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Sukumar Dandapat *et al.*,

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Antipathogenic efficacy of *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer. and *Ganoderma applanatum* (Pers.) Pat.

Sukumar Dandapat*[1], M. P. Sinha[1], Bharti Singh Raipath[2], T. C. Sarma[3], G. C. Sarma[3], Manalee Paul[3] and Jyoti Prova Barman[3]

[1]*Department of Zoology, Ranchi University, Ranchi - 834 008, Jharkhand, INDIA*
[2]*Department of Zoology, St. Xavier's College, Ranchi - 834 001, Jharkhand, INDIA*
[3]*Department of Botany, Gauhati University, Guwahati - 781 014, Assam. INDIA*
**e-mail: scholar.sukumar27@gmail.com*

Abstract. Anipathogenic efficacy of metanolic and aqueous extract of *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer. and *Ganoderma applanatum* (Pers. Ex Wallr.) Pat. has been tested against *P. mirabilis* and *S. typhi*. *Pleurotus tuber-regium* significantly ($p<0.001$) showed higher antipathogenic efficacy due to presence of significantly ($p<0.001$) higher pytochemical constituents than *Ganoderma applanatum*. Methanolic extract of *P. tuber* regium contain alkaloids (30.54 ± 0.32 mg/100g) and tannins (3.21 ± 0.30mg/100g) significantly ($p<0.001$) in higher quantity and saponine (20.19 ± 0.21mg/100g) content of aqueous extract of *P. tuber-regium* significantly ($p<0.001$) higher among the studied phytochemicals in both macrofungi. However, aqueous extract of *G. applanatum* contain alkaloids (0.75± 0.01mg/100g) tannins (1.28± 0.06mg/100g) significantly ($p<0.005$) in lower quantity and phenols (20.81± 0.14mg/100g) significantly ($p<0.05$) higher quantity among all the studied phytochemicals. Highest ZOI of aqueous extract of *P. tuber-regium* (9.01 ± 0.24 mm) was observed against *S. typhi* in agar disc diffusion method. Howevere, in broth dilution 100% inhibition observed against all the pathogens.

*Keywords:*Fungi, Disease, Nutritional, Phytochemical.

1. Introduction

Infectious diseases are the major cause of death in developing countries of the world and pathogenic microorganisms such as bacteria, viruses, protozoa and multi cellular parasites are the major agent of pathogenesis which is transmitted from one person to another person by vectors [1, 2]. Two most common pathogenic bacteria are *Salmonella typhi* and *Proteus*

*Corresonding author.

mirabilis have been studiedby several workers. *P. mirabilis* is known to cause disease like urethitis, cystitis, pylonephritis, prostatitis and pneumonia [3, 4]. Typhoid fever is a global infection which is predominantly caused by *S. typhi* [4, 5].

Synthetic antimicrobial drug and antibiotic chemotherapy has been used as most important weapon used against pathogenic bacterial infections since their introduction in pharmacology [6, 7]. However, over the past few decades commonly used antibiotics such as streptomycin, amoxicilin, tetracycline, ampicillin, kanamycin and chloramphenicol, cephalosporin etc. has became less effective in pathogenic diseases due to indiscriminate use in the treatment of infectious diseases which leads to emergence of multi drug resistant bacteria [8, 9]. Fungi have been used as a potential source of antibacterial agent since the first discovery of antibiotic penicillin from *Penicillium chrysogenum.* Recently Inouye *et al*. [10] reported that among 12, 000 known antibiotics approximately 55% are produced by *Streptomyces*, 11% by other Actinomycetes, 12% from other bacteria and 22% from filamentous fungi.

Worldwide, mushrooms have been used as regular diet for their nutritional and medicinal efficacy by most ethnic group of peoples of developing countries [12, 13]. Mushroom contains various bioactive primary and secondary metabolites which possess therapeutic efficacy against diseases and disorders [13].

Edible macro fungi or mushrooms belongs to the phylum Basidiomycota which includes 80 families, 550 genera and 10,000 species among which approximately 700 species have been reported for their significant pharmacological activity [14-16].

Pleurotus tuber-regium commonly called as king tuber mushroom, is an edible gilled fungus belonging to family Pleurotaceae and *Ganoderma applanatum* belonging to family Ganodermataceae has been used in typhoid, tuberculosis, pneumonia, hepatitis, renal infection and other diseases [17-19].

Therefore, present study was undertaken to analyse the present phytochemicals and antipatogenic efficacy of *Pleurotus tuber-regium* and *Ganoderma applanatum* extracts against *P. mirabilis* and *S. typhi*.

2. Materials and methods

2.1 *Collection of Macrofungi*

Fresh fruiting bdies of *Pleurotus tuber-regium* and *Ganoderma applanatum* were collected (Fig. 1 and 2) from different sites of three National Parks (Orang National Park, Kaziranga National Park and Manas National Park) of Assam and were identified in laboratory of Department of Botany, Gauhati University, Guwahati, Assam.

2.2 *Extract preparation*

The fresh mushrooms were washed and disinfected by treating with $HgCl_2$ and washed again. The mushrooms were dried in shade under room temperature for six to seven days, powered and sieved [20] 50g of the fine powder was subjected to extraction by soxhlet using methanol and distilled water separately for methanolic and aqueous extract. The extract obtained was filtered, concentrated and dried in rotary flash evaporator maintained at 45ºC for proper dehydration methanol free because methanol induce toxicity to living organisms. Percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies [21].

Figure 1. Fruiting bdies of *Pleurotus tuber-regium*(1A*) and *Ganoderma applanatum*(1B**)
* Source: http://greenzaku.deviantart.com/art/Pleurotus-tuber-regium-152260229
** Source: http://hongosgalicianportugal.blogspot.in/2010/06/aphyllophorales-b.html

2.3 *Phytochemical screening*

Estimation of total phenol, falavonoids, tannins, saponins and alkaloids content of fungal extracts were done followed [22]. The details have been described elsewhere by Dandapat *et al*. [23].

2.4 *Antipathogenic efficacy*

Antipathogenic efficacies of fungal extracts were carried out against *Salmonella typhi*(MTCC 3216) and *Proteus mirabilis* (MTCC 1429) by agar disc diffusion method and by broth dilution method comparing with standard antibiotic Gentamycin. The details have been described elsewhere Kumar *et al.* [24].

2.5 *Statistical analysis*

All results were expressed as mean ± standard error of mean (S. E. M.). Data was analysed using Student's t-test, $p< 0.05$ was considered as statistically significant.

3. Results and Discussion

3.1 *Phytochemical screening*

Results of phytochemical screening of aqueous (Aq) and methanolic extract (Me) of *P. tuber-regium* and *G. applanatum* are presented in Table 1. The results revealed that methanolic extract of *P. tuber regium* contain alkaloids (30.54 ± 0.32 mg/100g) and tannins (3.21±0.30mg/100g) significantly ($p<0.001$) in higher quantity and saponine (20.19±0.21mg/100g) content of aqueous extract of *P. tuber-regium* significantly ($p<0.001$) higher among the studied phytochemicals in both macrofungi (Table 1). However, aqueous extract of *G. applanatum* contain alkaloids (0.75± 0.01mg/100g) tannins (1.28± 0.06mg/100g) significantly ($p<0.005$) in lower quantity and phenols (20.81± 0.14mg/100g) significantly ($p<0.05$) higher quantity among all the studied phytochemicals (Table 1).

Laganathan *et al*. [25, 26] reported ethanol extract of *P. sajor-caju, P. florida* and *P.*

Table 1. Phytochemical composition of *P. tuber-regium* and *G. applanatum* (M ± SD; n=6).

Phytochemicals	*P. tuber-regium*		*G. applanatum*	
(mg/100g)	Aqueous	Methanolic	Aqueous	Methanolic
Alkaloids	28.14 ± 0.50 [a]	30.54 ± 0.32[b]	0.75± 0.01[a]	0.83± 0.04[a]
Saponins	20.19 ± 0.21 [a]	16.34 ± 0.27[a]	0.06± 0.005[a]	0.04± 0.005[a]
Flavonoids	13.78 ± 0.52 [a]	19.67 ± 0.22[a]	18.56± 0.28[a]	23.89± 0.38[a]
Tannins	2.74 ± 0.28[a]	3.21 ± 0.30[a]	1.28± 0.06[b]	2.31± 0.09[ns]
Phenols	20.19 ± 0.25 [a]	19.50 ± 0.31[c]	20.81± 0.14[c]	14.63± 0.21[a]

a = (p<0.001); b= (p<0.005); c = (p<0.05); ns = non significant

aureovillosus contains 6.001 ± 0.04µm/g, 7.501 ± 0.10µm/g and 6.72 ± 0.05µm/g phenol components respectively. Udu-Ibiam *et al.* [27] 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 % tanninsin *Psalliota campestris.* Secondary metabolites such as phenols, tannins, saponins, alkaloids, flavonoids are responsible for inhibition of growth of pathogenic bacteria [23, 24]. In the present study phytochemical compositions of both *P. tuber-regium* and *G. applanatum* is higher than the above studied edible mushrooms. Tannins form irreversible complexes with prolene rich protein resulting in the inhibition of cell wall synthesis [7, 23 and 28]. Flavonoids act as antimicrobial agent by inhibiting activity of reverse transcriptase, RNA-directed DNA polymerase, anti integrase and antiprotease [7, 29 and 30]. Saponin kills pathogenic protozoa by forming complexes with sterols in the protozoa membrane surface due to which the membranes become impaired and eventually disintegrate [31]. Phytophenols inhibits the bacterial growth by the blockage of protein synthesis at transcription or at translation level and inhibition of peptido-glycan synthesis at membrane level [32].

3.2 *Antipathogenic efficacy*

Antipathogenic efficacy of aqueous and methanolic extract of *P. tuber-regium* and *G. applanatum*

Table 2. ZOI (in mm) of aqueous and methanolic extract of *P. tuber-regium, G. applanatum* and Gentamycin against *S.typhi* (M ± SD; n=6).

S.typhi					
Concentra-tion (µg/mL)	Gentamycin	*P. tuber-regium*		*G. applanatum*	
		Aq	Me	Aq	Me
25	2.02 ± 0.10 [a]	0	0	0	0
50	13.13 ±0.20 [a]	0	0	0	0
100	16.05 ± 0.21 [a]	0	0	0	0
200	21.21 ± 0.20 [a]	4.13 ± 0.08 [a]	0	0	0
400	25.03 ± 0.26 [a]	5.06 ± 0.20 [a]	0	0	0
800	27.11 ± 0.20 [a]	9.01 ± 0.24 [a]	2.31 ± 0.158[a]	0	3.21 ± 0.06 [a]

Aq = Aqueous extract; Me = Methanolic extract; a = (p<0.001)

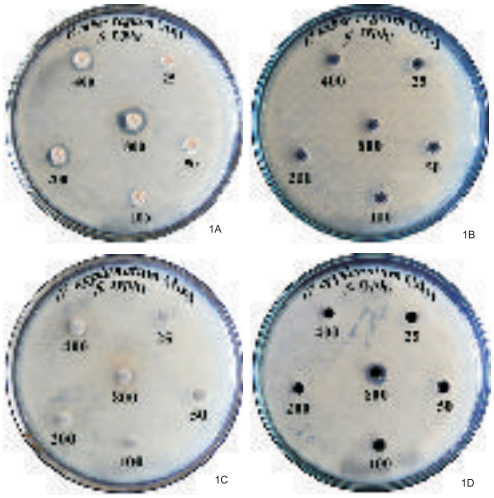

Figure 2. (a, b) ZOI of aqueous and (c, d) methanolic extract of *P. tuber-regium* and *G. applanatum* against *S. typhi.*

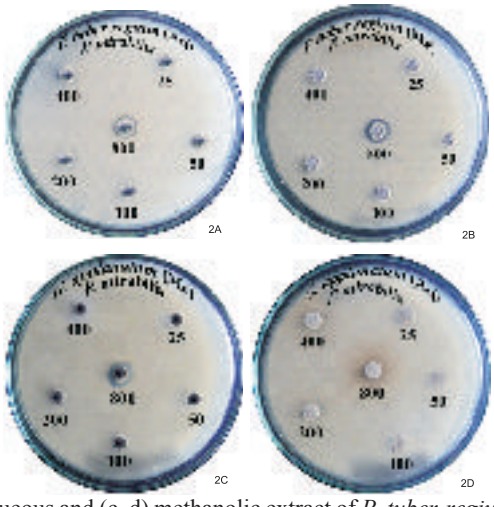

Figure 3. (a, b) ZOI of aqueous and (c, d) methanolic extract of *P. tuber-regium* and *G. applanatum* against *P. mirabilis*

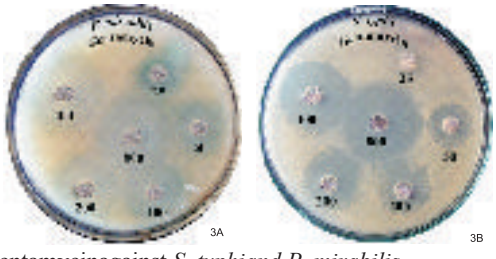

Figure 4. (a, b) ZOI of Gentamycinagainst *S. typhiand P. mirabilis*

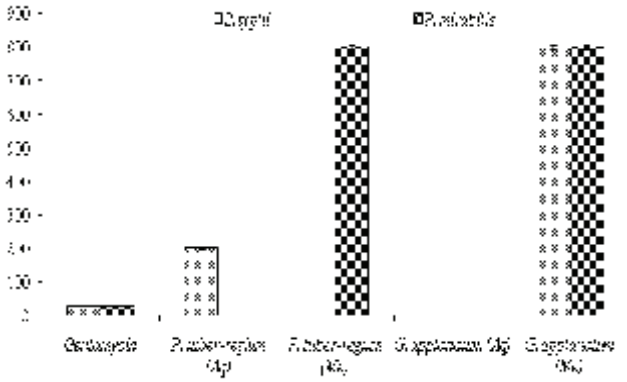

Figure 5. MIC (µg/mL) of Gentamycin, *P. tuber-regium* and *G. applanatun* against *S. typhi* and *P. mirabilis* (M ± SD; n=6; a = p<0.001).

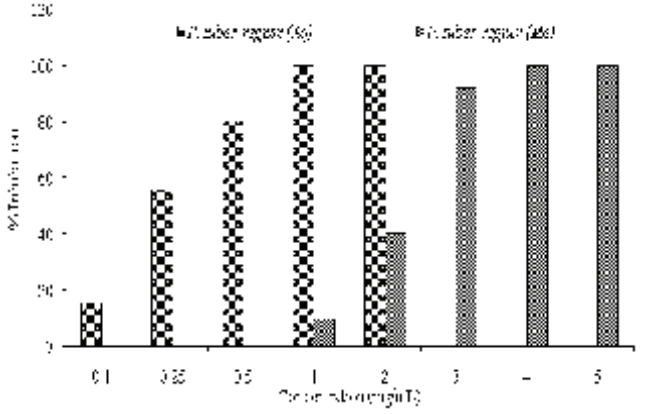

Figure 6. % Inhibition of *P. tuber-regium* aqueous (Aq) and methanolic (Me) extract against *P. mirabilis*

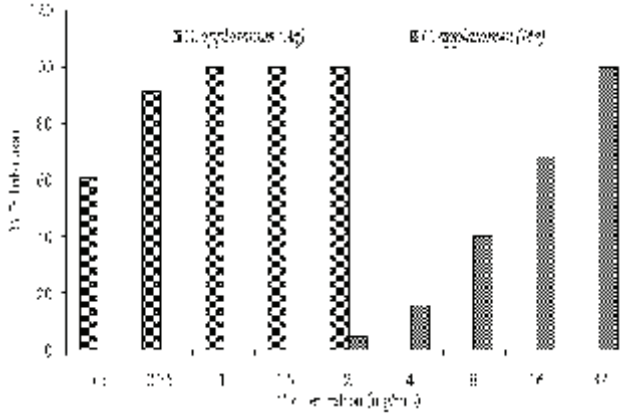

Figure 7. % Inhibition of *G. applanatumaqueous* (Aq) and methanolic (Me) extract against *P. mirabilis*

Table 3. ZOI (in mm) of aqueous and methanolic extract of P. tuber-regium,*G. applanatum* and *Gentamycin against P. mirabilis*(M ± SD; n=6).

P. mirabilis					
Concentration (µg/mL)	*Gentamycin*	*P. tuber-regium*		*G. applanatum*	
		Aq	Me	Aq	Me
25	9.04 ± 0.18 [a]	0	0	0	0
50	13.07 ± 0.12 [a]	0	0	0	0
100	18.02 ± 0.28 [a]	0	0	0	0
200	21.12 ± 0.24 [a]	0	0	0	0
400	25.03 ± 0.16 [a]	0	0	0	0
800	27.10 ± 0.20 [a]	0	3.02 ± 0.17[a]	0	3.02 ± 0.03[a]

Aq = Aqueous extract; Me = Methanolic extract; a = (p<0.001).

were quantitatively analysed on the basis of zone of inhibition (ZOI) in mm (Table 2 and 3; Fig. 2, 3 and 4) and minimum inhibitory concentrations (MICs) against the pathogenic bacteria were calculated (Fig. 5). The results reveal that synthetic antibiotic, Gentamycin showed significantly (p<0.001) higher ZOI than extracts of *P. tuber-regium* and *G. applanatum*. However, aqueous extract of *P. tuber-regium*shows significantly (p<0.001) higher ZOI than aqueous extract of *G. applanatum* and methanolic extracts of both the fungi (Table 1 and Fig. 2 and 3).

Antipathogenic efficacy of methanolic extracts *G. applanatum* and *P. tuber-regium* shows significantly (p<0.001) equivalent efficacy against *S. typhi* which were significantly low (p<0.001) as compared to gentamycin. The aqueous extracts does not show any ZOI aginst *S. typhi* (Table 3 and Fig. 2, 3).

The MICs of Gentamycin is significantly (p<0.001) higher than aqueous and methanoli extracts of both the macrofungi against studied pathogenic bacteria. However, the MIC of aqueous extract of *P. tuber-regium* significantly (p<0.001) higher than methanolic extract of *G. applanatun* against *S. typhi*and aqueous extract of *P. tuber-regium* and *G. applanatun* did not possess MICs against *P. mirabilis* (Fig. 4).

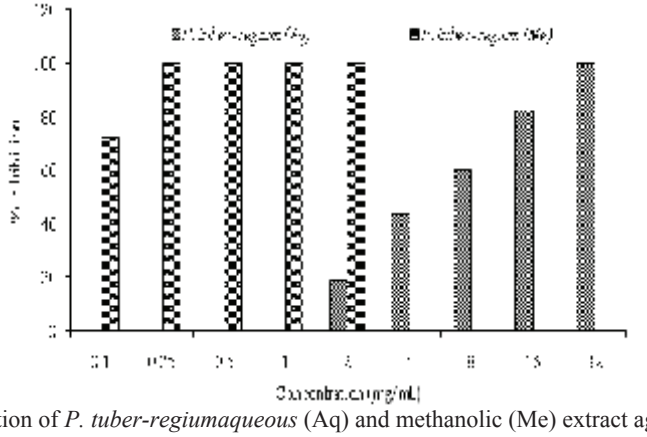

Figure 8. % Inhibition of *P. tuber-regiumaqueous* (Aq) and methanolic (Me) extract against *S. typhi*

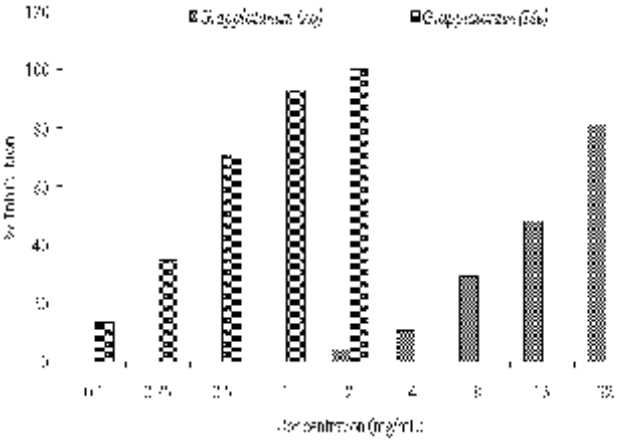

Figure 9. % Inhibition of *G. applanatumaqueous*(Aq) and methanolic (Me) extract against *S. typhi*

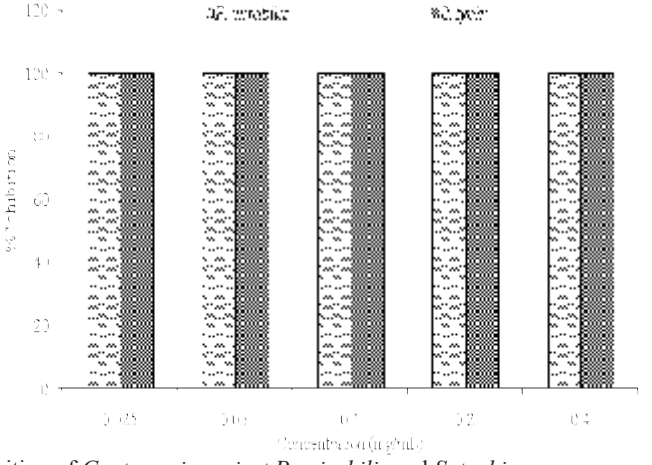

Figure 10. % Inhibition of *Gentamycinagainst P. mirabilis* and *S. typhi.*

Broth dilution method provides more pronounced antipathogenic efficacy of *P. tuber-regium, G. applanatum* extracts and Gentamycin against *S. typhi* and *P. mirabilis* by showing 100% inhibition (Fig. 6 to 10). Aqueous and methanolic extracts of both the macrofungi shows 100% inhibition against *S. typhi* and *P. mirabilis* except aqueous extract of *G. applanatun*, which does not show 100% inhibition against *S. typhi* (Fig. 9). However, gentamycin shows much higher efficacy than extracts of *P. tuber-regium* and *G. applanatun* by 100% inhibition of *S. typhi*and *P. mirabilis* (Fig. 10).

The MICs of Gentamycin against *P. mirabilis* and *S. typhi*in broth significantly (p<0.001) higher than the extracts of macrofungi. However, extracts also possess high MICs against the studied pathogenic bacteria. Aqueous extract of *P. tuber-regium* possess significantly (p<0.001) higher MIC (0.25 ± 0.01mg/mL) against *S. typhi* and methanolic extract of *G. applanatum* possess significantly (p<0.001) higher MIC against *P. mirabilis* compare to other extracts of both the fungi (Table 4).

Table 4. Minimum inhibitory concentration (in mg/mL) of different extract of *P. tuber-regium* and *G. applanatum* against *P. mirabilis* and *S. typhi*(M± SD; n=6; a = p<0.001)

Microbes	Gentamycin	P. tuber-regium		G. applanatum	
		Aqueous	Methanolic	Aqueous	Methanolic
P. mirabilis	0.025 ± 0.001 [a]	4.02 ± 0.20[a]	1.03 ± 0.10[a]	32.14 ± 0.20[a]	1.02 ± 0.11[a]
S. typhi	0.025 ± 0.001 [a]	0.25 ± 0.01[a]	32.21 ± 0.23[a]	2.05 ± 0.05[a]	NF

NF= not found; a = (p<0.001)

Akyuz *et al.* [33] studied the growth inhibitory impact of methanolic extract of *Pleurotus* species compared to standard anti biotic Streptomycin and Nystatinagainst pathogenic bacteria *Bacillus megaterium*, *S. aureus*, *E. coli*, *Klebsiella pneumoniae*, *C. albicans* and *C. glabrata* and they reported inhibitory impact of extract of *Pleurotus*species were lower than theused synthetic antibiotics. Iwalokun *et al.* [34] reported antimicrobial activity of mushrooms depended upon the nature of the solvent. Similarly, in the present study the inhibitory efficacy of Gentamycin is higher than the fungal extracts. Oyetayo and Ariyo [35] studied the antibacterial activity of ethanolic extract of *Pleurotus ostreatus* reported the MICs 15 mg/mL and 6.25 mg/mL against *S. typhi* and *P. mirabilis* respectively. Nagaraj *et al.* [36, 37] 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 MICs (200 ± 4.63µg/mL and 0.25 ± 0.01mg/mL in agar disc diffusion and broth dilution method respectively) of aqueous extract of *P. tuber-regium*ag ainst *S. typhi*and (1.02 ± 0.11 mg/mL in broth dilution method) of methanolic extract of *G. applanatum* against *P. mirabilis* were much higher than the above studied bacteria by various workers.

In the present study both *P. tuber-regium* and *G. applanatum* possess higher antipathogenic efficacy against *P. mirabilis* and *S. typhi* due to presence of phytochemicals. Therefore, *P. tuber-regium* and *G. applanatum* extract can be used in the diseses caused by *P. mirabilis* and *S. typhi*.

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