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Isolation, purification and characterization of antibacterial bioactive compounds from *Bougainvillea spectabilis* Leaf.

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Abstract
Bacterial diseases are the major threats for the mankind and silkworm. Hence, present study was initiated to evaluate the antibacterial potential of different solvent extracts of *Bougainvillea spectabilis* leaves. The initial screening of different solvents against mulberry silkworm infecting bacteria, *Bacillus thuringenesis* revealed that, chloroform extract showed more inhibition of test pathogen. Further, the chloroform extract was evaluated for the antibacterial potential against *Salmonella typhi* and *Shigella flexneri*, human pathogens. Chloroform extract of *B. spectabilis* inhibited the both pathogens, which confirms the antibacterial character of extract on both gram negative and positive bacteria. TLC fingerprinting of chloroform extract revealed presence of five major bands with different retardation factor value (RF value). The screening of individual bands for antibacterial activity revealed that, the band with Rf value of 0.84. The HPLC profiling of showed the reduction in number of compounds in partially purified extract comparison with crude extract. GC-MS analysis of antibacterial band revealed the presence of Nineteen different compounds having different important biological activity viz., antibacterial, anti-inflammatory, antidiabetic, antifungal, antioxidant and antiprotozoal characters. The results of the present work confirms that, the chloroform extract of *B. spectabilis* leaf can be used for the management of bacterial diseases of humans and silkworm.

Keywords: *Bougainvillea spectabilis*, Antibacterial activity, Silkworm, TLC, HPLC, GC-MS

Introduction
Plant based medicines has become a part of traditional healthcare in majority parts of the world for thousands of years. Several medicinal plants are being used daily in Ayurvedic practices. In India more than 7,000 medicinal plant species are known, which contains many biological active compounds having antimicrobial property and were used as an antimicrobial drugs in traditional medicines. As per the report of World Health Organization, nearly 80% of world's populations depends on traditional medicine for their primary health care needs. Recently, medical practitioners are switching over to such plant based medicines, as there is a high rate of developing of resistant pathogens due to excessive selection pressures created by misuse and rampant use of classical antimicrobials like antibiotics (Bayane *et al*., 2016) [4].
Bougainvillea is a genus of flowering plant belonging to the family of Nyctaginaceae native in South America. It has about 18 species and generally used in the arid landscapes for beautification, horticulture, pharmaceutical industries, agriculture and environmental industries on account of the large flexibility in different agro climatic regions of the world. *Bougainvillea spectabilis* is another widely used species from Brazil. It grows to 6 feet. Its leaves are darker than the rest of bougainvilleas, with hairy leaves, covered with very prominent hairs on the underside. It flowers during summer and its bracts are bright red or purple.
They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as antibacterial, anti-inflammatory, antiulcer, anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity were reported.
In this study, *Bougainvillea* plant was investigated for the bioactive compounds present in its leaf. Extraction of the plant material was done with n-butanol, methanol, chloroform and distilled water and the bioactivity of each extract was tested against mulberry silkworm pathogen, *Bacillus thuringiensis* and human pathogens like *Salmonella typhi, Shigella flexneri.* The separation and characterization of bioactive compounds present in the leaf was carried out by using TLC, HPLC and GC-MS.

Materials and Methods

Maintainance of pathogens

Pre-maintained culture of bacterial pathogens viz., *Bacillus thuringiensis* was collected by culture collection center of CSRTI, Mysore, where as *Salmonella typhi* and *Shigella flexneri* were collected from the Department of Biotechnology, University of Mysuru, Mysuru. The sub-culturing of the pathogens was carried out under a-septic condition to evaluate the antibacterial activity aganist leaf extract. The strains were grown in NB medium and incubated at 37 ºC for overnight. After incubation the culture were centrifuged and the pellets were collected to check the antimicrobial sensitivity test.

Collection of plant material

The fresh green leaves of *Bougainvillea spectabilis* visibly free from disease were collected from the garden area of Central Sericulture Research and Training Institute, Central Silk Board, Mysore.

Preparation of extract

The leaves of *B. spectabilis* were washed thoroughly several times with potable water and then allowed to dry naturally on room temperature. Dried leaves were crushed by using liquid nitrogen by using pestle and mortar. The fine powder was soaked separately in 50ml of n-butanol, 50ml of methanol, 50ml of chloroform and 50ml of distilled water for 24hours. The extracts were filtered through Whatman No.1 filter paper and freshly prepared extracts were used for analysis.

Determination of Antimicrobial activity

Antimicrobial susceptibility test of the selected pathogens was done by Disc diffusion method. Whatman filter paper disc with diameter about 6mm was used for preparing discs. Each disc was immersed in the respective leaf extracts and placed on the surface of pathogenic bacteria inoculated plates. These freshly prepared discs were used for the determination of antibacterial activity. All the tests were performed on nutrient agar plates under aseptic condition in Laminar Air Flow (LAF). Each pathogenic bacterial suspension was prepared in sterile distilled water. Suspension of microbial cultures (50µl) was inoculated on the surface of the nutrient agar media in a petriplate by spread plate method using L shaped glass rod. The sterile discs of diameter 6mm were immersed in the respective leaf extract and positive control (ampicillin) solutions placed onto inoculated Nutrient agar plates. The experimental Nutrient agar plates were incubated at 37 ºC for 24-48hours. After incubation the plates was observed for the presence of inhibition zone around each disc. The diameter of zone of inhibition of bacteria was recorded in milli metres.

Thin layer chromatography

The concentrated fractions collected from the column chromatography were subjected to thin layer chromatography (TLC). Briefly, a spot of each fraction was carefully applied onto a thin layer chromatographic plate (coated with silica) and left to dry. After about five minutes, the plates were dipped in a suitable solvent (methanol: chloroform: acetic acid in the ratio 9:1:1drop) which allowed the compounds in the spot to move upwards by capillary attraction. The plate was then removed from the solvent and left to dry. The position of different compounds was observed by fluorescence under UV-light and Rf value was calculated by using the following formula:

Rf = distance travelled by centre of component /distance travelled by solvent front.

The different bands from TLC plate were detached (scrapped) separately. The individual bands were dissolved in chloroform and the obtained supernatant was subjected to antibacterial activity against B. thuringenesis. The antibacterial activity exhibiting bands was further used for purity and analyis of compounds.

Analytical HPLC of crude and partially purified chloroform extract of *B. spectabilis*

The partially purified chloroform extract was subjected to HPLC for purity analysis. Samples were filtered through an ultra-membrane filter (pore size 0.45 µm) prior to injection in the sample loop. The HPLC system (Spinco laboratories) was used for analysis. Chromatographic analysis was carried out using a C-18 column at ambient temperature under isocratic condition with mobile phase of Acetonitrile: Methanol in the ration of 80:20 at flow rate 0.500 ml/min; and detection at 260nm. The total volume of the sample injected to HPLC was 100µl.

GC-MS analysis

The GC-MS MS analysis was carried out using Varian 4000 ion trap GC-MS with fused silica 15m×0.2mm 1D×1µm of capillary column. The instrument was set to an initial temperature of 70 ºC, and maintained at this temperature for 2min. At the end of this period the oven temperature was rose up to 260 ºC, at the rate of an increase of 6ºC/min, and maintained for 9min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1ml/min. The ionization voltage was 70CV. The samples were injected in spilt mode as 10:1. Mass spectral scan range was set at 45-450(m/z). Using computer searches on a NIST ver 2.1ms data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

Results

Initial evaluation of different solvent extracts of *B. spectabilis* leaf on *B. thuringiensis*

The antibacterial activity of different solvent extracts of *B. spectabilis* was evaluated against *Bacillus thuringiensis*. Based on the presence of clear zone of inhibition, among the four organic solvents used in the study, chloroform extract markedly inhibited the growth of organism. The zone of inhibition was measured. Standard antibiotic, Ampicillin showed the more inhibition zone in comparison with other solvent extracts. (Table 1 & Fig 2).

Evaulation of antibacterial activity against the bacterial pathogens of silkworm and humans.

The antibacterial activity of chloroform extract was evaluated by under *in vitro* condition against *Salmonella typhi, Shigella flexneri* (gram negative bacteria) *and Bacillus thuringiensis* (gram positive bacteria). Clear zone of inhibition was noticed against the three pathogen used in the study. Among the three organism tested, *S. typhi* showed more sensitivity towards chloroform extract of *B. spectabilis* followed by *Shigella flexneri* and *Bacillus thuringiensis* (Table 2 & Fig 3).

Characterization of compounds by using TLC

The seperation of compounds on TLC revealed the presence of different compounds in the chloroform extract of *B. spectabilis*. The Retardation factor value (Rf value) of

different seperated compounds was calculated by using the formula.

$$Rf = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent front}}$$

The different compounds seperated in TLC were collected by scrapping the band from TLC and evaluation of their antibacterial potential was carried out against *B. thuringenesis.* The promising compound present in Rf value 0.84 was further characterization (Table 3 & Fig 5).

Evaluation of purity of partially purified compounds by using HPLC

The isocratic sepeartion of crude and partially purified chloroform extract revealed the presence of different compounds. The HPLC chromatogram of crude extract revealed the presence of 10 compounds (peaks) (Fig.5), where as partially purified extracts showed the presence of 3 compounds (peaks) (Fig.6).

Characterization of compounds present in the partially purified extract by using GC-MS

The characterization of compounds present in the partially purified extract has revealed the presence of nineteen antimicrobial compounds (Table 4 & Fig 7). The majority of compounds identified in the present study have antimicrobial activity. Some of the compounds identified possess anti-inflammatory, antidiabetic, antifungal, antioxidant and antiprotozoal characters.

Discussion

The medicinal plants are of greatest interest pertaining to human health. Medicines derived from plants have been a component of traditional healthcare in most parts of the world for centuries or thousands of years. In Ayurveda practices, exploitation of many medicinal plants is being carried out. More than 7,000 medicinal plant species are reported from India. Due to application in pharmaceutical, cosmetic, agricultural and food industry medicinal plants gained major attention from different scientific corners of the world. Due to presence of numerous biologically active compounds in plants, many of these have been shown to exhibit antimicrobial properties and hence, they have been exploited as antimicrobial drugs in traditional medicines. As per the report of World Health organization, still 80% of world's populations for their primary health needs depends on traditional medicine. The screening of plant extracts or plant derived substances systematically still remains an attention-grabbing strategy to find new lead compounds (Maheshwari *et al.*, 2006) [14].

Bougainvillea spectabilis belonging to family Nyctaginaceae is an important horticultural plant. Leaf extracts of B. specatibilis showed strong antiviral activity against plant viruses (Madhusudhan *et al.*, 2011) [13]. The antiviral protein present in *B. spectabilis* was characterized by Balasaraswathi *et al.* (1998) [3] and anti-inflammatory activities were also observed by Mandal *et al.* (2015) [15].

Several reports says that, B. spectabilis have factors responsible for controlling and preventing diabetes (Jwala *et al.*, 2012) [9]. *In vitro* antibacterial activity of Bougainvillea spectabilis leaves extracts is been reported by Umamaheswari *et al.* (2008) [24]. The considerable antimicrobial activity was observed in *B. spectabilis*. All flower extracts of *B. spectabilis* inhibited the growth of few of the bacterial and fungal strains tested with varied effectiveness. The ethanol and chloroform extracts have shown relatively greater activity than that of any other extracts at 40µl concentration (Swamy *et al*., 2012). The results of our study revealed that, among different solvent extracts utilized the chloroform extract showed more inhibition of B. thuringenesis (Fig. 2). Subsequent evaluation of chloroform extract on the human pathogens *Salmonella typhi, Shigella flexneri* (gram negative bacteria) under *in vitro* condition showed clear zone of inhibition (Table 2 & Fig 3). These results confirms that, chloroform extract of *B. spectabilis* can be used as potent antibacterial extract for inhibiting bacterial infections in silkworm as well as human beings. Similarly, Dhankhar *et al*. (2013) [5] evaluated the antibacterial activity of various solvent extracts (water, methanol, acetone, chloroform, petroleum ether) of the leaf of *B. spectabilis*.

Natural products, such as plants extract, either as pure compounds or as standarized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity. Earlier study carried out by Maheshwari *et al.* (2008) [24] have analyzed qualitatively by phytochemical screening for the presence of amino acids, proteins, anthroquinones, saponins, triterpenoids, flavonoids, carbohydrates, alkaloids, phytosterols, glycosidal sugars, tannins, phenols and furanoids in different solvent extracts of *B. spectabilis*. The authors opinied that, phytochemicals present in exatracts may be responsible for the antibacterial acitvity of the plant leaf extract. Hajare *et al*. (2015) [7] evaluated the antibacterial activity of various solvent extracts of the leaf of *B.spectabilis*. Similarly, in the present study, the characterization of compounds present in the partially purified extract has revealed the presence of nineteen antimicrobial compounds which are having antimicrobial activity (Table 4 & Fig 7). The a number of compounds identified from GC-MS possess anti-inflammatory, antidiabetic, antifungal, antioxidant and antiprotozoal characters.

Table 1: Initial evaluation of different solvent extracts of *B. spectabilis* leaf on *B. thurengenisis*.

Sl. No.	Sample	Inhibition zone (in cm)
1.	Positive control (Ampicillin)	0.5±0.57
2.	Chloroform	0.5±0.27
3.	Methanol	0.2±0.43
4.	n- Butanol	0.1±0.33

The values are the mean of three independent experiments ± Standar error.

Table 2: Antibacterial activity of leaf extract against human bacterial pathogens

Sl No.	Organism	Positive control(cm)	Chloroform extract(cm)
1.	*B. thuringiensis*	0.8±0.17	0.2±0.07
2.	*Shigella flexneri*	0.5±0.13	0.2±0.17
3.	*Salmonella typhi*	1.0±0.23	0.5±0.21

The values are the mean of three independent experiments ± Standar error.

Table 3: The Retardation factor value (Rf) of different seperated compounds

Sl No.	Rf value
1.	0.59
2.	0.68
3.	0.84
4.	0.89
5.	0.93

Table 4: GC-MS Analysis of chloroform extract of *Bougainvillea spectabilis*

Sl. No	Compound name	Remarks	References
1.	Cyclotetradecane	Antibacterial, antifungal	Afrouzan *et al*., (2018) [1]
2.	5-Isopropyl-4-(Trifluoro methyl)-1H-pyrimidin-2-one	Antibacterial	Sengottuvel *et al.*, (2015) [20]
3.	2-tert-butyl-4-triflurомethyl-1-methylimidazole	Antibacterial	Sangeetha *et al.*,(2015) [19]
4.	2-tert-butyl-4-isopropyl-5-methylphenol	Antibacterial	Sangeetha *et al*., (2015) [19]
5.	2-Allyl-5-t-butylhydroquinone	Antibacterial	Pradheesh *et al*., (2017) [17]
6.	15-Methyltricyclo pentadeca-1,3,5,7	Antibacterial	Mohana Priya and Senthilkumar (2014) [16]
7.	9,11,13-Heptene	Antibacterial, antifungal and anti-inflammatory	Veena *et al*., (2016) [25]
8.	Isochiapin- B	Antibacterial	Senthilkumar *et al*., (2012) [21]
9.	2(1H)Naphthalenone	Antibacterial	Senthilkumar *et al*.,(2012) [21]
10.	Octahydro-1-methyl-1-(2-propenyl)	Antibacterial	Kusuma and Mahfed (2017) [12]
11.	Olean-18-ene	Antiproliferation and anti-inflammatory	Hussain *et al*.,(2016) [18]
12.	D-Galactopyranoside, methyl	Antibacterial	Kawsar *et al*., (2016) [11]
13.	1,4-Naphthoquinone-2-acetyl-5,8-dihydroxy-3-methoxy	Antibacterial	Chahra *et al*., (2016) [6]
14.	1,2-Benzenedicarboxylic acid	Antibacterial	Shoge *et al*.,(2016) [22]
15.	Celidoniol, deoxy	Anti-inflammatory and Anti-cancer	Kanimozhi *et al*., (2012) [10]
16.	Pthalic acid	Antibacterial	Akter *et al*., (2017) [2]
17.	2,3-bis-o-(trimethylsilyl)-, cyclic butylboronate (CAS)	Antibacterial	Rzhepishevska *et al*., (2011) [18]
18.	Germanicol	Antiplasmodial	Zahid *et al*., (2013) [26]
19.	7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	Antibacterial	Pradheesh *et al*., (2017) [17]

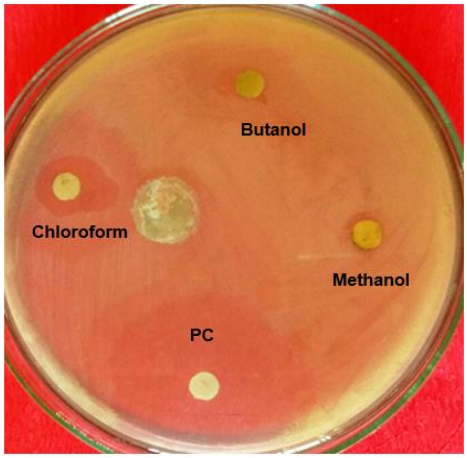

Fig 1: Initial screening of different solvent extracts of B. spectabilis leaf on *B. thuringiensis*

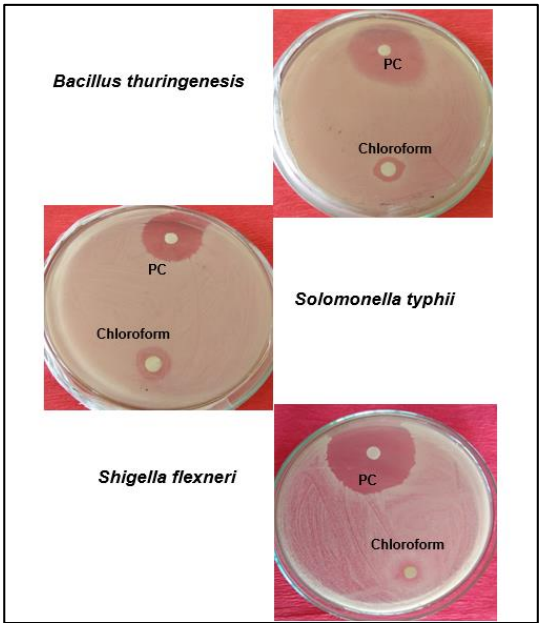

Fig 2: Inhibition of different pathogens by chloroform extract of *B. spectabilis* leaf.

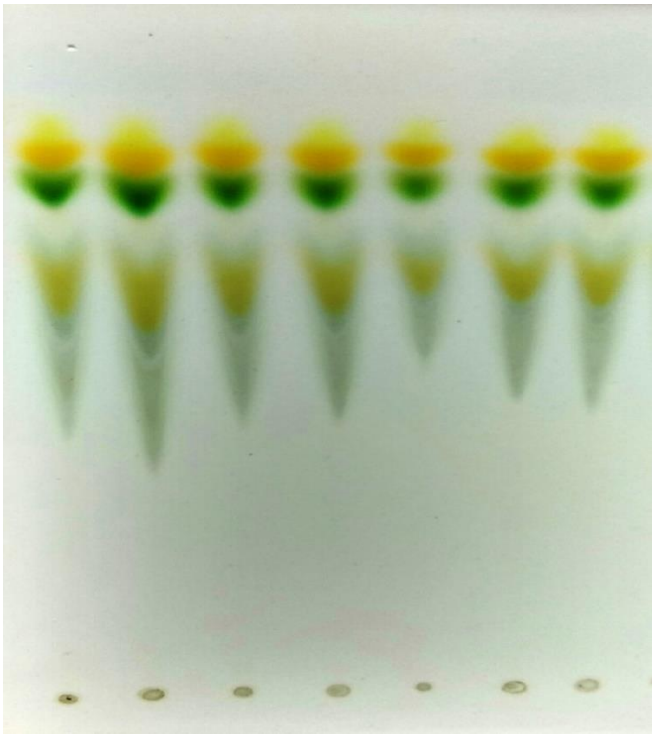

Fig 3: TLC fingerprinting of chloroform extract of *B. spectabilis* leaf

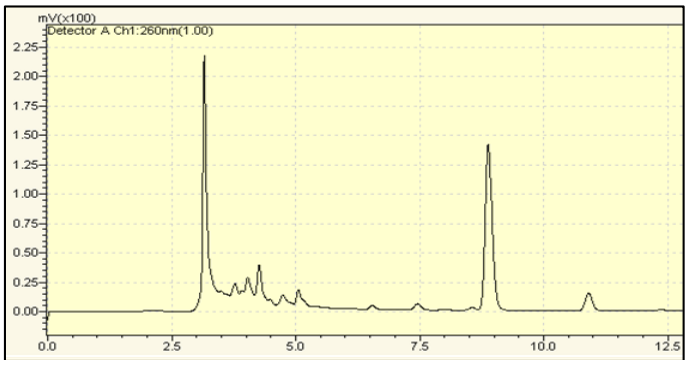

Fig 4: HPLC-Chromatogram of crude chloroform extract of *B. spectabilis* leaf.

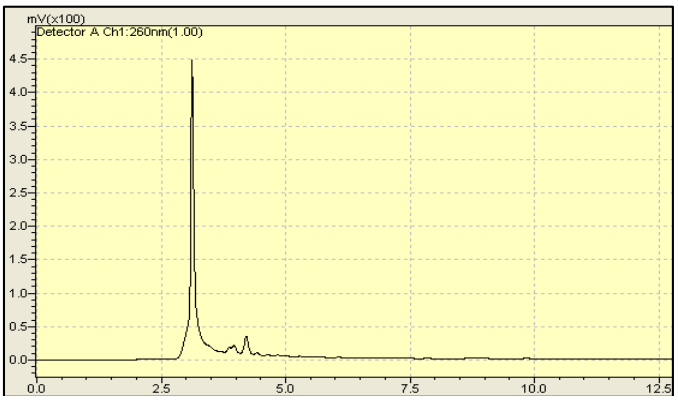

Fig 5: HPLC-Chromatogram of partially purified chloroform extract of *B. spectabilis* leaf.

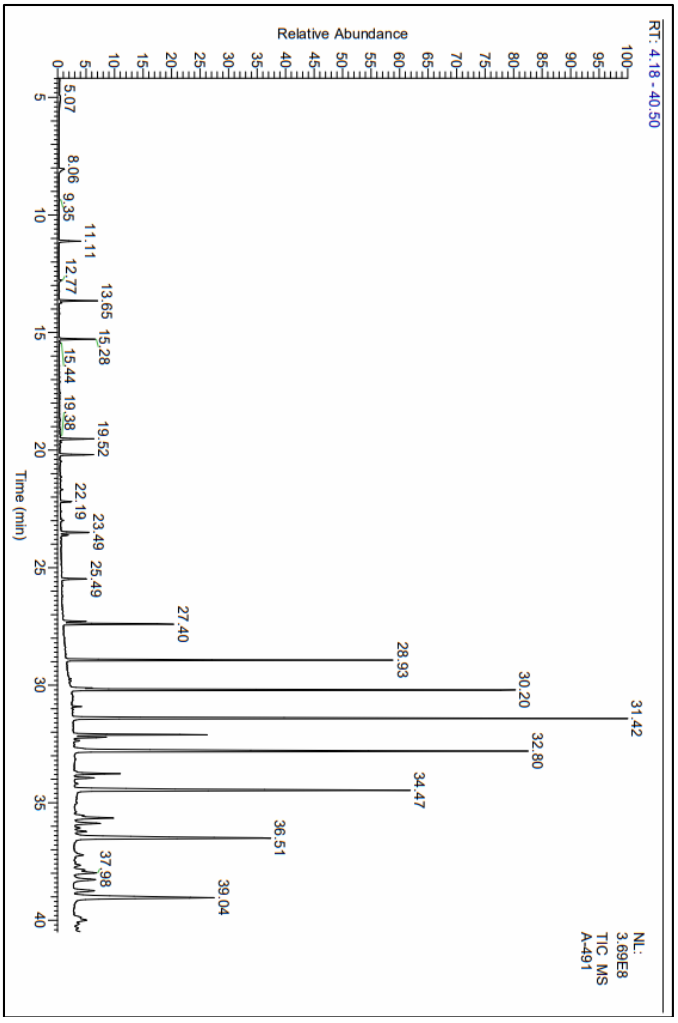

Fig 6: GC-MS spectra and fragmentation pattern of partially purified chloroform extract of *B. spectabilis* leaf.

Conclusion

The results of the present study suggested that, the different solvents antibacterial action of chloroform extract of *Bougainvillea spectabilis* leaves may signify their prospective as antibacterial herbal remedies. The compounds extracted from *Bougainvillea spectabilis* showed the antibacterial activity against bacterial pathogens viz., *Salmonella typhi, Shigella flexneri and Bacillus thuringiensis.* The study also shows that, bioactive compounds isolated from *Bougainvillea spectabilis* showed inhibition against both gram positive and gram negative bacteria. Hence, further studies are suggested to be undertaken the mechanism of such actions scientifically. This will emphasize on the isolation and characterization of active principles responsible for these activities of *B. spectabilis*. Since, *B. spectabilis* possess significant antimicrobial activity that, following additional studies, the potential antimicrobial compound could replace commercially known antibiotics.

References

1. Afrouzan H, Tahghighi A, Zakeri S, Es-haghi A. Chemical Composition and Antimicrobial Activities of Iranian Propolis. Iranian biomedical journal. 2018; 22:50.
2. Akter M, Graham H,. Iji PA. Interactions between phytase and different dietary minerals in *in vitro* systems. South African journal of animal science. 2017; 47:5.
3. Balasaraswathi R, Sadasivam S, Ward M, Walker JM. An antiviral protein from Bougainvillea spectabilis roots; purification and characterisation. Phytochemistry 1998; 47:1561-1565.
4. Beyene B, Beyene B, Deribe H. Review on application and management of medicinal plants for the livelihood of the local community. Journal of Resources Development and Management. 2016; 22:33-39.
5. Dhankhar S, Sharma M, Ruhil S, Balhara M, Kumar M, Chhillar AK. Evaluation of antimicrobial and antioxidant activities of *Bougainvillea spectabilis.* International Journal of Pharmceutical Sciences. 2013; 34:178-182.
6. Chahra D, Ramdani M, Lograda T, Chalard P, Figueredo G. Chemical composition and antimicrobial activity of Evernia prunastri and Ramalina farinacea from Algeria. Issues in Biological Sciences and Pharmaceutical Research. 2016; 4:35-42.
7. Hajare CN, Inamdar FR, Patil RV, Shete CS, Wadkar SS, Patil KS *et al*. Antibacterial activity of the leaves of Bougainvillea spectabilis against E. coli NCIM 2832 and M. aureus NCIM 5021. Int J Pharm Sci Rev Res 2015; 34:194-196.
8. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of Adiantum Capillus- Veneris using GC-MS and FT-IR spectroscopy. International Journal of Pharmacognosy and Phytochemical Research 2016; 8:369-385.
9. Jawla S, Kumar Y, Khan MSY. Hypoglycemia activity of *Bougainvillea spectabilis* stem bark in normal and alloxan induced diabetic rats. Asian Pac J Trop Biomed 2012; 2:19-23.
10. Kanimozhi D, Ratha bai V. Evaluation of Phytochemical antioxidant antimicrobial activity determination of bioactive components of ethanolic extract of aerial and underground parts of *Cynodon dactylon* L. International Journal of Scientific Research and Reviews. 2012; 1:33-48.
11. Kawsar SM, Nishat SS, Manchur MA, Ozeki Y. Benzenesulfonylation of Methyl [alpha]-D-Glucopyranoside: Synthesis, Characterization and Antibacterial Screening. International Letters of Chemistry, Physics and Astronomy. 2016; 64:95.
12. Kusuma HS, Mahfud M. GC-MS analysis of essential oil of Pogostemon cablin growing in Indonesia extracted by microwave-assisted hydrodistillation. International Food Research Journal. 2017; 24:1525-1528.
13. Madhusudhan KN, Vinayarani G, Deepak SA, Niranjana SR, Prakash HS, Singh GP *et al*. Antiviral Activity of Plant Extracts and other Inducers against Tobamoviruses Infection in Bell Pepper and Tomato Plants. International Journal of Plant Pathology. 2011; 2:35-42.

14. Maheshwari RK, Singh AK, Gaddipati J, Srimal RC. Multiple biological activities of curcumin: a short review. Life sciences 2006; 78:2081-2087.
15. Mandal G, Chatterjee C, Chatterjee M. Evaluation of anti-inflammatory activity of methanolic extract of leaves of *Bougainvillea spectabilis* in experimental animal models. Pharmacogonsy Research. 2015; 7:18-22.
16. Mohana Priya R, Senthilkumar P. Pharmacological Study of *Trichoderma indicum*. International Journal of Biotech Trends and Technology. 2014; 4:1-5.
17. Pradheesh G, Suresh J, Suresh S, Alex Ramani V, Hong SI. Antimicrobial activity and identification of potential compounds from the chloroform extract of the medicinal plant *Cyathea Nilgirensis Holttum*. World Journal of Pharmacy and Pharmaceutical Sciences. 2017; 6:1167-1184.
18. Rzhepishevska O, Ekstrand-Hammarström B, Popp M, Björn E, Bucht A, Sjöstedt A *et al*. The antibacterial activity of Ga3+ is influenced by ligand complexation as well as the bacterial carbon source. Antimicrobial agents and chemotherapy. 2011; 55:5568-5580.
19. Sangeetha C, Krishnamoorthy AS, Nakkeeran S, Ramakrishnan S, Amirtham D. Evaluation of bioactive compounds of *Ophiocordyceps sinensis sacc.* Against *Fusarium spp*. Biochem Cell Arch. 2015; 2:431-435.
20. Sengottuvel T, Kannappan N, Sivakumar T, Pytochemical and GC-MS analysis of bioactive compounds present in the *Adiantum incisum* forsk. Der Pharma Chemica. 2015; 7:137-142.
21. Senthilkumar N, Murugesan S, Vijayalakshmi KB. GC-MS-MS analysis of Trichilia connaroides (Wight and Arn.) Bentv (Meliaceae): A tree of ethnobotanical records. Asian Journal of Plant Science and Research. 2012; 2:193-197.
22. Shoge M, Garba S, Labaran S. Antimicrobial activity of 1,2-Benzenedicarboxylic acid, butyldecal ester isolated from the seeds and pods of Acacia nilotica Linn. Basic research Journal of Microbiology. 2016; 3:8-11.
23. Swamy MK, Sudipta KM, Lokesh P, Neeki MA, Rashmi W, Bhaumik SH *et al*. Pythochemical screening and in vitro antimicrobial activity of *Bougainvillea spectabilis* flower extracts. International Journal of Phythomed 2012; 4:375-379.
24. Umamaheshwari R, Shreevidya A. Nuni, *In vitro* antimicrobial activity of *Bougainvillea spectabilis* leaves extracts. Advances in Biological Research. 2008; 2:01-05.
25. Veena ME, Niranjana P, Rajeshwara Gas NA. chromatography-Mass spectrometry analysis of bioactive components from the rhizome extract of *Nardostachys Jatamansi*. Asian Journal of Pharmaceutical and Clinical Research. 2016; 9:115-118.
26. Zahid IA, Habib-ur-Rehman, Fiaz AM, Ashfaq AA. Antiplasmodial activity of compounds isolated from *Viburnum nervosum*. International Journal of Pharmaceutical Science Invention. 2013; 2:19-24.