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Antimicrobial and antioxidant analysis of *Strobilanthes glutinous*: an unexplored medicinal plant

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The antimicrobial and antioxidant activity of leaf and bark extract of *Strobilanthes glutinous* Nees was evaluated by using different protocols. The petroleum ether, chloroform, methanol and aqueous were used as solvents for maceration process. The inhibition zones were measured via agar well diffusion method. Plant crude extract were tested using bacterial and fungal strains and also compared with standard antibiotic discs. Among antibiotics tetracycline exhibited higher zone revealed (20±0.6 mm) against *S. aureus*. The methanolic bark extract revealed maximum inhibition zone (18±0.5 mm) against *P. aeruginosa* whereas the minimum inhibition zone (10±0.2 mm) was examined in the bark extract of chloroform against *P. aeruginosa*. The highest antifungal potential was revealed by chloroform extract of bark (14±0.5 mm) against *A. niger* and lowest was observed by the leaf extract of petroleum ether against *A. oryzae* i.e. 9 ± 0.4 mm. The *S. glutinosus* Nees bark and leaf extract of aqueous could not show any effect against fungal pathogens. The total phenolic contents were observed maximum in methanolic bark extract, i.e. 1.573±0.31 µg/mL GAE. The methanolic leaf extract have maximum absorbance of flavonoid content, i.e. 1.27±0.24 µg/mL. The highest percentage value of DPPH was showed with chloroform leaf, i.e. 77.12% and lowest, i.e. 14.02% in the bark extract of aqueous. The plant extracts of *S. glutinosus* Nees revealed significant results in antimicrobial and antioxidant activities so it was concluded that more investigation is needed for further isolation and screening of chemical constituents present in this plant.

Keywords: Antimirobial, Antioxidant, *Strobilanthes glutinous* Nees

INTRODUCTION

The plants have been used as a source of traditional remedy in various human cultures around the world for medicinal purposes and play significant role in health care. The plants have been used for medicinal purposes due to lower cost, high accessibility and tolerability. The plants are used for extraction of verities of drugs by World health organization.

It has been considering that almost 25% of advanced nations and 75-80% of the urbanized nations used indigenous plants for remedy sources (Burkill et al.1966). The Pakistani people livelihood have mostly reliant on plants, as people acquired a lot of remuneration through utilizing of plants in many activities of their life such as in folklore medicines, manufacturing functions, bonfire, animal fodders, grocery and so many others (Altaf et al., 2109). A person of rustic regions depends upon folklore medicines due to

unavailability of proper facilities and enduring a lot of trouble related to health (Maqbool et al., 2019).

Human beings are ubiquitously affected by various pathogenic bacteria and microbes causing different diseases. The rural population of the world are being affected by bacterial and fungal strains due to malnutrition and unhygienic conditions. So there is need to treat these diseases with antibiotics and affective compounds that fight against their resistance property (Baravalia et al., 2009; Ajaib et al., 2019). According to estimation, almost 3 million people died due to *Salmonella typhimurium*. *E. coli* has also influenced the human life. *Staphylococcus aureus* has created several diseases such as pneumonia and urinary troubles (Sarbadhikary et al., 2015). Antioxidants are those chemical compounds which reduce the oxidative stress because free radicals are producing constantly in body and responsible for cell damage and causing various health disease, diabetes, and cancer. The natural antioxidants such as flavonoids, vitamin C and other phenolic compounds are present in many medicinal plants which are affective against many diseases (Dehshari et al., 2012)

Acanthaceae is a family of dicotyledonous flowering plants having about 250 genera and 4,000 species. The members of this family are tropical but some are found in temperate region.

Figure 1: *Strobilanthes glutinosus*

This family is widely used in drug formation and plantof this family are known for medicinal importance (Malik and Ghafoor, 1988). *Strobilanthes* is a genus belongs to family Acanthaceae having 350 species (Moylan et al., 2004). *S. glutinosus* Nees (Fig. 1) is a perennial plant, 1.5 m high, having stalked leaves covered with hairs and pale blue flowers. The plant is generally located in Western Himalayas, and spread from Kashmir to Nepal (Rahman et al., 2012).

MATERIALS AND METHODS

Plant Material

Strobilanthes glutinosus Nees was collected from the region of Kotli Azad Jammu & Kashmir (AJK) and identified from Herbarium with voucher specimen number MUST.BOT. 5356. from Department of Botany.

Test organisms

Both gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) were selected for antibacterial activity. Whereas *Aspergillus niger* and *Aspergillus oryzae* were used for investigation of antifungal potential of *S. glutinosus* Nees. The standard antibiotics of bacterial strains such as Tetracycline, Erythromycin, and Cefoperazon whereas standard for antibiotics of fungal strains (Ampicillin and Terbinafine) were tested for comparing the potential of plant extract with them.

Methodology adopted

The whole plant was washed with cold water for removing of mud. After washing the plant parts such as leaves and bark were separated and kept for drying under room temperature. After drying, the powder was then exposed for maceration process.

Maceration of plant material

The plant parts (leaf and bark) were shade dried and then subjected for maceration by using Chloroform, Petroleum Ether, Methanol and distilled water as a solvent to get crude extracts.

Evaluation of antimicrobial activity

Agar well diffusion method of Cruick-Shank et al., (1975) in which Nutrient agar media was used for the antimicrobial activity of *S. glutinosus* Nees. For antifungal activity following the method of (Johansen, 1940) in which Potato Dextrose Agar media were used.

Evaluation of antioxidant activity

For estimation of Antioxidant activity of leaf and bark extract of *S. glutinosus* Nees following parameters were used i.e. Total Phenolic Contents, Total Flavonoid Content and DPPH radical scavenging action.

Total Flavonoid Contents (TFC)

The flavonoids contents of *S. glutinosus* were estimated by using the procedure of Dewano et al., (2002).

Total Phenolic Contens

For the determination of phenolic contents, the methodology of Makker et al., (1993) was used.

DPPH radical scavenging activity

The assay of **(**Wang et al., 2011) was employed for the investigation of DPPH radical scavenging action.

RESULTS

Antimicrobial activity

The antimicrobial action was evaluated in contrast to bacterial strains by employing agar well diffusion method. For comparing the potential of *S. glutinosus* extracts against standard antibiotics with bacterial strains three antibiotics tetracycline, erythromycin and cefoparazon were applied. The inhibition zones of standard antibiotics were measured with all strains of bacteria. Tetracycline exhibited higher zone of inhibition against *S. aureus* (20.6 ± 0.8 mm) and lower was examined in erythromycin 15 ± 0.7 mm against *E. coli.* Cefoparazone has highest inhibition against *B. subtilis* 19 ± 0.5 mm than other strains (Table 1).

The methanolic extract of bark showed Maximum zone of inhibition against *P. aeruginosa* 18 ± 0.5 mm. The lower inhibition zone was measured in the bark extract of chloroform with *P. aeruginosa* 10 ± 0.4 mm and with leaf extracts of P. ether against *P. aeruginosa* 10.7 ± 0.4 mm. The bark extract of methanol exhibited highest inhibition zone 17.5 ± 0.2 mm against *S. aureus*. The chloroform extract of plant parts confirmed very close inhibition zones. Aqueous extract did not perform any activity with all microbes (Table 2).

The maximum inhibition potential in bacterial strain of *E. coli* was displayed in the *S. glutinosus* Nees. bark extract of methanol with 14 ± 0.5 mm and lower zone of inhibition 10.7 ± 0.4 mm was exhibited by leaf extract of p. ether 10.7 ± 0.4 mm. Both leaf and bark extracts of chloroform showed closed inhibition against *E. coli* (Figure 2).

The methanol bark extract of *S. glutinosus* Nees displayed maximum inhibition against *P. aeruginosa* (18 ± 0.5 mm) and lowest was exhibited by chloroform bark i.e. 10 ± 0.4 mm. The bark extract of p. ether has lowest inhibition 11 ± 0.8 mm rather than the leaf extracts (Figure 3).

Table 1: Zone of Inhibition exhibited by antibiotics against bacterial strains

Antibiotics standard disc	Zone of inhibition (mm)			
	E. coli	S. aureus	P. aeruginosa	B. subtilis
Tetracycline	19 ±0.8	20.6 ± 0.8	20.1± 0.6	16.2 ± 0.7
Erythromycin	15 ± 0.7	16 ± 0.5	16 ± 0.5	17 ± 0.8
Cefoparazon	16 ± 0.7	17.8±0.7	18.2 ± 0.4	19 ± 0.5

Table 2: Zone of Inhibition exhibited by bark and leaf extract of *S. glutinosus* against bacterial strains

Plant parts	Solvents	Zone of inhibition(mm)			
		E. coli	S. aureus	P. aeruginosa	B. subtilis
Bark	**P. ether**	12.1 ± 0.7	15 ± 0.7	11 ± 0.8	12.8 ± 0.7
	Chloroform	12.33 ± 0.8	13 ± 0.7	10 ± 0.4	11.66 ± 0.4
	Methanol	14 ± 0.5	17.5 ±0.2	18 ± 0.5	13 ± 0.5
	Aqueous	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Leaf	**P. ether**	10.7 ± 0.4	13.2 ± 0.4	12.66 ± 0.8	13.8 ± 0.9
	Chloroform	12 ± 0.5	11.3 ± 0.8	12.5 ± 0.5	11.16 ± 0.6
	Methanol	12.16 ± 0.4	17.2 ± 0.7	14.16 ± 0.6	14 ± 0.5
	Aqueous	0 ± 0	0 ± 0	0 ± 0	0 ± 0

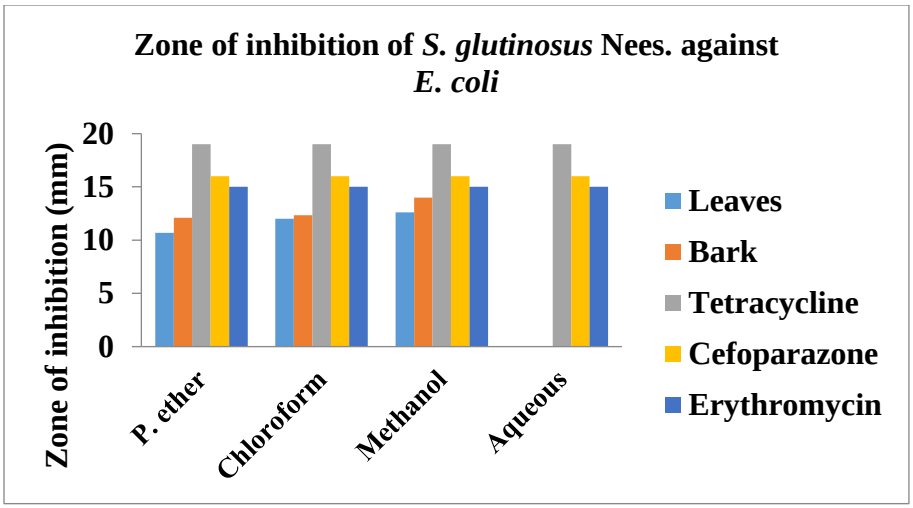

Figure 2: zone of inhibition produced by leaves and bark extracts of *S. glutinosus* against *Escherichia coli*

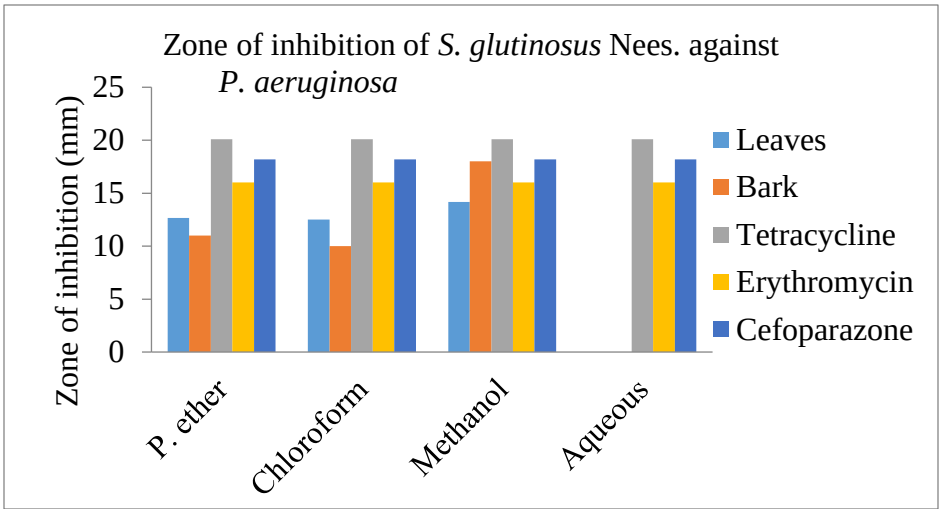

Figure 3: zone of inhibition produced by leaves and bark extracts of *S. glutinosus* against *Pseudomonas aeruginosa*

The methanolic bark extracts of *S. glutinosus* Nees. having maximum antibacterial action 17.5 ± 0.2 mm against *S. aureus* and very close results were displayed with leaf extract of methanol that were 17.2 ± 0.7 mm. The bark extract of p. ether has lowest inhibition (Figure 4).

The methanol extract of bark showed highest inhibition against *B. subtilis* with 13 ± 0.5 mm and lower was examined in the leaf extract of chloroform that was 11.16 ± 0.6 mm. The leaf extract of petroleum ether displayed highest inhibition that was 13.8 ± 0.9 mm in contrast to bark extract (Figure 5).

The antifungal activity was evaluated by using two fungal strains. The plant extract of both bark and leaves were compared against two standard antibiotics. Terbinafine revealed maximum antifungal potential against *A. niger*, i.e. 16 ± 0.5 mm in contrast to Ampicillin having minimum inhibition zone of 13 ± 0.8 mm against *A. oryzae* (Table 3).

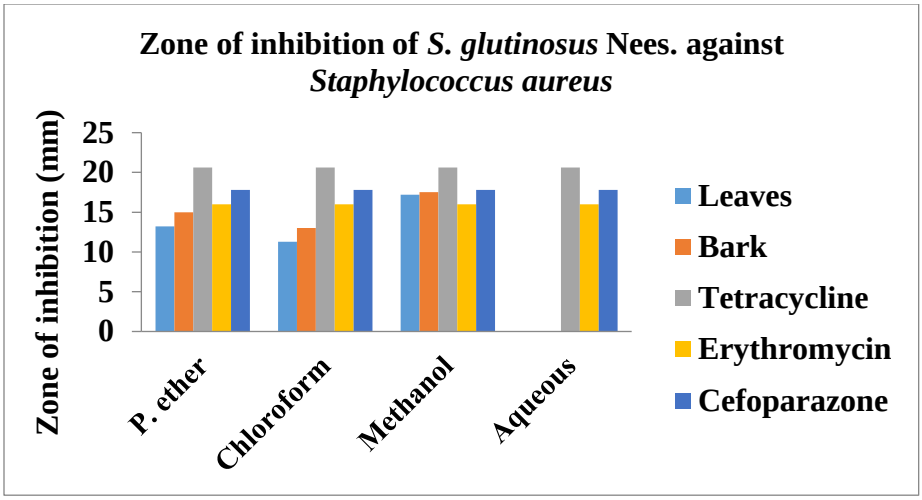

Figure 4: zone of inhibition produced by leaves and bark extracts of *S. glutinosus* against *Staphylococcus aureus*

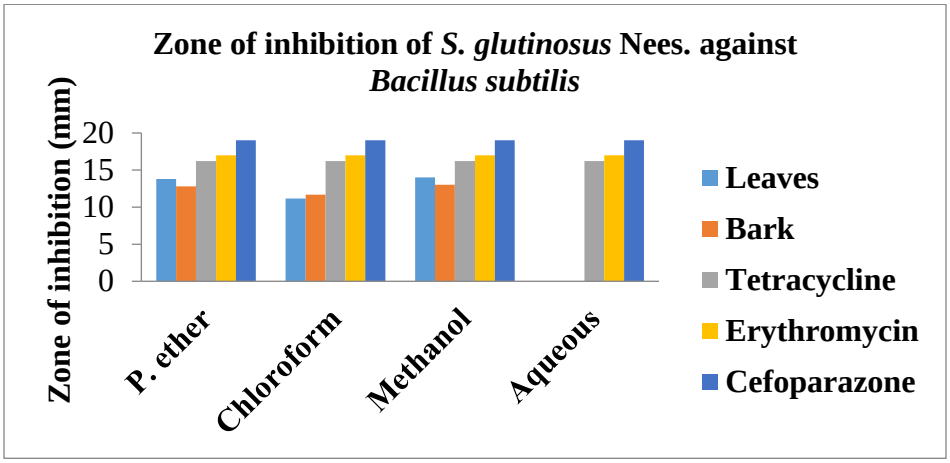

Figure 5: Graphical representation of zone of inhibition produced by leaves and bark extracts of *S. glutinosus* Nees against *Bacillus subtilis*

Table 3: Zone of inhibition exhibited by fungal strains against standard antibiotics

Antibiotics standard Disc	Zone of inhibition (mm)	
	A. oryzae	***A. niger***
Terbinafine	14 ± 0.6	16 ± 0.5
Ampicillin	13 ± 0.8	15 ± 0.4

The assessment of inhibition zones of *S. glutinosus* Nees. in the bark extract lies from 10.2 ± 0.6 to 14 ± 0.5 mm and in leaf extracts the potential vary from 9 ± 0.4 to 12 ± 0.7 mm. The bark extract of chloroform exposed maximum inhibition potential against *A. niger* i.e. 14 ± 0.5mm that was higher than the leaf extract of chloroform i.e. 11 ± 0.6 mm. The bark extract of methanol exposed inhibition potential of 13.3 ± 0.4 mm against *A. niger* and that of leaf extract was 12 ± 0.7 mm. The leaf extract of petroleum ether against *A. oryzae* displayed lower potential 9 ± 0.4 mm as compared to all other extracts. The water extract of both bark and leaves could not exhibit any zone (Table 4).

The maximum inhibition zone against fungal

strain of *A. oryzae* was exposed with leaf extract of methanol that was11.83 ± 0.9 mm and lower potential was revealed with leaf extract of p. ether i.e. 9 ± 0.4 mm (Figure 6).

The *S. glutinosus* Nees. With bark extract of chloroform has highest inhibition potential i.e. 14 ± 0.5 mm and less inhibition (10 ± 0.5 mm) was examined in the leaf extract of P. ether against *A. niger* (Figure 7).

Table 4: Zone of inhibition exhibited by bark and leaf extract of *S. glutinosus* against fungal strains.

Plant parts	Solvents	Zone of inhibition (mm)	
		Aspergillus oryzae	*Aspergillus niger*
Bark	P. ether	10.2 ± 0.6	11.16 ± 0.6
	Chloroform	10.8 ± 0.7	14 ± 0.5
	Methanol	11.16 ± 0.7	13.3 ± 0.4
	Aqueous	0 ± 00 ± 0	
Leaf	P. ether	9 ± 0.4	10 ± 0.5
	Chloroform	10.16 ± 0.4	11 ± 0.6
	Methanol	11.83 ± 0.9	12 ± 0.7
	Aqueous	0 ± 0	0 ± 0

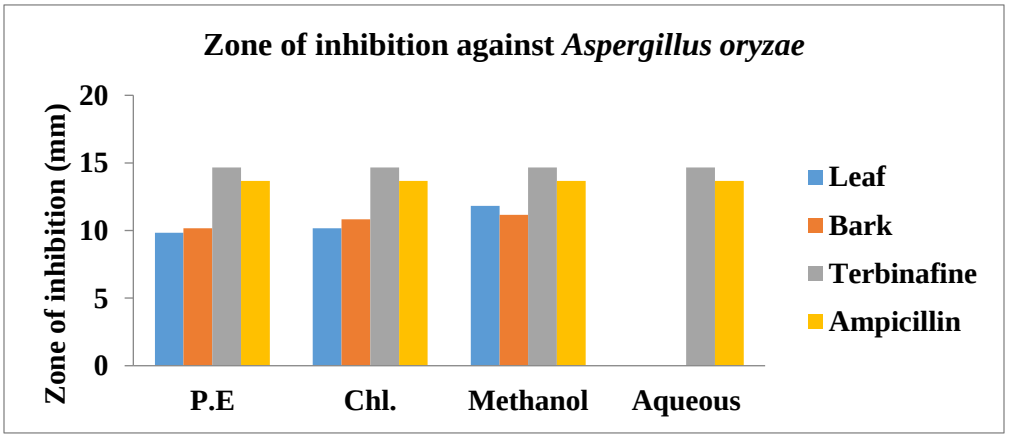

Figure 6: zone of inhibition produced by leaves and bark extracts of *S. glutinosus* against *Aspergillus oryzae*

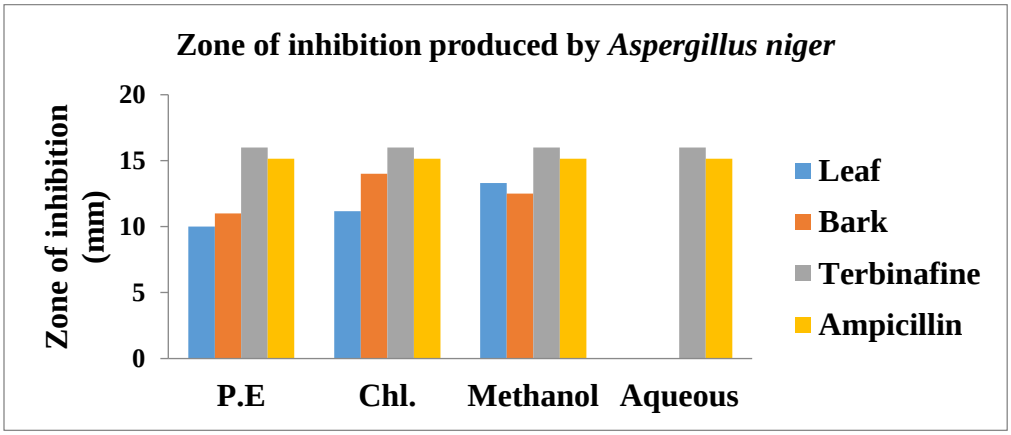

Figure 7: Zone of inhibition produced by leaves and bark extracts of *S. glutinosus* against *Aspergillus niger*

The leaf extracts of *S. glutinosus* revealed the following potential values of total phenols in the leaves extracts were from 0.143 ± 0.04 to 1.24 ± 0.31 µg/mL GAE while in the bark extracts that were within 0.223 ± 0.07 to 1.573 ± 0.31 µg/mL GAE (Table 5). The highest potential value was revealed by methanol bark i.e. 1.573 ± 0.31 at 500 µg/mL GAE by comparing with chloroform, petroleum ether and aqueous extracts (Figure 8). The maximum phenolic contents in the leaf extracts were revealed by chloroform (Figure 9). These results were compared with standard gallic acid and its standard curve is drawn in Figure 10.

Table 5: Total Phenolic Contents in the leaf and bark of *Strobilanthes glutinosus*

Plant parts	Extract	Absorbance at different concentrations (µg/mL)			
		60	125	250	500
	Methanol	0.243± 0.12	0.603 ± 0.05	0.796 ± 0.04	0.99 ± 0.08
Leaf	P. ether	0.143 ± 0.04	0.496 ± 0.04	0.626 ± 0.06	0.893 ± 0.01
	Chloroform	0.208 ± 0.06	0.525 ± 0.06	0.667 ± 0.05	1.24 ± 0.31
	Aqueous	0.286 ± 0.11	0.493 ± 0.04	0.706 ± 0.05	0.866 ± 0.04
	Methanol	0.223 ± 0.07	0.613 ± 0.06	0.793 ± 0.04	1.573 ± 0.31
Bark	P. ether	0.273 ± 0.13	0.546 ± 0.06	0.756 ± 0.06	0.886 ± 0.04
	Chloroform	0.296 ± 0.14	0.539 ± 0.10	0.688 ± 0.08	0.963 ± 0.66
	Aqueous	0.383 ± 0.06	0.573 ± 0.05	0.843 ± 0.03	0.973 ± 0.04
GAE	Standard	0.413 ± 0.14	0.66 ± 0.04	0.973 ± 0.05	1.896 ± 0.15

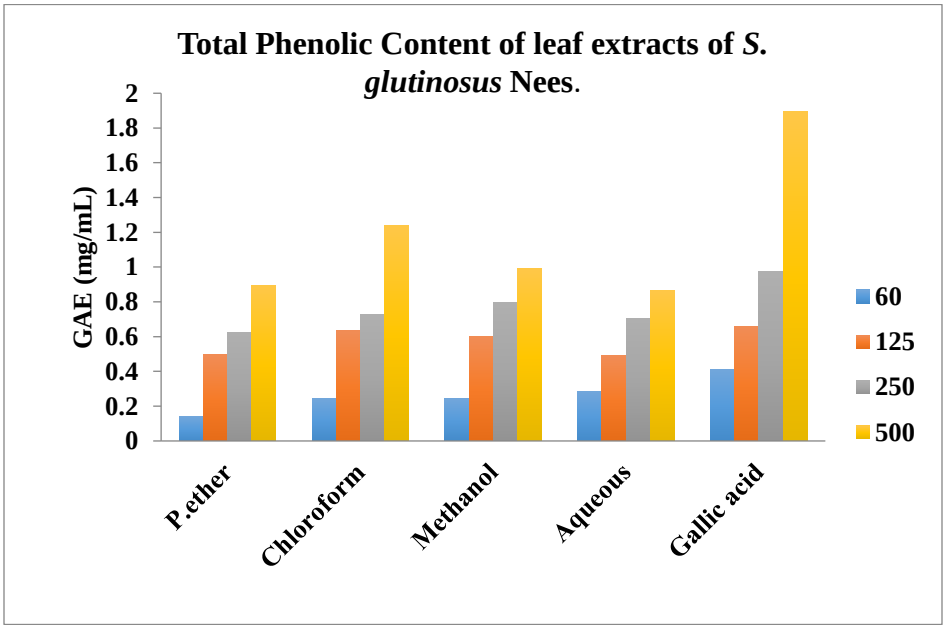

Figure 8: Total Phenolic Contents of leaf extracts of *S.Glutinosus*

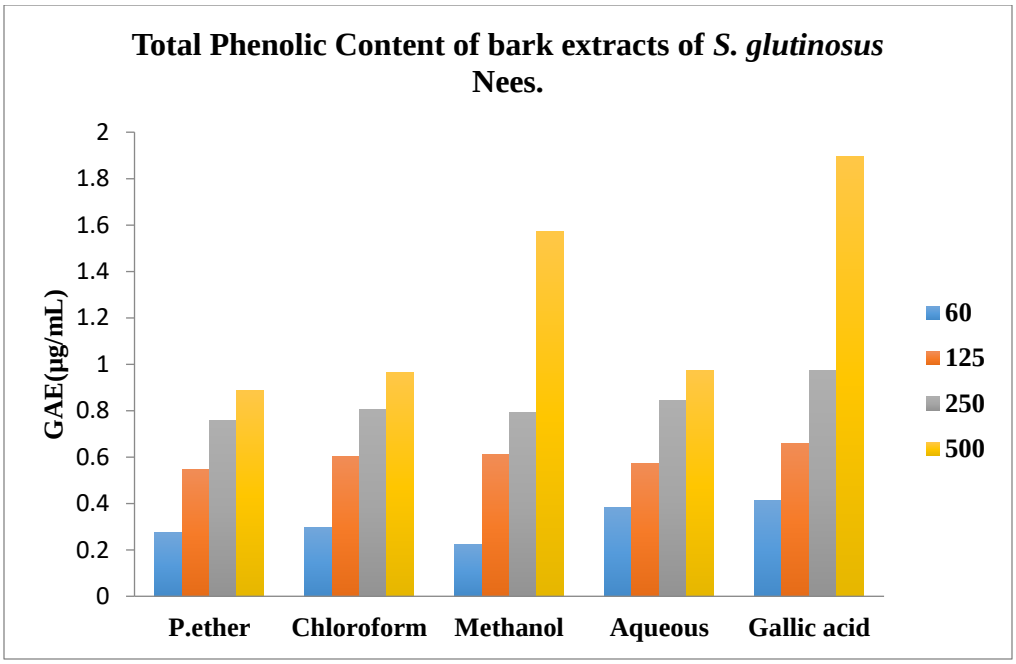

Figure 9: Total Phenolic Content of bark extracts of *S. glutinosus*

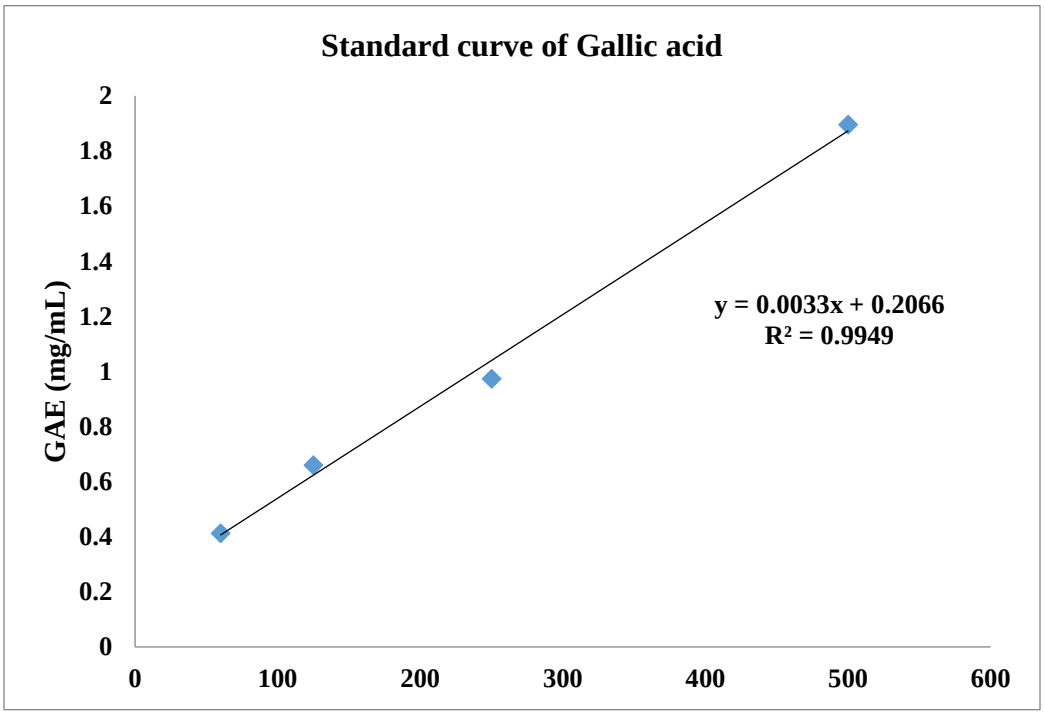

Figure 10: Standard curve of gallic acid

The overall flavonoid content values ranged within 0.203 ± 0.10 to 1.27 ± 0.24 catecin (µg/mL). The leaf extracts revealed the total flavonoid content from 0.207 ± 0.07 to 1.27 ± 0.24 catecin (µg/mL) while the bark extracts values were from 0.203 ± 0.10 to 0.956 ± 0.04 catecin (µg/mL) (Table 6). The highest flavonoid content was exposed by methanol extract of leaf at concentration of 500 µg/mL with 1.27 ± 0.24 catecin (µg/mL). The lower flavonoid absorbance was examined via leaf extract of petroleum ether with 0.203±0.10 catecin (µg/mL) at concentration of 60 µg/mL (Figure 11). The absorbance of methanol leaves confirmed good flavonoid content than all extracts. The bark extract of methanol and aqueous revealed highest flavonoid contents 0.956 ± 0.04 and 0.903 ± 0.05 catecin (µg/mL) respectively (Figure 12). Ascorbic acid was kept as a standard and its standard curve showed in Figure 13.

Table 6: Total Flavonoid Contents in the bark and leaf extract of *Strobilanthes glutinosus*

	Extract	Absorbance at different concentrations (µg/mL)			
		60	125	250	500
Leaf	Methanol	0.366 ± 0.15	0.453 ± 0.04	0.623 ± 0.05	1.27 ± 0.24
	P. ether	0.207 ± 0.07	0.416 ± 0.06	0.690 ± 0.04	0.823 ± 0.04
	Chloroform	0.294 ± 0.15	0.352 ± 0.18	0.77 ± 0.07	0.886 ± 0.09
	Aqueous	0.333 ± 0.05	0.726 ± 0.11	0.792 ± 0.03	0.885 ± 0.16
Bark	Methanol	0.395 ± 0.06	0.553 ± 0.04	0.743 ± 0.07	0.956 ± 0.04
	P. ether	0.203 ± 0.10	0.407 ± 0.17	0.687 ± 0.09	0.8 ± 0.03
	Chloroform	0.266 ± 0.11	0.428 ± 0.07	0.647 ± 0.04	0.864 ± 0.05
	Aqueous	0.289 ± 0.12	0.67 ± 0.03	0.713 ± 0.07	0.903 ± 0.05
Catecin (µg/Ml) Ascorbic acid	Standard	0.40±0.19	0.64 ±0.03	0.986±0.02	1.99 ±0.14

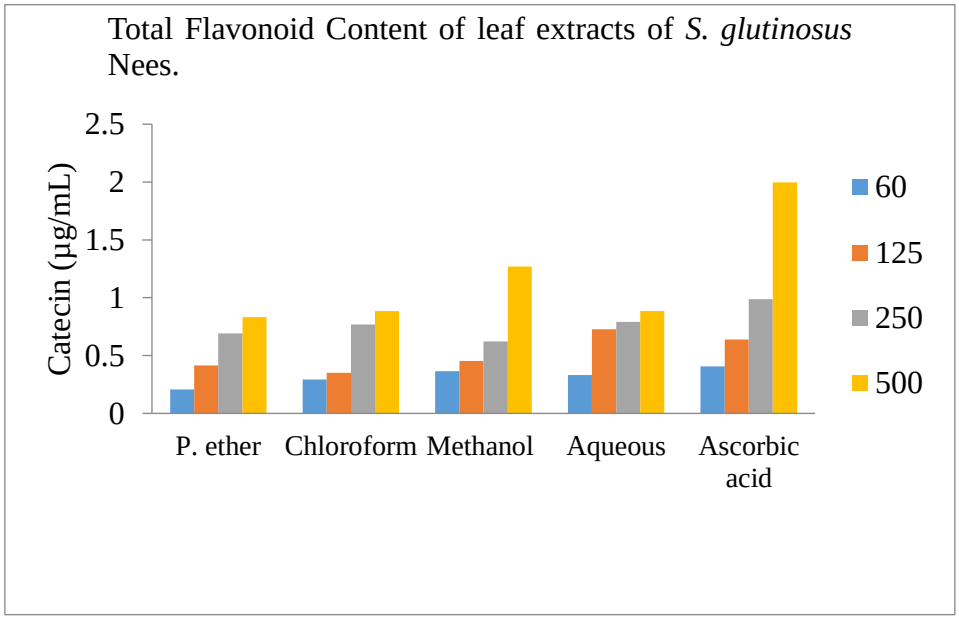

Figure 11: Total Flavonoid Content of leaf extracts of *S. glutinosus*

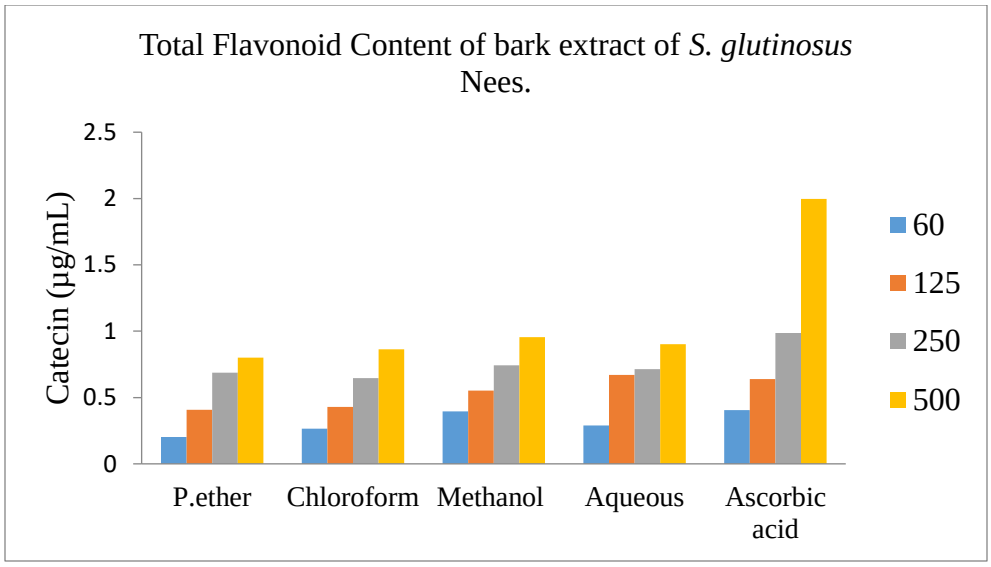

Figure 12: Total Flavonoid Content of bark extracts of *S.glutinosus*

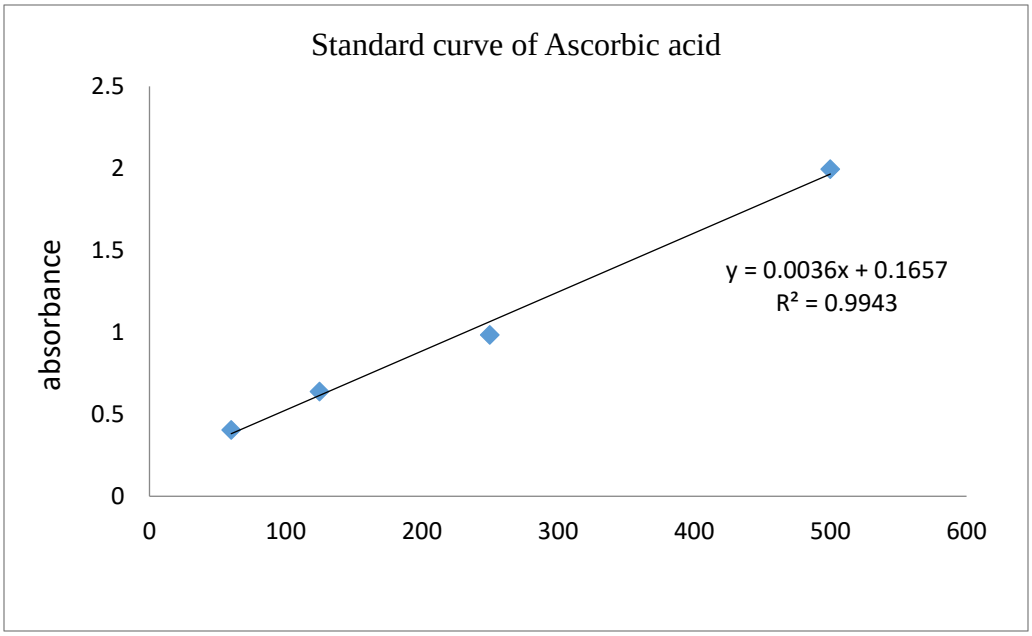

Figure 13: Standard curve of Ascorbic acid

The DPPH method was employed to investigate the antioxidant potential of *S. glutinosus* Nees. The overall % DPPH values range within 12.17 to 77.12%. The maximum DPPH value was exposed by leaf extract of chloroform and lower was in the bark extract of aqueous (Table 7). The maximum % DPPH value was given out by leaf extract of chloroform i.e. 77.12% at 500 µL (Figure 14). The chloroform bark was DPPH value of 57.56%. The petroleum ether extract of bark has also high percentage 44.64% after chloroform extract (Figure 15). BHT was kept as a standard for DPPH method and its standard curve has been drawn in Figure 16.

Table 7: Absorbance and free radical scavenging of *S. glutinosus*

Plant part	Extract	Concentration (µL)	Absorbance	% scavenging of DPPH
Leaf	Methanol	500 250 125 60	1.25 ± 0.15 0.86 ± 0.04 0.58 ± 0.08 0.38 ± 0.16	46.12 31.73 21.40 14.02
	Petroleum ether	500 250 125 60	0.96 ± 0.04 0.85 ± 0.07 0.79 ± 0.02 0.64 ± 0.03	35.42 31.36 29.15 23.61
	Chloroform	500 250 125 60	2.09 ± 0.05 1.03 ± 0.04 0.83 ± 0.05 0.72 ± 0.06	77.12 38.00 30.60 26.50
	Aqueous	500 250 125 60	0.97 ± 0.01 0.77 ± 0.07 0.67 ± 0.05 0.39 ± 0.17	35.79 28.41 24.72 14.39
Bark	Methanol	500 250 125 60	1.17 ± 0.16 0.90 ± 0.04 0.61 ± 0.07 0.41 ± 0.18	43.17 33.21 22.50 15.35
	Petroleum ether	500 250 125 60	1.21 ± 0.06 0.98 ± 0.01 0.84 ± 0.03 0.64 ± 0.06	44.64 36.16 30.99 23.61
	Chloroform	500 250 125 60	1.56 ± 0.2 1.05 ± 0.07 0.73 ± 0.06 0.70 ± 0.04	57.56 38.74 26.93 25.83
	Aqueous	500 250 125 60	0.91 ± 0.03 0.74 ± 0.06 0.58 ± 0.09 0.33 ± 0.14	33.57 27.30 21.40 12.17
BHT		Standard		2.71 ± 0.15

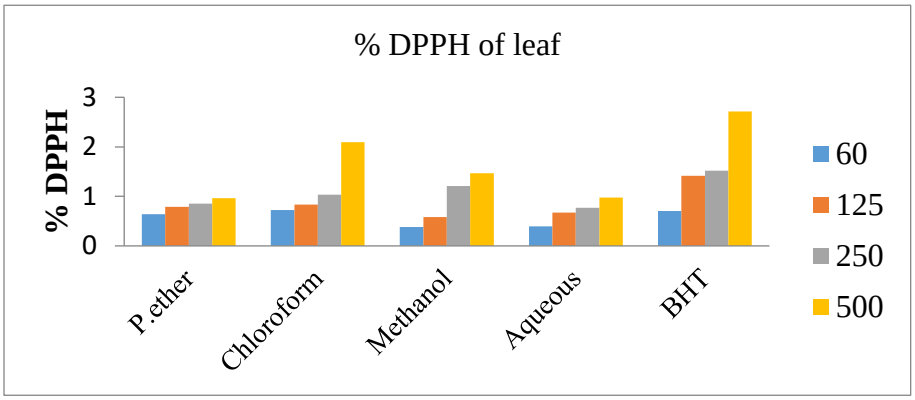

Figure 14: DPPH scavenging activity of leaf extracts of *S. glutinosus*

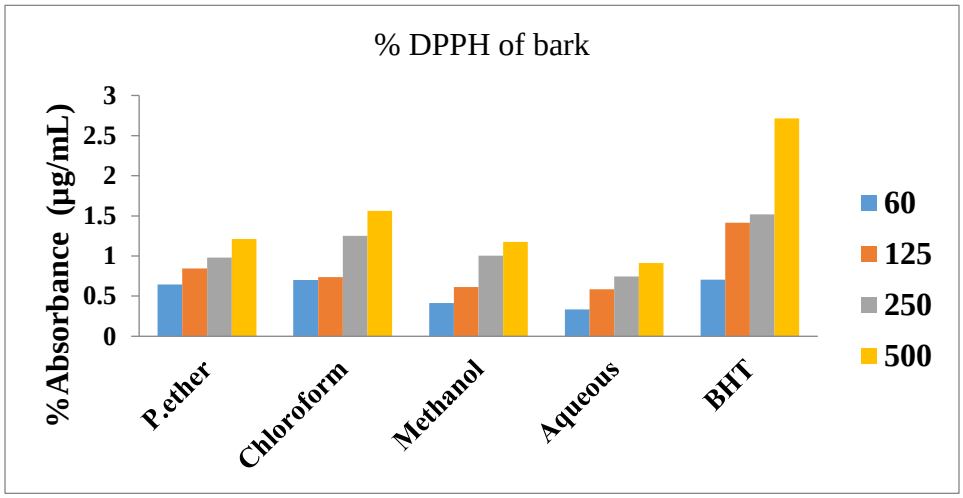

Figure 15: DPPH scavenging activity of bark extracts of *S. glutinosus*

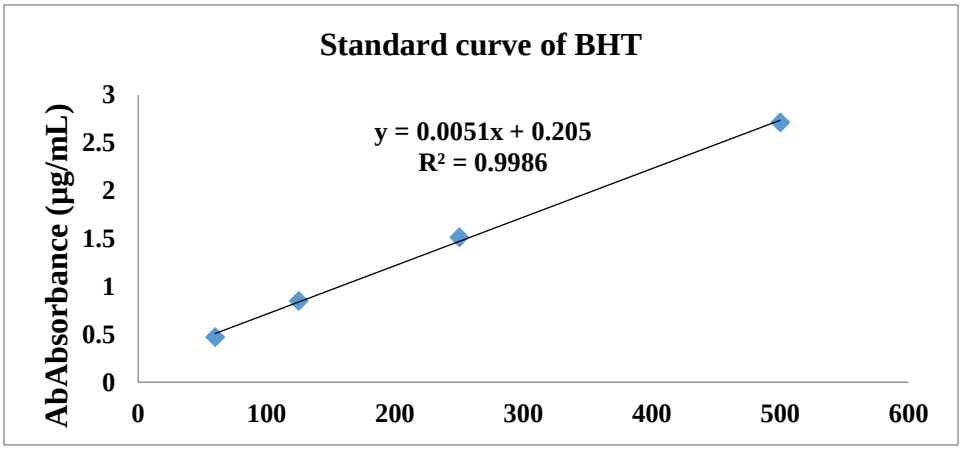

Figure 16: Standard curve of BHT

DISCUSSION

The methanol extract of plants have significant results of antimicrobial action as compared to other extracts and the same documentation was reported by the work of Khond et al., (2009). The overall inhibition zones that were examined with all bacterial strains vary from 18 ± 0.5 mm to 10 ± 0.4 mm. The maximum inhibition zone of methanolic bark extract was exposed against *P. aeruginosa* (18 ± 0.5 mm) and these results were similar to the findings of (Ajaib et al., 2015) while working on antimicrobial screening of *Helinus lanceolatus*.

The overall antifungal potential of extracts of *S. glutinosus* in opposition to two fungal strains was in following order *A. niger* > *A. oryzae*. The chloroform extract of bark revealed maximum inhibition potential against *A. niger* (14 ± 0.5 mm). The same results were reported by the work of (Ajaib et al., 2014) on antimicrobial activity of *Clerodendrum splenders*.

The phenolic content of *S. glutinosus* were maximum in the methanolic bark extract 1.573 ± 0.31 GAE (µg/mL) that were resembled with the results of (Bukke et al., 2013). The results obtained by DPPH radical scavenging activity indicate that chloroform extract of leaf showed better results (77.12%) for DPPH. These results were similar to the results of (Ajaib et al., 2016) regarding antimicrobial and antioxidant status of *Chenopodium ambrosioides*.

The highest flavonoid content was showing by

methanol extract of leaf i.e.1.27 ± 0.24 µg/mL. The results were close to those findings reported by Muhammad and Saeed (2011) during the biological screening of *Viola betonicifolia*. The antioxidant and antimicrobial potential of *S. glutinosus* Nees. was evaluated and the similar results noted by (Ajaib et al., 2013) during the estimation of antioxidant and antimicrobial activities of *Rivina humilis*. The results reveal good antioxidant activity and strong antimicrobial potential than standard drug.

CONCLUSION

The antibacterial potential was analysed against gram positive and gram-negative bacterial strains and the antimicrobial potential was exhibited by both leaf and stem extract of *S. glutinosus* Nees. The highest antibacterial potential was revealed with bark extract of methanol whereas the lowest potential was examined by chloroform extract of bark against *P. aeruginosa.* The antifungal activity was better performed by the bark extract of chloroform against *A. niger* and lower was examined in the leaf extract of p. ether against *A. oryzae*. Both the bark and leaf extracts of *S. glutinosus* Nees did not expose any results in aqueous against bacterial and fungal pathogens.

The antioxidant potential was exhibited by all plant extracts with TPC, TFC and DPPH methods. The methanolic bark extract exposed higher absorbance for total phenolic content, methanol leaf extract has higher value toward TFC and chloroform extract of leaf showed maximum percentage of DPPH. All the activities of *Strobilanthes glutinosus* Nees. have significant results.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

M Ajaib Plant collection, Experimental design and write up (as supervoisor); F. Shafi, Ishtiaq M, Zahid MT and Bhatti KH Experimental evaluation and data collection

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