ANTIMICROBIAL AND ANTIOXIDANT SCREENING OF *Gardenia tetrasperma*

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ABSTRACT: Antimicrobial and antioxidant activities of an unexplored medicinal plant, i.e., *Gardenia tetrasperma* Roxb. was investigated. The antimicrobial potential of extracts of *G. tetrasperma* was determined using human pathogenic four bacteria and two fungi. The maximum antibacterial potential was recorded, in chloroform extract of leaf, i.e., 91.66±0.4 mm zone of inhibition against *E. coli*. Chloroform extract of leaves showed 62±1.1 mm zone of inhibition against *S. aureus*. The methanolic leaf extract showed maximum potential against *A.niger* and *F. solani* with the values 42.42±1.5 and 36.22±1.11 mm respectively, whereas the lowest value showed by water extract of the leaf against *F.solani*, i.e. 16.43±1.37. The methanolic extract of seed also showed satisfactory results against *F. solani*, i.e. 42±1.75. In seed extracts lowest value was revealed by the water macerates of seeds i.e. 12.33±0.8. The MIC assay was performed for further study which presented the significant MIC value i.e. 0.02±0.1 at 0.8 mg/mL of leaf extract against *E. coli* where as it was 0.02±0. 9 at 0.2 mg/mL against *A. niger*. Antioxidant potential was determined using DPPH scavenging potential. The uppermost value of % DPPH was observed as s95.51±1.7 at 500 µL concentration in petroleum ether extract of bark. The maximum values of total antioxidant activity (TAA) were 1.29±0.11 and 1.19±0.5 in methanolic extract of bark and petroleum ether macerates of leaf respectively. Total Phenolic Content (TPC) was 1.702±0.3 and 1.07±3.7 in Petroleum ether macerates of leaf and water macerates of seed, respectively.

Keywords: *Gardenia tetrasperma*, Antimicrobial, *Aspergillus niger*, Antioxidant activity.

(Received 17-7-2018 Accepted 20-9-2018)

INTRODUCTION

Plant constituents differ widely in terms of their structure and biological properties and become a source of active natural products. Fresh fruits, vegetables and plant beverages are rich in natural antioxidants that prevents humans from many disorders such as cancer and cardiovascular diseases. The antioxidant and antimicrobial potential of plant products is due to the presence of several compounds in them which have distinct mechanisms of action in which some are enzymes and proteins while others are low molecular weight compounds such as vitamins, carotenoids, flavonoids, anthocyanins and other phenolic compounds (Ajaib *et al*., 2016).

According to the recent research,WHO has assessed that almost 80% world's population depend on herbal medicines for health care system (Amir *et al.*, 2018). Compounds include antioxidants not only lack the reactive free radicals especially ROS (reactive species of oxygen) but reduce or stop the improvement of deteriorating diseases including cancer, cardiovascular, inflammatory and other chronic diseases (Sreejayan and Rao, 1996; Ajaib *et al.*, 2015). Many plants of family Rubiaceae demonstrated antioxidant, antimicrobial, antimalarial activities due to presence of certain bioactive compounds. *Nauclea latifolia*, *Crossopteryx febrifuga* and *Mitragyna inermis* are important medicinal plants of family Rubiaceae. Many potent compounds are awaiting exploration in this family (Karou *et al.*, 2011). *G. tetrasperma* Roxb. (Figure 1) of family Rubiaceae commonly known as 'Guggle' in District Kotli, Azad Jammu and Kashmir is fairly a common species of open slopes, in the middle of rocks and dry river beds; arising upto 2000 m. It is a shrubby plant height about 2 m. Flowers are greenish-white, sweet scented; fruit is upto 8 mm in diameter, rounded dark purple or black (Nazimuddin and Qaiser, 1989).

Figure 1. ***Gardenia tetrasperma***

MATERIALS AND METHODS

Plant material: The plant material such as bark, leaves and seeds of *G. tetrasperma* was collected from Tehsil Khuiratta, District Kotli Azad Kashmir at N. 33°18´, E. 74°01.5´ with an elevation 850 m. The plant material was identified from Dr. Sultan A. Chaudhry Herbarium (SAH), Botany Department of GCU Lahore with a voucher no. BOT. 2747.

Plant Extracts: 250 g powdered plant material was used for extraction, following maceration technique (Singh, 2008) using petroleum ether, chloroform, methanol and distilled water as sovents.

Antimicrobial activity

Test Organisms: For appraisal of antibacterial and antifungal potential of bark and leaf of *G. tetrasperma*, two gram-negative, two gram positive bacteria and two fungal strains were used following Cruick-shank *et al*. (1975).

Antimicrobial bioassay: Agar well diffusion method according to Ferriera *et al*. (1996) and Ortega *et al*. (1996) was employed to investigate the antimicrobial activity. Minimum Inhibitory Concentration (MIC) of *G. tetrasperma* was evaluated using Broth-dilution method following Murray *et al*. (1999).

Antioxidant assays: DPPH free Radical Scavenging Activity of *G. tetrasperma* was evaluated using Lee and Shibamoto (2001) method.Total Antioxidant Activity (TAA) of selected plant was studied by using phosphomolybdenum complex formation technique following Prieto *et al*. (1999). Total Phenolic Contents (TPC) of *G.tetrasperma* was assessed using the methodology applied by Makkar *et. al.* (1993). Ferric reducing antioxidant power (FRAP) assessment of the plant extracts was completed by employing methodology adopted by Benzie and Strain (1996) with slight amendments.

RESULTS AND DISCUSSION

Verification that the antibacterial and antifungal potential was displayed decently by crude extract of *G. tetrasperma* plant only and not by solvents, negative response was observed.

All plant parts possesses satisfactory results against bacterial and antifungal strains (Table 3). Methanolic extract of bark showed 44±0.5 mm against *Escherichia coli* as compared to standard disc Cephradine 26±0.3 mm. Methanolic extract of bark against *S.aureus* showed 36±2.2 mm as compared to standard disc Azithromycin, i.e. 15±0.8 mm where as methanolic extract of bark against *P.aeruginosa* showed 49.66±5.1 mm zone of inhibition. Methanolic extract of bark showed 45.67±2.9 mm zone of inhibition against *B.subtilis*. It was noticed that methanolic extracts of bark showed best results as compared to the standard disc. Methanolic extracts of seeds also showed best results, i.e. 36.66±1.73 mm against *E.coli*, 54.34±2.6 mm against *S.aureus*, 23±4.2 mm against *P.aeruginosa*, 20.66±1.7 mm zone of inhibiton against *B.subtilis*. Leaves extract of chloroform showed best result against *E.coli* 91.66±0.4 mm and against *S. aureus* 62±1.1mm. Seed extracts of methanol showed poor results as compared to methanolic extracts of leaf and bark.

Methanolic extracts of *G. tetrasperma* during MIC, displayed substantial confrontation against both types of bacteria. Furthermore significant results of seed extracts, i.e. 0.10±0.01 against *E.coli* and 0.29±0.04 against *P. aeruginosa* was recorded. *E.coli* showed susceptibility against leaf extracts which was 0.02±0.1. *P. aeruginosa* showed susceptibility against leaf extracts with a value 0.04±0.01. All part of plants showed good results against bacterial strains (Table 4). Methanolic extract of leaf have supreme results in contrast to *F.solani* and *A.niger* with the values 42.42±1.5 and 36.22±1.11 respectively. On the other hand, lowest value showed by aqueous extract of the leaf against *F. solani*, i.e., 16.43±1.37. The seed methanolic extract also displayed satisfactory results against *F. solani* 42±1.75. Whereas, lowest value is exposed by the water extract of the seeds, i.e., 12.33±0.8. Maximum value showed by seed methanolic extract, i.e. 42.42±1.5 against *A.niger*. Minimum zone displayed by aqueous extract, i.e. 15±0.62 against *A. niger* (Table 5). The MIC assay was performed for further study which presented the significant MIC value, i.e. 0.02±0.1 at 0.8 mg/mL of leaves extract against *E.coli* (Table 4) where as 0.02±0. 9 at 0.2 mg/mL concentration of leaves extract against *A. niger* (Table 6).

Best results of DPPH free radical scavenging was shown by bark extract in petroleum ether (PE) at absorbance value 95.51±1.7 at 500 µL/ml, then by chloroform extract with absorbance of 87.19±0.79 at 250 µL/ml, on the other side, a smaller amount of proficiency is revealed by the bark aqueous extract (43.19±0.99 at 125µL/mL). In all extracts, petroleum ether and chloroform extracts of leaf showed uppermost value, i.e., 91.36±0.77 and 81.6±0.77 respectively at 1000 µL/mL. Whereas, least value was shown by the aqueous extract of leaf, i.e. 31.11±0.89 at 125 µL/mL. Petroleum ether seed seed extracts of *G. tetrasperma* showed maximum absorbance at 1000 µL/mL with a value of 89.37±0.01. Alike, methanolic and chloroform extract exhibited the significant value at 1000 µL/mL, i.e., 84.3±19.1and 72.12±0.53 respectively. Overall least value is displayed by the water extract, i.e. 32.43±1.2 at 125 µL/mL (Table 7).

The total phenolic components (TPC) present in the leaf, bark and seed of the *G.tetrasperma* were

predictable in relevance to the Gallic acid, attained by linking the subsequent values with the standard curve of Gallic acid in GAE mg/mL (Table. 10). To determine TPC the plant extract was allowed to react with FC reagent and absorbance value was taken at 726 nm. The petroleum ether bark extract of plant exhibited maximum absorbance, i.e. 1.53±0.27 GAE mg/mL. Maximum value of absorbance exhibited by chloroform extracts of leaves 1.11±0.45 GAE mg/mL. Although the aqueous extracts of the seed showed absorbance of 1.8±1.26 GAE mg/ml which is the maximum value amongst all aqueous extracts of this assay. During whole experiment bark extracts of *G. tetrasperma* were found to have maximum phenolic substances as associated to the extracts of other parts (Table 10).

Ferric reducing antioxidant power assay is beneficial over all other extracts of *G.tetrasperma*. The assay was performed following Benzie and Strain (1996) and the results were measured in TE (µM/mL). Maximum reduction potential was recorded by the bark methanolic extract, i.e., 65.66±0.01 µM/mL (Table 12) whereas the minimum value was shown by the leaf water extract, i.e., 13.44±0.37 µM/mL.

The antioxidant potential of *G. tetrasperma* was assessed using Phoshomolybdenum method (Table-11). The results were paralleled with the known value of BHT. Greatest antioxidant power was displayed by the methanolic extract of bark, i.e., 1.29±0.11 mm whereas the minimal effectiveness was presented by the aqueous extract of seed, i.e., 1.01±0.77 mm.

Almost all plant parts of *G. tetrasperma* showed good antimicrobial results against bacterial as well as fungal strains. Methanolic extract of bark showed 44±0.5 against *E.coli* as compared to standard disc Cephradine 26±0.3mmmay be due antimicrobial compounds collected in the bark and the results were more significant than standard antibiotic discs, such findings are also testified by (Ajaib *et al*., 2015) throughout the determination of antimicrobial compounds in *Clerodendrum splendens.* Antioxidant potential, i.e. Total phenolic contents, DPPH, Total antioxidant assay FTC was evaluated. In whole process bark extract of Petroleum ether presented good results (95.51±1.7%) at 500 µL/ml (Table 7) followed by leaf extracts of Chloroform (91.44±0.6%) at 1000 µL/mL (Table 8) while less competency was disclosed by water extracts of Seed (32.43±1.2%) at 125 µL/mL (Table 9). Similar situations were also reported by Ebrahimzadeh *et al.* (2008) while investigating oxidative stress on Iranian Corn Silk.

The total phenolic contents were determined in comparison to the Gallic acid. Petroleum ether extract of bark of *G. tetrasperma* showed highest absorbance, i.e. 1.53±0.27GAEmg/mL. Maximum value shown by leaf extract in chloroform was 1.11±0.45 GAEmg/mL as compared to other solvents. Bark macerates of plant have significant phenolic contents as compared to other parts of *G. tetrasperma* (Table 10). Somewhat similar results were noticed by Ajaib *et al.* (2016) while working on *Chenopodium ambrosioides* for antimicrobial and antioxidant screening.

The significant TPC was possessed by methanolic and petroleum ether extracts of bark and leaf, i.e., 1.29±0.11 mm and 1.19±0.5 mm respectively very close to BHT standard with a value 1.2±0.1 (Table 11). The antioxidant potential was found in order Methanol >Petroleum ether >Chloroform > Water. Aqueous extracts presented minimum antioxidant activity as reported by Siddiqui *et al.* (2015) and Riaz *et al*. (2012) during investigations on *Cotinus coggyria* and *Pyrus pashia*.

Table-1: Inhibitory Zone formation by standard antibacterial discs (Positive control).

Antibacterial Standard Disc	Conc. (µg)	Bacterial Strains	Zone of Inhibition (mm)
Azithromycin	15	*S. aureus*	15±0.8
Amikacin	30	*B. subtilis*	17±0.41
Ampicillin	10	*P. aeruginosa*	22±0.2
Cephradine	30	*E. coli*	26±0.3

Table-2: Inhibitory Zone formation by standard antifungal discs (Positive control).

Antifungal standard disc	Conc. (µg)	Fungal strains	Zone of inhibition (mm)
Itraconale	100	***F. solani***	9±0.97
Voriconazole	100	***A. niger***	39±1.00

Table-3: Zone of Inhibition produced by bark, leaf and seed extracts of *G. tetrasperma* against bacterial strains.

Plant Parts	Extract	Zone of Inhibition (mm)			
		E.coli	***S.aureus***	***P.aeruginosa***	***B.subtils***
Bark	**Petroleum ether**	30.34±1.7	53±2.5	20±2.1	20.34±1.8
	Chloroform	50±3.2	38.67±3.2	43±0.5	30±2.0
	Methanol	44 ±0.5	36±2.2	49.66±5.1	45.67±2.9
	Aqueous	37.66±0.11	39±0.4	22.66±1.3	20.6±1.1
Leaf	**Petroleum ether**	33.66±0.3	52±1.4	75±0.3	15±1.1
	Chloroform	91.66±0.4	62±1.1	49±1.5	43.6±1.4
	Methanol	25.33±0.2	71.66±1.1	44.67±1.1	29±0.3
	Aqueous	51.33±2.6	49±2.6	34±1.4	27.67±4.1
Seed	**Petroleum ether**	35±1.7	43±1.2	32±0.2	24±4.2
	Chloroform	32.34±0.5	40±0.3	35±0.8	36.6±1.6
	Methanol	36.66±1.73	54.34±2.6	23±4.2	20.66±1.7
	Aqueous	32±4.2	46.3±1.5	26±3.2	26.33±0.2

Table-4: MIC values of bark , leaf and seed extracts of *G. tetrasperma* against bacterial strains.

Plant parts	Bacterial strains							
	E.coli		***P.aregenosa***		***B.subtilis***		***S.areus***	
	Conc. mg/mL	**MIC**	**Conc.**	**MIC**	**Conc.**	**MIC**	**Conc.** mg/mL	**MIC**
Bark	1	0.06±0.37	0.7	0.04±0.01	0.3	0.21±0.37	0.7	0.96±0.02
Leaf	0.8	0.02±0.1	0.9	0.53±0.37	0.7	0.25±0.21	0.9	1.08±0.1
Seed	0.9	0.10±0.01	0.8	0.29±0.04	0.8	1.22±0.01	0.4	0.09±0.03

Table-5: Zone of Inhibition produced by leaf, bark and seed extracts of *G. tetrasperma* against fungal strains.

Plant part	Solvent	Zone of inhibition (mm)	
		Fusarium solani	***Aspergilus niger***
Bark	**Petroleum Ether**	22.33±0.2	20±0.5
	Chloroform	37.3±0.16	21±0.5
	Methanol	49±0.6	42.3±1.4
	Aqueous	26.3±0.3	43±0.5
Leaf	**Petroleum Ether**	15.34±1.4	19±0.5
	Chloroform	21.33±1.8	16±1.5
	Methanol	42.42±1.5	36.22±1.11
	Aqueous	16.43±1.37	18.66±2.4
Seed	**Petroleum Ether**	18±0.5	28±1.5
	Chloroform	16±0.8	22±0.5
	Methanol	42±1.75	42.42±1.5
	Aqueous	12.33±0.8	15±0.62

Table-6: MIC values of bark, leaf and seed extracts of *G. tetrasperma* against fungal strains.

Plant parts	Fungal Strains			
	F.solani		***A.niger***	
	Conc. mg/mL	**MIC**	**Conc.** mg/mL	**MIC**
Bark	0.9	0.05±0.1	0.8	0.25±0.37
Leaf	1.00	0.30±0.04	0.2	0.02±0. 9
Seed	0.4	0.70±0.03	0.7	0.177±0.2

Table-7: % Free radical scavenging activity of bark of *G. tetrasperma.*

Plant Part	Extract	Concentrations (µL)	Absorbance In %
Bark	Petroleum ether	1000	67.66±1.5
		500	95.51±1.7
		250	89.37±0.5
		125	51.56±0.4
	Chloroform	1000	80.22±1.04
		500	70.11± 0.3
		250	90.21±0.5
		125	66.13±0.5
	Methanol	1000	59.86±0.9
		500	62.67±0.4
		250	70.24±0.4
		125	40.25±0.7
	Aqueous	1000	79.40±1.1
		500	68.29±0.4
		250	49.8±2.7
		125	43.19±0.99
BHT Standard			**91.35**

Table-8: % Free radical scavenging activity of leaf of *G. tetrasperma.*

Plant Part	Extract	Concentrations(µL)	Absorbance In %
Leaf	Petroleum ether	1000	81.6±0.77
		500	75.5±1.1
		250	58.566±1.3
		125	64.13±0.6
	Chloroform	1000	91.44±0.6
		500	82.84±1.2
		250	73.9±1.86
		125	43.45±0.5
	Methanol	1000	87.34±0.6
		500	61.64±0.67
		250	58.76±0.18
		125	53.34±1.5
	Aqueous	1000	78.14±1.12
		500	42.34±1.4
		250	67.76±1.3
		125	31.11±0.89
BHT Standard			**91.35**

Table-9: % Free radical scavenging activity of seeds of *G. tetrasperma.*

Plant Part	Extracts	Concentrations	Absorbance In %
Seeds	Petroleum ether	1000	89.37±0.01
		500	85.73±0.45
		250	76.66±1.45
		125	54.5±1.4
	Chloroform	1000	72.12±0.53
		500	39.54±0.9
		250	45.47±0.45
		125	44.1±0.2
	Methanol	1000	86.5±2.1
		500	52.6±1.2
		250	58.41±0.7
		125	44.1±0.7
	Aqueous	1000	51.64±0.9
		500	42.45±1.4
		250	45.87±1.3
		125	32.43±1.2
BHT Standard			**77.3±0.7**

Table-10: Total phenolic content of *G. tetrasperma.*

Plant Part	Extract	Absorbance
Bark	Petroleum ether	1.702±0.3
	Chloroform	0.63±0.1
	Methanol	1.43±.6
	Aqueous	0.4±1.4
Leaf	Petroleum ether	0.71±0.7
	Chloroform	1.11±0.45
	Methanol	0.84±2.4
	Aqueous	0.56±0.9
Seed	Petroleum ether	0.8±1.7
	Chloroform	0.95±2.3
	Methanol	1.032±0.1
	Aqueous	1.07±3.7

Table-11: Total antioxidant activity of *G. tetrasperma.*

Plant part	Extract	Absorbance
Bark	Petroleum ether	1.07±0.1
	Chloroform	1.03±0.6
	Methanol	1.29±0.11
	Aqueous	1.17±0.3
Leaf	Petroleum ether	1.19±0.5
	Chloroform	1.13±0.6
	Methanol	1.14±0.1
	Aqueous	1.175±0.4
Seed	Petroleum ether	1.09±0.1
	Chloroform	1.03±0.2
	Methanol	1.04±0.7
	Aqueous	1.01±0.77
Standard		**1.2**±0.1

Table-12: FRAP assay of *G. tetrasperma.*

Plant part	Extract	Absorbance
Bark	Petroleum ether	45.66±0.03
	Chloroform	24.33±0.09
	Methanol	65.66±0.01
	Aqueous	39.33±0.4
Leaf	Petroleum ether	45.23±0.02
	Chloroform	20.43±0.04
	Methanol	53.66±0.2
	Aqueous	13.44±0.37
Seed	Petroleum ether	41.2±0.3
	Chloroform	50.44±0.2
	Methanol	42.22±0.01
	Aqueous	18.63±0.05

Conclusion: it was concluded that *G. tetrasperma* contains bioactive compounds which are potent antimicrobial and antioxidants.

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