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Evaluation of antibacterial activity from stem bark and leaf extracts of *Gardenia gummifera* Linn

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Abstract
Currently, plant based drug production demand has increased for the curing of various diseases. This study investigates the phytochemical constituents screening and antibacterial property of *Gardenia gummifera* stem bark and leaf extracts. Preliminary phytochemical screening of various extracts using standard methods revealed the presence of alkaloids, phenolic compounds, terpenoids, tannins, glycosides and saponins. The antibacterial efficiency of the extracts were screened by agar well diffusion method against human pathogens viz, *Staphylococcus aureus, Streptococcus pneumoniae*, *Vibrio cholera, Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*. Ethanol stem bark extract showed the pronounced antibacterial effect with 12.67±0.33mm and 12.33±0.33mm inhibition zone against *S. typhi* and *S. aureus* respectively and leaf extract showed 12.33±0.33mm and 12.83±0.60mm inhibition zone against *S. typhi and S. aureus* respectively. Leaves ethanol extract exhibited highest inhibitory activity against *S, aureus* with MIC value of 8.33 µg. The obtained results have supported the traditional claim of *Gardenia gummifera* stem bark and leaves to control microbial infection.

Keywords: *Gardenia gummifera,* stem bark and leaf extarcts, antibacterial activity, MIC.

1. Introduction
The use of plant material to treat diseases is a traditional practice in large parts of the world, mainly in developing countries [1]. According to World Health Organisation (WHO) medicinal plants could be the better source to obtain different types of drugs [2]. Modern isolation and pharmacological screening techniques have emphasized the way to discover new drugs to modern medicines [3].
In recent years, attention has been given to traditional system of treatment for protection against human pathogens. Plant play a significant role in the inhibition of disease causing pathogens.
Gardenia gummifera is an endemic and endangered medicinal plant belonging to family Rubieacea [4]. It is commonly known as gummy gardenia. It is distributed in dry forest of Karnataka, Tamil Nadu, Andra Pradesh and Kerala. This plant is well known for its application in folk medicine [5]. This plant is claimed to have number of medicinal properties possessing carminative and astringent properties and are used in the treatment of dyspepsia and haemorrhoid. It is also claimed to be useful in flatulence for cleaning foul ulcers and wounds [6]. Additionally, the qualitative analysis has exposed that the *Gardenia gummifera* is very rich in phytoconstituents, which gives a very strong purpose to select this palnt for future pharmacological evaluation [7]. Keeping this in view, the present study was designed by selecting *Gardenia gummifera* to systematically screen the antibacterial potentials of different extracts of stembark and leaf on human pathogens.

2. Materials and Methods
2.1 Collection of plant material
The fresh leaf material and stem bark of *Gardenia gummifera* has been collected from the area of Sakarayapatna, Chikmagaluru District, Karnataka, India. The plant has been identified and authenticated by a taxonomist Dr. V. Krishna, Professor, Post Graduate Studies and Research in Biotechnology, Kuvempu University. Thereafter, the plant material was washed 2-3 times with running tap water followed by distilled water treatment and allowed to dry under the shade.

2.2 Extraction of plant material
Dried stem bark and leaves were crushed into powder form, subjected to successive solvent extraction from non-polar to polar solvents like petroleum ether, chloroform and ethanol respectively. The extraction has been carried out using soxhlet apparatus.

2.3 Preliminary phytochemical analysis
2.3.1 Qualitative Analysis
The extracts were analysed qualitatively for the presence of phytoconstituents such as Alkaloids, Flavonoids, Terpenoids, Glycosides, Phenolic compounds and Tannins [8-9].

2.3.2 Quantitative Analysis
Stem bark and Leaf extracts were allowed for quantitative estimation of alkaloids and total phenolic compounds.

2.3.3 Alkaloid estimation
1 gm of sample was added to 40 ml of 10% acetic acid, covered and allowed to stand for 4 h. The filtrate was then concentrated on a water bath to get 1/4th of its 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 collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed [10].

2.3.4 Phenolic compounds estimation
The amounts of total phenolic contents of extracts of *G, gummifera* were determined by the spectrophotometric method of Singleton [11]. 20 µl of extracts (5mg/ml) was mixed with 0.75ml of 20% sodium carbonate solution and 0.25 ml of Folin-Ciocalteau reagent. The reaction mixture was allowed to stand in light for 3 min and incubated for 2 h in dark. The absorbance was measured at 765 nm using UV-Visible Spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of known concentration of Gallic acid standard (0-100 µg/ ml). The concentrations were expressed as µg of Gallic acid equivalents per ml and all the determination were performed in triplicates.

2.4 Bacteiral cultures (collection and maintenance)
Pure cultures of *Staphylococcus aureus, Streptococcus pneumoniae*, *Vibrio cholera, Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae* used in this investigation were obtained from Institute of Medical Sciences, Shimoga, Karnataka, India**.** From these strains, loop full bacteria were inoculated into nutrient broth and inocubated over night at 37°C.

2.5 Agar well diffusion method
This method was adopted to determine the antibacterial activity of plant extracts according to standard protocol [12-13]. 20 ml of Muller's Hilton agar media was poured into petri plates and were allowed to solidify, followed by addition of 100µl of 24 h fresh culture (10^5 cells/ml) of each test organism and were spreaded over the sterile agar plates. Subsequently, wells were created in plates using sterile cork borer (6 mm diameter). In each plate four wells have been created, among which three wells were induced with 500µg, 1000µg and 1500 µg/ml of dissolved extract in 10% DMSO, and remaining one well with positive control streptomycin (20 µg/ml). These plates were incubated in the upright position at 37ºC for 24 h. The incubated plates were observed for the formation of clear zone of inhibition around the well which indicates the induction of antibacterial activity [14-15].

2.6 Resazurin micro titre-plate assay
Minimal inhibitory concentration (MIC) of the stem bark and leaves was evaluated by modified resazurin micro titre plate assay [16-17]. To all wells, 50 µl of nutrient broth was added. 50 µl of test sample containing 250 µg of extract sample dissolved in 10% dimethyl sulfoxide were added to first six wells of the first row of tritator plate. Seventh well of first row was added with standard streptomysin (1mg/ml) solution in 10% DMSO. Subsequently, eight well of first row was treated as negative control. Two fold serial dilution were performed using a multichannel pipette such that each well had 50 µl of the test material (except standard and control wells) in serially descending concentration. 30 µl of 3.3 times stronger hi-sensitivity broth and 10 µl of resazurin dye (dissolving 27mg/4ml) were added to each well. Finally, 10 µl of bacterial suspension was added to the appropriate wells to achieve a concentration of approximately 5×10^6 CFU/ml. DMSO was used as a negative control and streptomycin as positive control. The plates were incubated at 37^0C for 24 h and colour change was observed. The growth was indicated by colour changes from blue to pink and MIC was confirmed by visual observation where no change of color was observed at minimum concentration of plant extract [18-19].

2.7 Statistical analysis
The statistical analysis of variance (ANOVA) was performed using ezANOVA (version 0.98) software. The results are expressed as mean±sem.

3. Result and Discussion
3.1 Preliminary phytochemical analysis
Alkaloids, phenolic compounds, terpenoids, glycosides and tannins are the major phytoconstituents present in almost all stem bark and leaf extracts. whereas, flavanoids proved to be absent in all extracts but has shown its prenece in a single extract of LEE. The Bark petroleum ether extract did not show the presence of flavonoids, terpenoids and glycosides. Similarly, saponins are absent in BCE, LPE and LCE (Table-I)

Table I: Qualitative phytochemical analysis of extracts of *Gardenia gummifera*:

Tests	BPE	BCE	BEE	LPE	LCE	LEE
Alkaloids	+	+	+	+	+	+
Flavonoids	_	_	_	_	_	+
Phenolic compounds	+	+	+	+	+	+
Terpenoids	_	+	+	+	+	+
Glycosides	_	+	+	+	+	+
Tannins	+	+	+	+	+	+
Saponins	+	_	+	_	_	+

BPE-Bark petroleum extract, BCE-Bark chloroform extract, BEE-Bark ethanol extract, LPE-Leaf petroleum extract, LCE-Leaf chloroform extract, LEE – Leaf ethanol extract (+) indicate the presence and (–) the absence of the respective phytochemicals.

The quantitative analysis showed the presence of maximum concentration of phenolic compounds in LEE followed by LPE, BEE and BPE with almost same quantities. Lowest concentration is found to be present in BCE. Similarly alkaloids are present in maximum concentration in LEE, moderate concentration in LCE and BEE. BPE showed in lowest concentration of 16 µg/mg alkaloids(Table II).

Table II: Quantitative phytochemical analysis of extracts of *Gardenia gummifera*:

Tests	BPE	BCE	BEE	LPE	LCE	LEE
Phenolic compounds	30µg/mg	22µg/mg	32µg/mg	34µg/mg	24µg/mg	38µg/mg
Alkoloids	16 µg/mg	14µg/mg	22µg/mg	14µg/mg	20µg/mg	26µg/mg

BPE- Bark petroleum ether extract, BCE- Bark chloroform extract, BEE- Bark ethanol extract**,** LPE- Leaf petroleum ether extract, LCE- Leaf chloroform extract, LEE- Leaf ethanol extract.

3.2 Evaluation of antibacterial activity

The antibacterial capacity of both the experimental plant parts were screened according to their zone of inhibition against selected clinical pathogens and the results (zone of inhibition) were compared with the standard streptomycin (10µg/disc).

The results showed that all the extract acted against all the microorganism studied in present investigation. Among the different solvents extracts used, ethanol showed more inhibition followed by petroleum ether and chloroform extracts. Stem bark and leaf extract were screened by the agar well plate method and the mean value of zone of inhibition was assessed in milli meter. The extract showing very good inhibition against growth of bacteria at a concentration of 1500µg/ml. For all the screened pathogens ethanol extracts showed more activity. In stem bark ethanol extract, maximum zone of inhibition was obtained in *S. typhi* and *S. aureus* with 12.67±0.33mm and 12.33±0.33mm diameter respectively, and in leaf ethanol extract maximum zone of inhibition was recorded in *S. typhi* and *S. aureus* with 12.33±0.33mm and 12.83±0.60mm diameter respectively.

In stembark chloroform extract, maximum zone of inhibition was obtained in *P. aeruginosa* and *V. cholerae* with 11.17±0.17mm and 10.23±0.15mm diameter respectively, and in leaf chloroform extract maximum zone of inhibition was recorded in *P. aeruginosa* and *S. typhi* with diameter of 11.17±0.17mm and 11.00±0.00mm respectively

In stem bark petether extract, maximum zone of inhibition was obtained in *S. aureus* and *S. pneumoniae* with diameter 10.33±0.33mm and 09.50±0.29mm respectively, and in leaf petether extract maximum zone of inhibition was recorded in *S. typhi* and *V. cholerae* with 10.90±0.20 mm and 10.50±0.00mm diameter respectively.

Table III: zone of inhibition measurement of antibacterial activity:

Sl. No.	Extracts	Microorganisms	Zone of inhibition (Z.I) (1500 µg/well)	Activity index (A.I)
1	BPE	*S. aureus*	10.33±0.33	0.459
		S. pneumoniae	09.50±0.29	0.459
		V. cholerae	09.33±0.33	0.351
		P. aeruginosa	08.83±0.17	0.420
		S. typhi	09.00±0.00	0.443
		K. pneumoniae	08.67±0.17	0.376
2	BCE	*S. aureus*	08.57±0.23	0.381
		S. pneumoniae	09.17±0.17	0.443
		V. cholerae	10.23±0.15	0.385
		P. aeruginosa	11.17±0.17	0.531
		S. typhi	09.50±0.00	0.467
		K. pneumoniae	09.33±0.33	0.405
3	BEE	*S. aureus*	12.33±0.33	0.548
		S. pneumoniae	12.00±0.58	0.580
		V. cholerae	10.67±0.33	0.402
		P. aeruginosa	12.17±0.33	0.579
		S. typhi	12.67±0.33	0.624
		K. pneumoniae	11.00±0.00	0.478
4	LPE	*S. aureus*	08.90±0.21	0.396
		S. pneumoniae	09.00±0.29	0.435
		V. cholerae	10.50±0.00	0.395
		P. aeruginosa	09.83±0.17	0.468
		S. typhi	10.90±0.20	0.536
		K. pneumoniae	10.23±0.15	0.444
5	LCE	*S. aureus*	09.83±0.17	0.437
		S. pneumoniae	09.23±0.15	0.446
		V. cholerae	08.57±0.23	0.322
		P. aeruginosa	11.17±0.17	0.531
		S. typhi	11.00±0.00	0.541
		K. pneumoniae	09.00±0.58	0.391
6	LEE	*S. aureus*	12.83±0.44	0.571
		S. pneumoniae	11.67±0.33	0.564
		V. cholerae	11.33±0.33	0.426
		P. aeruginosa	12.00±0.58	0.571
		S. typhi	12.33±0.33	0.607
		K. pneumoniae	11.00±0.58	0.478

AI (Activity Index) = ZI of Test/ZI of Standard. BPE-Bark petroleum ether extract, BCE-Bark chloroform extract, BEE-Bark ethanol extract**,** LPE-Leaf petroleum ether extract, LCE- Leaf chloroform extract, LEE – Leaf ethanol extract. Readings are presented in Mean ± S.D. ZI of Streptomycin for SA, SP, VC, PA, ST and KP is 22.46±0.00, 20.67±0.33, 26.54±0.58, 21.00±0.33, 20.30±0.00 and 23.00±0.58 respectively.

3.3 Minimum inhibitory concentration

The modified resazurin assay (Fig.1) indicated that the stem bark ethanol extract exhibited highest minimum inhibitory activity against *S. typhi* with a significant inhibition zone of 12.67±0.33 mm and MIC value of 5.66 µg. Leaves ethanol extract exhibited highest inhibitory activity against *S, aureus* with prominent zone of inhibition of 12.83±0.44 mm with MIC value of 8.33 µg.

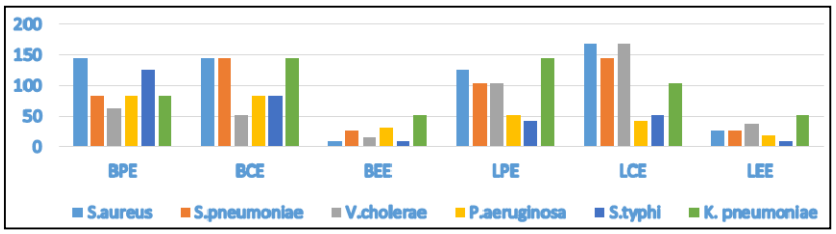

Fig 1: Minimum inhibitory concentration of bark and leaf extracts

4. Discussion

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents [20]. During the last several years, natural products with antimicrobial effect were explored in order to eliminate the use of synthetic antibiotics which cause the resistance of microorganisms and can exhibit side effects to human health [21]. Scientific investigations for medicinal plants have been initiated in many countries because of their contributions to health care. It was cleared that the primary benefits of using plant derived medicines are relatively safer than synthetic alternatives. So that, the exploration for antimicrobials from natural basis has received much attention in these days. Many studies have been conducted with the extracts of various plants assessing antimicrobial activity as well as for the discovery of new antimicrobial compounds [22]. Phytochemicals derived from the plant products serve to develop less toxic and more effective medicines in controlling the growth of microorganisms and curing of many diseases [23].

The traditional claim indicated that the *G. gummifera* plant parts are used to treat wounds, roundworms, cough, respiratory diseases fever and skin diseases [24]. Moreover, the related plants of this genus *Gardenia latifolia* and *Gardenia resinifera*, have good antibacterial efficacy against bacterial strains namely *Bacillus cereus, Bacillus megatherium, Staphylococcus aureus, Enterobacter aerogenes* and *Salmonella paratyphi* [25]. *Gardenia coronaria* also have very good antibacterial activity against *Streptococcus agalactiae, Bacillus cereus, Shigella sonnei*, *Shigella boydii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis* [26]. However, there are no scientific reports available for antibacterial activity against human pathogens and further the quantitative estimation has not been performed for alkaloids and phenolic components in the stem bark and leaf extracts of *G. gummifera*. While considering the above reports this study was planned to explore the antibacterial efficacy of *G. gummifera.*

The result of the current phytochemical and quantitative screening showed that the stem bark and leaves of *G. gummifera* extracts were rich in phytoconstituents such as alkaloids, phenolic compounds, terpenoids, glycosides and tannins. Among these groups alkaloids and phenolic compounds are found to be very rich followed by tannins. While as terpenoids and glycosides are present in all the extracts except BPE. Earlier reports on medicinal plants suggest that alkaloids [27] and phenolic compounds [28] extensively play a vital role against the pathogens.

The quantitative analysis showed the presence of maximum concentrations of phenolic compounds in LEE (38 µg/mg) and alkaloids in BEE (26 µg/mg). In this study, different extracts of *G, gummifera* were assessed for exploration of their antimicrobial activities against certain gram positive and gram negative bacteria.

The preliminary investigation showed that ethanol, chloroform and pet ether extracts in both stem bark and leaves are active against the human pathogens like *S, aureus, S, pneumoniae*, *V, cholera, P, aeruginosa*, *S, typhi,* and *K, pneumoniae*. The alcoholic extracts shows significant antibacterial activity against all the pathogens compared to chloroform and pet ether extracts. Among the tested extracts highest inhibition is observed in BEE exhibiting 12.67±0.33mm inhibition zone against *S. typhi* and similarly LEE exhibited 12.83±0.60mm inhibition zone against *S. aureus*.

In the present study, the comparison of the samples with the reference standard have emphasized that the samples used exhibited moderate activity in terms of activity index method. The sample BEE showed 0.624 A.I against *S. typhi* and in LEE sample is found be that 0.601 A.I against *S. aureus*. The lowest MIC value has been observed against *S. typhi* after treating with BEE and also in *S. aureus* when exposed to LEE. This observation confirms that the *G. gummifera* plant extracts possess have an antibacterial efficacy.

Thus, the present investigation indicates that *G. gummifera* contains important phytochemicals. That may be responsible for the inhibition of growth of pathogens. The present results are promising for the isolation and characterization of biologically active constituents as anti-microbial agents.

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6. References

1. Hosseinzadeh S, Jafarikukhdan A, Hosseini A, Armand R. The Application of Medicinal Plants in Traditional and Modern Medicine. Int J Clin Med. 2015; 6(9):635-642.
2. Shakya AK. Medicinal plants: Future source of new drugs. Int J Herb Med IJHM. 2016; 59(44):59-64.
3. Wermuth CG. The practice of medicinal chemistry. Academic Press, 2003.
4. Sivakamasundari PR, SK, RP. Survey on the RET-listed Medicinal Plants in Thadagamalai Range of Kanyakumari District, Tamilnadu. J Biodivers Endanger Species. 2015; 3(1).
5. Tambekar H, Khante BS. Antibacterial evaluation of medicinal plants used by korkusin melghat forest against

gastrointestinal infections Int J Phar sc res. 2011; 2(3).
6. Vindhya K, Leelavathi S. Evaluation of Antioxidant Properties and Total Phenolic Content of *Gardenia gummifera* Linn. 2015; 32(42):255-261.
7. Vindhya K, Kk SK, Hs N, Leelavathi S. Research Journal of Pharmaceutical, Biological and Chemical Sciences Preliminary Phytochemical Screening of Gardenia latifolia Ait. and Gardenia. Res J Pharm Biol Chem Sci. 2014; 5(2):527-32.
8. Prabhavathi RM, Prasad MP, Jayaramu M. Studies on Qualitative and Quantitative Phytochemical Analysis of *Cissus quadrangularis*. Pelagia Res Libr Adv Appl Sci Res. 2016; 7(4):11-17.
9. Ira S, Manisha M, GPS, Anirudha R. International journal of drug development & research. International Journal of Drug Development & Research. 2009; 6(2).
10. Prabhavathi RM, Prasad MP, Jayaramu M. Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis. Pelagia Res Libr Adv Appl Sci Res. 2016; 7(4):11-7.
11. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, 1999, 152-78.
12. Sen A, Batra A. Determination of antimicrobial potentialities of different solvent extracts of the medicinal plant: Phyllanthus amarus Schum. and Thonn. Int J Green Pharm. 2012; 6(1).
13. Murray PR, Baron EJ, American Society for Microbiology. Manual of clinical microbiology. 8th ed. Washington D. C: ASM Press, 2003.
14. Saleem U, Saleem M, Ahmad B, Hussain K, Ahmad M, Bukhari NI, *et al*. *In-vitro* antimicrobial susceptibility testing of leaves methanol extract and latex of euphorbia helioscopia using agar well diffusion and broth dilution methods. J Anim Plant Sci. 2015; 25(1):261-267.
15. Sudhesh L, Shastri V, Krishna, Ravi Kumar S, Santosh Kumar S, R Venkatesh, *et al*. Phytochemical analysis, antibacterial property and molecular docking studies of *Mammea suriga* Kosterm., World Journal Pharmaceutical sciences. 2017; 4(9):331-340.
16. Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. Methods. 2007; 42(4).
17. Arunodaya HS, Krishna V, Shashikumar R, Girishkumar K. International journal of pharmacy and pharmaceutical sciences. IJPPS. 2016; 8(12):258-264.
18. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant Staphylococcus aureus activity. BMC Complement Altern Med. 2005; 5(1):6.
19. Hasselmann C, Diseases I. Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. Clin Microbiol Infect. 2003; 9(8).
20. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J Ethnopharmacol. 1998; 61(1):57-65.
21. Alabi OA, Haruna MT, Anokwuru CP, Jegede T, Abia H, Okegbe VU, *et al*. Comparative studies on antimicrobial properties of extracts of fresh and dried leaves of *Carica papaya* (L) on clinical bacterial and fungal isolates. Pelagia Res Libr Adv Appl Sci Res. 2012; 3(5):3107-3114.
22. Sen A, Batra A. Determination of antimicrobial potentialities of different solvent extracts of the medicinal plant: Phyllanthus amarus Schum. and Thonn. Int J Green Pharm. 2012; 6(1).
23. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol. 2001; 74(2):113-123.
24. Maitreya BB. Int J, Life P, An overview of Ethnomedicinal plants of Family Rubiaceae from Sabarmati River of Gujarat state, India. IJPLS 2015; 6(5):4476-80.
25. Jhansi LB, Jaganmohanreddy k. *In Vitro* Anti-Bacterial Studies of Gardenia Resinifera Roth and Gardenia Latifolia Ait. Research journal of biotechnology. 2011; 6:26-30.
26. Chowdhury A, Azam S, Jainul MA, Faruq KO, Islam A. Antibacterial Activities and *In Vitro* Anti-Inflammatory (Membrane Stability) Properties of Methanolic Extracts of Gardenia coronaria Leaves. Int J Microbiol, 2014.
27. Debnath B, Uddin MJ, Patari P, Das M, Maiti D, Manna K. Estimation of alkaloids and phenolics of five edible cucurbitaceous plants and their antibacterial activity. Int J Pharm Pharm Sci. 2015; 7(12):223-227.
28. Baba SA, Malik SA. Evaluation of antioxidant and antibacterial activity of methanolic extracts of Gentiana kurroo royle. Saudi J Biol Sci. 2014; 21(5).