methanol, ethanol and chloroform extract of *G. applanatum* against *P. mirabilis*; I and j ZOI of Gentamycin against *S. typhi* and *P. mirabilis*.

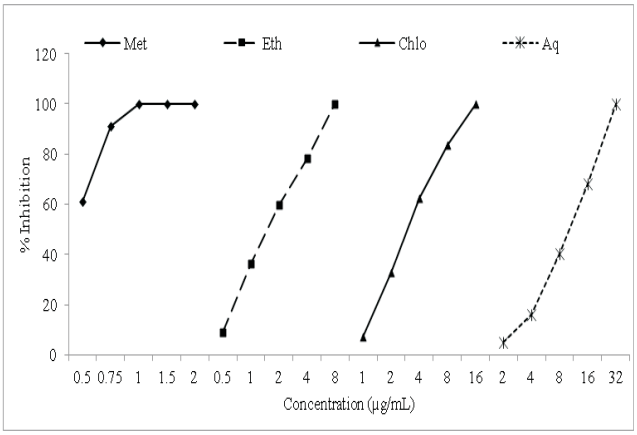

Figure 2: % Inhibition of *G. applanatum* methanolic (Me), ethanolic (Eth), chloroform (Chlo) and aqueous (Aq) extract against *P. mirabilis*.

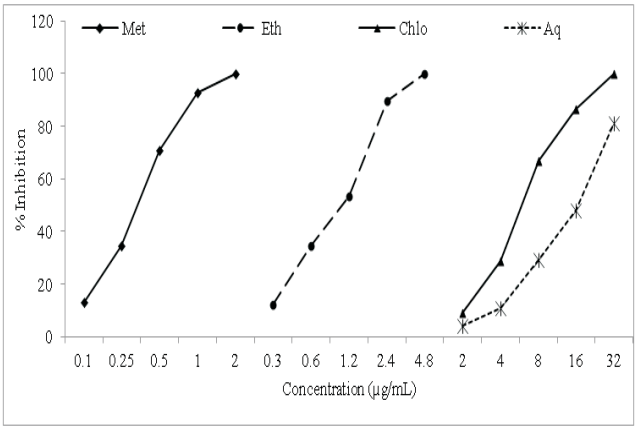

Figure 4: % Inhibition of *G. applanatum* methanolic (Me), ethanolic (Eth), chloroform (Chlo) and aqueous (Aq) extract against *S. thyphi*

antipathogenic activity of *G. applanatum* is much better than the antipathogenic efficacy of studied macrofungi against the above pathogenic bacteria.

Nutritional potentiality

The result of nutritive potentiality is presented in table 6. The result reveals carbohydrate content is significantly ($p < 0.001$) and fat content significantly ($p < 0.001$) low among the all nutritional components of *G. applanatum*. Jha and Tripathi (2012) studied nutritional composition of three ethnomycologically edible fungi and reported *Ramaria botrytis* possess higher nutritional composition (13.55% protein, 72.88% carbohydrate, 4.22% lipid, 7.25% ash, 5.0% fibre and 87.30%moisture) and *Laccaria lacata* possess lowest amount of nutritional components (25.71% protein, 58.50% carbohydrate, 3.30% lipid, 11.75% ash, 11.0% fibre and 87.88% moisture) and *Lycoperdon pyriforme* possess moderate amount of nutritional components. The ash content of any organic matter represents the inorganic constituents (minerals such as Na, K, Ca, Mg, Zn, Fe *etc.*) which are essential to human body for various metabolic processes (Stolen *et al.*, 2010; Vermani *et al.*, 2010). Fibre reduces the cholesterol absorption, carcinogenic toxins and free radical damage but major primary metabolites such as carbohydrate, protein and lipid are essential to provide energy to the body (Ebun-Oluwa and Alade, 2007; Vermani *et al.*, 2010).

Kakon *et al.* (2012) studied the nutritional potentiality of nine edible macrofungi and reported metabolize energy of *Trcinclla*

Table 3: ZOI (in mm) and MIC (µg/mL) of Gentamycin against *S. typhi* and *P. mirabilis* (M ± SD; n = 6).

Concentration (µg/mL)	Zone of Inhibition (mm)	
	S. typhi	*P. mirabilis*
25	2.02 ± 0.10*	9.04 ± 0.18*
50	13.13 ±0.20 ns	13.07 ± 0.12 ns
100	16.05 ± 0.21*	18.02 ± 0.28*
200	21.21 ± 0.20 ns	21.12 ± 0.24 ns
400	25.03 ± 0.26 ns	25.03 ± 0.16 ns
800	27.11 ± 0.20 ns	27.10 ± 0.20 ns
MIC (µg/mL)	25	25

* = ($p < 0.001$); ns = non significant

Table 4: MIC of Gentamycin and *G. applanatun* against *S. typhi* and

MIC(μg/mL)	Methanol	Ethanol	Chloroform	Aqueous	Gentamycin
S.typhi	800	800	0	0	25
P. mirabilis	800	800	800	0	25

Table 5: % Inhibition of gentamycin against *S. thyphi*and *P. mirabilis*

Organism	25 μg	50 μg	100 μg	200 μg	400 μg	800 μg	MIC μg
P. MirabilisS. typhi	92	1313	1816	2121	2525	2727	2525

Table 6: Minimum inhibitory concentration (in mg/mL) of different extract of *G. applanatum* against *P. mirabilis* and *S. typhi* (M± SD; n=6; a = $p<0.001$)

Microbes	Methanolic	Ethanolic	Chloroform	Aqueous	Gentamycin
P. mirabilis	1.02 ± 0.11*	2.53 ±	16.03 ±	32.14 ± 0.20*	0.025 ± 0.001*
S. typhi	2.05 ± 0.05*	4.83 ±	32.05 ±	NF	0.025 ± 0.001*

NF = not found; * = ($p<0.001$).

Table 7: Composition of nutritional components and nutritional value of *G. applanatum* (M± SD; n=6; a = $p<0.001$)

Attributes	Values in g%
Total ash	2.68 ± 0.21
Moisture	5.80 ±0.1
Crude protein	11.72 ±0.25
Crude fat	0.48 ±0.03*
Total carbohydrate	42.72 ±0.54*
Crude fibre	15.53 ±0.61
Metabolizable energy (K Cal/100g)	222.08 ± 2.26

fuciforinis (dried) is higher (412 K Cal) and *Agaricus bisporus* (fresh) is lower (328 Kcal) among all the studied macrofungi. In the present study the nutritional components and calorific value of *G. applanatum* is god compare to above studied fungi. Therefore, consumption of *G. applanatum* during and after pathogenic infection can improve the strength the immunity against the pathogenic infection by providing other essential metabolites.

In the present investigation *G. applanatum* extracts possess high growth inhibitory efficacy against *P. mirabilis and S. typhi* and possess high calorific value. There fore, *G. applanatum* can be used an antibiotic supplement against the diseases caused by pathogenic bacteria and can be used as food supplement.

ACKNOWLEDGEMENT

The authors acknowledge the financial assistance received from DBT, NER-BPMC, New Delhi (BT/462/NE/TBP/2013) under the Twinning Project to Department of Zoology, Ranchi University, Jharkhand and Department of Botany, Gauhati University, Guwahati, Assam.

REFERENCES

Aruoma, I. O. 1998. Free radicals, oxidative stress and antioxidant in human health and disease. *J. Amr. Oil Chem. Soc.* **75(2):** 199-212.

Crump, J. A., Okoth, G. O., Slutsker, L., Ogaja, D. O., Keswick, B. H. and Luby, S. P. 2004. Effect of point-of-use disinfection, flocculation and combined flocculation disinfection on drinking water quality in western Kenya. *J. Appli. Microbiol.* **97:** 225-231.

Dandapat, S. and Sinha, M. P. 2015. Antioxidant and anti-inflammatory activity of *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer. *Adv. Biol. Res.* **9(3):** 140-145.

Dandapat, S., Kumar, M. and Sinha, M. P. 2014. Nanobio pharmaceutical technology, In: S. Latha, S. Palanisamy, S. Natesan (Eds.). Nanomaterials: Synthesis and characterization of green silver nanoparticles mediated by *Aegle marmelos* (L.) leaf extract. ISBN: 978-935-107-293-5, *Elsevier India Pvt. Ltd, India,* pp. 31-37.

Dandapat, S., Kumar, M. and Sinha, M. P. 2014. Therapeutic efficacy of *Cinnamomum tamala* (Buch.-Ham.) and *Aegle marmelos* (L.) leaf. *Balneo Res. J.* **(5)3:** 113-122.

Dandapat, S., Kumar, M., Kumar, A. and Sinha, M. P. 2013. Antipathogenic efficacy of methanolic leaf extract of *Cinnamomum tamala* (Buch.-Ham.) and *Aegle marmelos* (L.) with their nutritional potentiality. *The Bioscan. Supplement on Medicinal Plants.* **8(2):** 635-641.

Dandapat, S., Kumar, M., Kumar, A. and Sinha, M. P. 2013. Therapeutic efficacy and nutritional potentiality of *Cinamomum tamala* (Buch.-Ham). In *Int. J. Pharma.* **3(4):** 779-785.

Ebun-Oluwa, P. O. and Alade, A. S. 2007. Nutritional potential of Berlandier nettle spurge (*Jatropha cathatica*) seed. *Pak. J. Nutrition*. **6:** 345-348.

Harborne, J. 1973. *Phytochemical Methods*. Chapman and Hall, London.

Helmja, K., Vaher, M., Gorbatsova, J. and Kaljurand, M. 2007. Characterization of bioactive compounds contained in vegetables of the Solanaceae family by capillary electrophoresis. *Proc. Estonian Acad. Sci. Chem.* **56:** 172-186.

Jha, S. K. and Tripathi, N. N. 2012. Comparative nutritional potential of three dominant edible and medicinal macrofungi of Kathmandu valley, Nepal. *Am. J. Pharma. Technol. Res.* **2(3):** 1037-1042.

Kakon, A. J., Choudhury, M. B. K. and Saha, S. 2012. Mushroom is an ideal food supplement. *J. Dhaka. National Med. College and Hospital*. **18(1):** 58-62.

Karaman, M., Vesic, M., Stahl, M., Novakovic, M., Janjic, L. and Matavuly, M. 2012. Bioactive Properties of wild-growing Mushroom Species *Ganorderma napplanatum* (Pers.) Pat. from Fruskagora forest (Serbia). *Ethnomed. Therap. Valid.* **32:** 361-377.

Kumar, A., Dandapat, S., Kumar, M. and Sinha, M. P. 2013. Evaluation of genotoxicity and cytotoxicity of *Tinospora cordifolia* (Thunb.). *The Bioscan. Supplement on Toxicology.* **8(3):** 1083-1087.

Kumar, A., Dandapat, S., Kumar, M. and Sinha, M. P. 2013. Antipathogenic efficacy and aemolytic activity of *Calotropis procera* leaves. *World J. Zool.* **8(4):** 366-370.

Kumar, A., Kumar, M., Dandapat, S. and Sinha, M. P. 2013. Antioxidant activity and pharmacological screening of *Tinospora cordifolia* (Thunb.). *The Bioscan. Supplement on Medicinal Plants.* **8(2):** 689-693.

Kumar, M., Dandapat, S., Kumar, A. and Sinha, M. P. 2013. Anti-

typhoid activity of *Adhatoda vasica* and *Vitex negundo*. *Persi. Gulf. Crop Protect.* **2(3):** 64-75.

Kumar, M., Dandapat, S., Kumar, A. and Sinha, M. P. 2013. Determination of nutritive value and mineral Elements of Five- Leaf Chaste Tree (*Vitex negundo* L.) And Malabar Nut (*Adhatoda vasica* Nees). *Academ. J. Plant Sci.* **6(3):** 103-108.

Kumar, M., Dandapat, S., Kumar, A. and Sinha, M. P. 2014. Pharmacological screening of leaf extract of *Adhatoda vasica* for therapeutic efficacy. *Global J. Pharmacol.* **8(4):** 494-500**.**

Kumar, M., Kumar, A., Dandapat, S. and Sinha, M. P. 2013. Growth inhibitory impact of *A. vasica* and *V. negundo* on some human pathogen. *The Ecoscan*. **4(Spl. issue):** 241-245.

Malick, C. P. and Singh, M. B. 1980. *Plant Enzymology and Histoenzymology*. Kalyani Publishers, New Delhi.

Mizuno, T. 1995. Bioactive biomolecules of mushrooms: Food function and medicinal effect of mushroom fungi. *Food Rev. Int.* **11:** 7-21.

Nagaraj, K., Mallikarjun, N., Naik, R. and Venugopal, T. M. 2013. Phytochemical analysis and *in vitro* antimicrobial potential of *Ganoderma applanatum* (Pers.) Pat. of Shivamogga District-Karnataka. In *Ind. Int. J. Pharma. Sci. Rev. Res*. **23(2):** 36-41.

Nagshetty, K., Channappa, S. T. and Gaddad, S. M. 2010. Antimicrobial susceptibility of *Salmonella typhi* in India. *J. Infect. Develop. Countr*y. **4(2):** 70-73.

Nile, S. H. and Khobragade, C. C. N. 2009. Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. *J. Med. Plants*. **8(5):** 79-88.

Oyetayo, V. O. and Ariyo, O. O. 2013. Antimicrobial and antioxidant Properties of *Pleurotus ostreatus* (Jacq: Fries) cultivated on different tropical woody substrates. In *J. Waste Conve. Bioprod. Biotechnol*. **1(2):** 28-32.

Sharma, A. K., Gupta, M., Shrivastav, A., Jana, A. M. 3013. Antioxidant and anticancer therapeutic potentiality of mushrooms: a review. *Int. J. Pharma. Sci. Res*. **4(10):** 3795-3802.

Soetan, K. O., Olaiya, C. O. and Oyewole, O. E. 2010. The importance of mineral elements for humans, domestic animals and plants: A review. *Afr. J. Food Sci.* **4(5):** 200-222.

Sofowara, A. 2008. Screening plants for boactive agents (3rd Eds.) In medicinal plants and traditional medicinal in Africa, ISBN-13: 978-0471103677, Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria pp.134-156.

Solanki, R. 2010. Some medicinal plants with antibacterial activity. *Int. J. Comprehen. Pharma.* **4(10):** 1-4.

Taylor, E. and Webster, T. J. 2011. Reducing infections through nanotechnology and nanoparticles. *Int J Nanomed.* **6:** 1463-1473.

Threlfall, E. J., Fisher, I. S. T., Ward, L., Tschape, H. and Gernersmidt, P. 1999. Harmonization of antibacterial susceptibility testing for Salmonella: Result of a study by 18 national reference laboratories within the Europian Union-funded Enter-Net group. *Microbiol. Drug Resistant*. **5:** 195-199.

Udu-Ibiam, O. E., Ogbu, O., Ibiam, U. A., Nnachi, A.U., Agah, M. V., Ukaegbu, C. O., Chukwu, O. S., Agumah, N. B. and Ogbu, K. I. 2014. Phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria. *J. Pharma. Biolo. Sci.* **9(3):** 86-91.

Vermani, A., Prabhat, N. and Chauhan, A. 2010. Physico-chemical analysis of ash of some medicinal plants growing in Uttarakhand, India. *Nature and Sci.* **8(6):** 88-91.

Walker, R. D. 2000. Antimicrobial therapy in veterinary medicine, In: I.F. Prescott, I.D. Baggot, R.D. Walker, I.A. Ames, (3rd Eds.). Antimicrobial susceptibility testing and interpretation of results. *ISBN*: 9780-8138-2206-8. Iowa State University Press, pp. 12-26.

Wasser, S. P. and Weis, A. 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (review). *Int. J. Medi. Mushrooms*. **1:** 31-62.

World Health Organization (WHO). 1998. Quality Control Methods for Medicinal Plant Materials. *WHO Library Cataloguing in Publication Data, Geneva*.