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

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## ABSTRACT

In the present study antibacterial activity of medicinal mushrooms *G. applanatum* was carried out by agar well diffusion 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 ( $0.06 \pm 0.05$ mg/100g) and methanolic extract ( $0.04 \pm 0.005$  mg/100g) significantly ( $p < 0.001$ ) low among all the studied phytochemicals and phenolic ( $20.81 \pm 0.14$  mg/100g) and flavonoid content ( $23.89 \pm 0.38$ mg/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  $3.21 \pm 0.06$  mm ZOI and  $3.02 \pm 0.03$  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 ( $222.08 \pm 2.26$ ; K Cal/100g) and contains highly nutritional components (11.72  $\pm$  0.25% crude protein, 0.48  $\pm$  0.03% crude fat and 42.72  $\pm$  0.54% carbohydrate). Therefore, *G. applanatum* can be used in pathogenic disease caused by *S. typhi* and *P. mirabilis*.

## 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, urethritis, 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<sub>2</sub> and washed again. The basediocarps were dried in shade under room temperature for six to seven days, powdered 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 45°C. Percentage yield of each

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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-Ciocalteu 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 boiling 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 µg/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

10µg/mL, 50µg/mL and 100µg/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 10µg/mL, 50µg/mL and 100µg/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 105°C 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 105°C 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 105°C and weighed ( $T_0$ ). The quantity of tannins was calculated as percentage using the formula suggested in the Quality control methods (WHO, 1998):

$$\text{Tannin(\%)} = \frac{[T_1 - (T_2 - T_0)] \times 500}{W}$$

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 37°C 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 (100µL 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 37°C 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 (100µL 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 37°C 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):

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

### Statistical analysis

All results were expressed as mean  $\pm$  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 ( $0.06 \pm 0.05$ mg/100g) and methanolic extract ( $0.04 \pm 0.005$  mg/100g) is significantly ( $p < 0.001$ ) lower among all the studied phytochemicals. However, phenolic content ( $20.81 \pm 0.14$  mg/100g) of methanolic extract and flavonoid content ( $23.89 \pm 0.38$ mg/100g) is significantly ( $p < 0.001$ ) higher among the studied phytochemicals quantity presented in Table 1.

Udu-Ibiam *et al.* (2014) reported  $64.12 \pm 1.2$  mg/g phenols,  $0.016 \pm 0.001$  mg/g flavanoid,  $0.28 \pm 0.04$ mg/g saponins,  $0.1 \pm 0.04\%$  alkaloids and  $0.014 \pm 0.003$  % tannins in *Tricholoma nudum* and  $6.012 \pm 0.91$  mg/g phenols,  $0.031 \pm 0.02$  mg/g flavanoid,  $0.27 \pm 0.008$ mg/g saponins,  $2.0 \pm 0.01\%$  alkaloids and  $0.014 \pm 0.001$  % 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.

### Antipathogenic activity

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  $\pm$  SD; n=6).**

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

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

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

Concentration ( $\mu$ g/mL)	Zone of Inhibition (mm) against <i>S. typhi</i>			
	Methanolic	Ethanol	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	$3.21 \pm 0.06^*$	$2.65 \pm 0.01^*$	0	0
MIC ( $\mu$ g/mL)	800	800	800	0
Concentration ( $\mu$ g/mL)	Zone of Inhibition (mm) against <i>P. mirabilis</i>			
	Methanolic	Ethanol	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	$3.02 \pm 0.03^*$	$2.31 \pm 0.02^*$	$1.98 \pm 0.01^*$	0
MIC ( $\mu$ g/mL)	800	800	800	0

\* = ( $p < 0.001$ ).

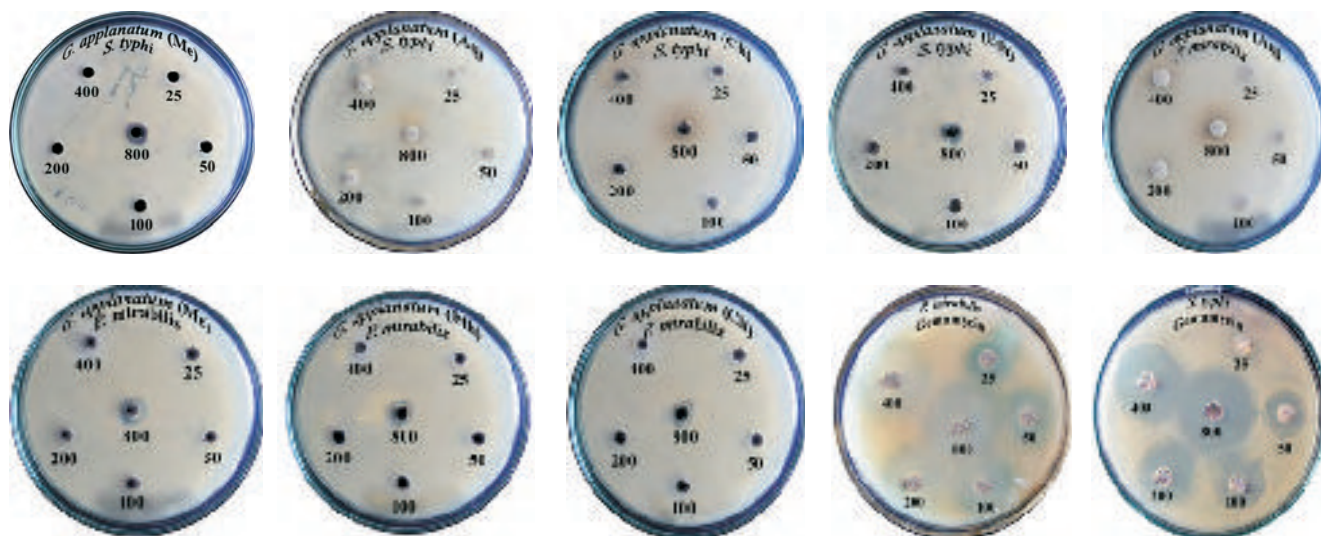


Figure 1: a, b, c and ZOI of methanol, aqueous, chloroform and ethanol extract of *G. applanatum* against *S. typhi*; e, f, g and h ZOI of aqueous methanol, ethanol and chloroform extract of *G. applanatum* against *P. mirabilis*; i and j ZOI of Gentamycin against *S. typhi* and *P. mirabilis*.

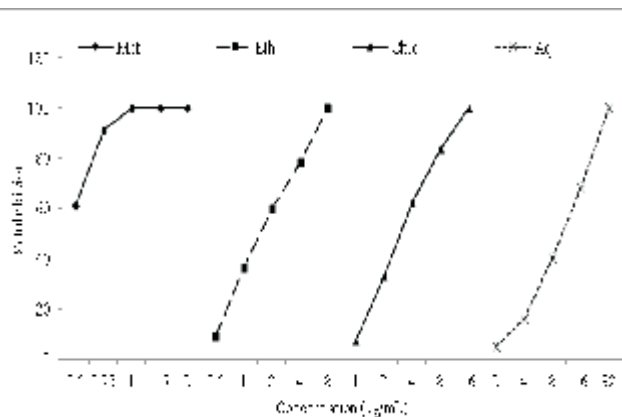


Figure 2: % Inhibition of *G. applanatum* methanolic (Me), ethanolic (Eth), chloroform (Chlo) and aqueous (Aq) extract against *P. mirabilis*.

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.,

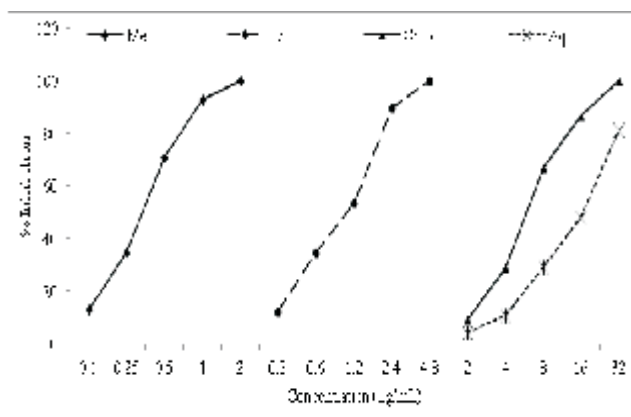


Figure 4: % Inhibition of *G. applanatum* methanolic (Me), ethanolic (Eth), chloroform (Chlo) and aqueous (Aq) extract against *S. typhi*

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 *Trcinlla*

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

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

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

MIC( $\mu$ g/mL)	Methanol	Ethanol	Chloroform	Aqueous	Gentamycin
<i>S.typhi</i>	800	800	0	0	25
<i>P. mirabilis</i>	800	800	800	0	25

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

Organism	25 $\mu$ g	50 $\mu$ g	100 $\mu$ g	200 $\mu$ g	400 $\mu$ g	800 $\mu$ g	MIC $\mu$ g
<i>P. Mirabilis</i> / <i>S. typhi</i>	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* ( $M \pm SD$ ; n=6;  $\alpha = p < 0.001$ )**

Microbes	Methanolic	Ethanolic	Chloroform	Aqueous	Gentamycin
<i>P. mirabilis</i>	1.02 $\pm$ 0.11*	2.53 $\pm$	16.03 $\pm$	32.14 $\pm$ 0.20*	0.025 $\pm$ 0.001*
<i>S. typhi</i>	2.05 $\pm$ 0.05*	4.83 $\pm$	32.05 $\pm$	NF	0.025 $\pm$ 0.001*

NF = not found; \* = ( $p < 0.001$ ).**Table 7: Composition of nutritional components and nutritional value of *G. applanatum* ( $M \pm SD$ ; n=6;  $\alpha = p < 0.001$ )**

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

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