Annals of Biology **40** (1) : 52-60, 2024

Impact Score : 0.32 (Scopus)

Phytochemical Analysis and Antibacterial Efficacy of *Sphagneticola trilobata* Extracts

ASHMITA CHHIKARA, PREETY ROHILLA AND PUSHPA DAHIYA*

Department of Botany, Maharshi Dayanand University, Rohtak-124 001 (Haryana), India
**(e-mail: puspha.dahiya@hotmail.com; Mobile: 94168 63575)*

(Received: June 11, 2023; Accepted: July 19, 2023)

ABSTRACT

The demand of in use medications and the urge to search for new storehouse of bioactive compounds have led the scientists to explore more plants for their therapeutic potential. The present investigation was planned to assess qualitative as well as quantitative phyto-compounds of *Sphagneticola trilobata* extracts prepared in aqueous, methanol, chloroform and petroleum ether solvent. Furthermore, antibacterial efficacy of all four plant extracts was assessed against seven bacterial strains i.e. *Bacillus subtilis* (MTCC-2057), *Chromobacterium violaceum* (MTCC-2656), *Escherichia coli* (MTCC-41), *Klebsiella pneumoniae* (MTCC-109), *Mycobacterium smegmatis* (MTCC-992), *Pseudomonas aeruginosa* (MTCC-2453) and *Staphylococcus aureus* (MTCC-96) employing disc diffusion method. MIC was also estimated for the plant extracts using micro-broth dilution assay. The methanol extract showed the highest percentage yield and presence of maximum number of phytoconstituents in addition to maximum TPC and TFC. Splendid antibacterial potential of *S. trilobata* was recorded due to its inhibitory activity against two bacterial strains i.e. *M. smegmatis* and *P. aeruginosa* where ampicillin was found ineffective. This shows the vast antibacterial potential of the plant against ampicillin resistant strains. These promising results showed the great potential of this plant in pharmaceutical industries.

Key words: Antibacterial, *Sphagneticola trilobata*, phytoconstituents, percentage yield, MIC

INTRODUCTION

Mankind has been using plants for treating diverse conventional diseases since antiquity. This is due to secondary metabolites found in plants well known for their biological properties. Plants based medications not only fight against microbes through their active bio-constituents but also boost the immune system of the consumer. However, progressively expanding population is amplifying disorders with time which is directly increasing the search for more plants with curative properties. Countless numbers of plants having active bio-constituents are still unexplored for their medicinal properties (Cheek *et al.*, 2020). Emergence of new diseases and old disease with more vigour yet again desires new and improved medications. The rising demand of prevailing medications straightaway weighs down their accessibility and manifests the invention of new pharmaceutics. Conjointly, adverse effects of allopathic medications make it more challenging for the investigators to meet the demand for new and effective plant based drugs. As a consequence, scientists throughout the world are looking for safe and effective bio active plant products to amplify the production (Iqbal *et al.*, 2017; Kashyap *et al.*, 2021).

Sphagneticola trilobata usually called as "trailing daisy" is a member of Asteraceae family. It is a broadleaved perennial plant showing mat-forming property and can grow up to a height of 60 cm. This plant was barely seen around the world before last few decades but now is making a headway gradually being naturalized in most countries. Wide range of environment tolerance makes this plant self-sowed in different parts of the world mainly with low elevations. The plant has traditional therapeutic value according to literature survey as the flowers and leaves were used by females to heal childbirth and amenorrhea (Suchantabud *et al.*, 2017; Luyen *et al.*, 2019; Afzal and Rajesh, 2021). In addition, the plant is reported to have pharmacological properties like antimicrobial, antioxidant, antitumor, analgesic, wound healing, anti-inflammatory, hepatoprotective, trypanocidal, larvicidal, uterine contraction, diabetes and even for treating reproductive and menstrual problems in women (Husain and Kumar, 2017; Luyen *et al.*, 2019; Prasanna *et al.*, 2019; RV, 2019;

Borghi *et al.*, 2021; Gowtham *et al.*, 2023). Traditional knowledge regarding medicinal herbs has always helped the modern world for new and effective medical formulations (Awuchi, 2019; Salmerón-Manzano *et al.*, 2020). Looking at the vast medicinal potential of the plant, the present investigation was designed to scrutinize the plant extracts prepared in different solvents for their phytoconstituents and to figure out their contribution in hampering bacterial growth.

MATERIALS AND METHODS

Sphagneticola trilobata was selected to explore its phytoconstituents composition and antibacterial properties due to its shortfall of literature studies. Plant material was collected from the coordinates 28.6055°N, 76.6538°E of Jhajjar district, Haryana. Reaffirmation was done by Prof. Bhoo Dev Vashishtha, Botany Department, Kurukshetra University, Kurukshetra.

Freshly collected plants were cleansed thoroughly using tap water to takeoff dust and soil. Vegetative aerial parts were washed further using double distilled water. Cleaned up plant portions were then dehydrated in shade for 20-22 days. After drying, plant material was grounded into powdered form and stored in refrigerator for further use. Soxhlet apparatus was used to prepare plant extracts in four solvents viz., aqueous, methanol, chloroform and petroleum ether in ratio 1:5 (weight/volume). Extraction process was completed in 28-30 cycles once the dark green solvent in extraction chamber became colourless. Prepared extracts were filtered through Whatmann filter paper no. 1, thereafter rotary evaporator was used to evaporate excess solvent. Plant extract was collected in a sealed container at 4°C till further experiments.

The concentrated plant extract was weighed and its yield was calculated as:

Percentage yield (PV) = 100 x [(Weight of crude plant extract)/(weight of powdered plant material)]

The extracts prepared in different solvents were screened to confirm the existence of phenols, flavonoids, alkaloids, saponins, glycosides, steroids, tannins and terpenoids according to the standard techniques (Sujatha *et al.*, 2019; Verma and Singh, 2020).

Total phenol content (TPC) was estimated using Folin-Ciocalteu Colorimetric Method with slight modifications (Pico *et al.*, 2020). 2.5 ml of freshly prepped FC reagent (10%) was poured in a test tube containing 1 ml of plant extract followed by addition of 2 ml Na_2CO_3 (2%). The solution was preserved in dark corner for 30 min and its absorbance was assessed at 765 nm using UV-Vis spectrophotometer. Gallic acid (1 mg/ml) was considered as standard.

Total flavonoid content (TFC) was estimated using Aluminum Chloride Colorimetric Method (Tristantini and Amalia, 2019) with minor modifications. 0.3 ml aluminum chloride (10%) and 0.3 ml potassium acetate (1M) were poured in a test tube containing 1 ml of plant extract followed by addition of 3 ml methanol and 3.5 ml distilled water. This solution was preserved in dark corner for 30 min. Its absorbance was assessed at 417 nm. Quercetin (1 mg/ml) was considered as standard.

Antibacterial property of the plant extracts was evaluated against seven bacterial strains using disc diffusion assay. Microbroth dilution assay was performed using 96 welled microtitre plates to determine the MIC values of different plant extracts.

Distinct concentrations of plant extracts were prepped i.e. 100, 50, 25 and 12.5 mg/ml. Four different plant extracts (methanol, chloroform, petroleum ether and aqueous) were reconstituted in DMSO to obtain desired concentrations.

Bacterial strains owned in the present investigation were *Bacillus subtilis* (MTCC-2057), *Chromobacterium violaceum* (MTCC-2656), *Escherichia coli* (MTCC-41), *Klebsiella pneumoniae* (MTCC-109), *Mycobacterium smegmatis* (MTCC-992), *Pseudomonas aeruginosa* (MTCC-2453) and *Staphylococcus aureus* (MTCC-96). The strains were purchased from CSIR-IMTECH (Institute of Microbial Technology), Chandigarh, India.

Bacterial inoculum was prepared using nutrient broth purchased from HiMedia. Mentioned grams of lyophilized powder were dissolved in distilled water to make 12-15 ml broth for each bacterium and autoclaved at 121°C for 20 min. After sterilization of nutrient broth, each tube was cultured with respective bacterium by adding loopful of particular

bacteria. Bacterial culture tubes were then kept in B.O.D. shaker at 37°C for 20 h. For experimental studies, the turbidity of bacterial cultures was checked and calibrated to standard i.e. 0.5 McFarland (1.5×10^8 CFU/ml). To access the antibacterial potentiality of the plant extracts, disc diffusion method was practised (Saedi *et al.*, 2020). Mentioned grams of nutrient agar were mixed in distilled water and kept in autoclave at 121°C for 20 min. 22-25 ml of sterilized nutrient agar media was poured in aseptic Petri plates purchased from Tarsons. 100 ml of calibrated bacterial culture (1.5×10^8 CFU/ml) was evenly spread over the solidified media using L-shaped spreader. Petri plates were top-dressed with six sterile discs placed equidistant including one in the center. 10 ml of plant extract with four different concentrations was loaded on the discs and named A, B, C and D, respectively. Disc loaded with DMSO was used as negative control and specified as E, whereas ampicillin (0.1 mg/ml) was used as positive control and disc loaded with it was labelled as F on the Petri plates. Prepared Petri plates were then incubated in B.O.D. at 37°C for 24 h. ZOI obtained was measured using antibacterial scale purchased from Himedia. The experiment was performed in triplicates to record the mean value specifying the actual ZOI for a particular solvent.

Minimum concentration of plant extract required to inhibit the growth of a specific bacterium was calculated using micro broth dilution assay employing two-fold serial dilutions of the distinct plant extract (Fajarningsih *et al.*, 2018). Firstly, 100 ml of nutrient broth was added up to 8×6 wells of microtiter plate having dimensions 12×8 wells. 100 ml of plant extract starting from concentrations undermost on the Petri plates plus dropping further viz. 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195 and 0.097 mg/ml was added, respectively, up to eight wells for each plant extract. 10 ml of calibrated bacterial culture (1.5×10^8 CFU/ml) was added in each well and consequently 10 ml of resazurin dye (0.4% weight/volume in double distilled water) was added in every well. These Petri plates were then shielded with parafilm to avoid drying up and placed in B.O.D. incubator at 37°C for 24 h. MIC value for a particular bacterium was analyzed and no change in colour from purple to pink was noted as MIC value.

RESULTS AND DISCUSSION

In the present study, *Sphagneticola trilobata* was selected for investigating its phytochemical analysis and antibacterial properties due to its long history as potential therapeutic plant. Vegetative aerial parts of the plant were used to prepare the plant extract in aqueous, methanol, chloroform and petroleum ether for the present investigation.

The percentage yield obtained from different plant extracts varied considerably. Methanol extract was found to show maximum percentage yield (14.36%) followed by chloroform extract (8.12%). Lowest percentage yield was observed in aqueous extract (3.17%). Variation in the percentage yield clearly showed the significant role of solvent in extracting phytoconstituents from the plant material (Table 1). Workers from different parts of the world have also reported variable percentage yield in different solvent and maximum percentage yield in methanol and ethanol extracts (Dhawan and Gupta, 2017; Muhamad *et al.*, 2019; Akinmoladun *et al.*, 2022).

Table 1. Percentage yield of *S. trilobata* plant extracts prepared in different solvents

Solvent used for preparation of plant extract	% Yield obtained
Methanol	14.36
Chloroform	8.12
Petroleum ether	5.48
Aqueous	3.17

Plant extracts prepared in different solvents were screened for the existence of eight phytochemicals, namely, phenols, flavonoids, alkaloids, saponins, glycosides, steroids, tannins and terpenoids (Table 2). Methanol extract showed the occurrence of maximum number of phytochemicals tested. Phenols, flavonoids, glycosides and steroids were found to be present in every plant extract screened. Alkaloids were detected uniquely in methanol and aqueous extract, whereas terpenoids were found in methanol and chloroform extract only. On paying attention to previous studies, presence of maximum number of phytocompounds in methanol extract was also noticed by various researchers (Karthika and Manivannan, 2018; Kaur *et al.*, 2019; Kumari *et al.*, 2019; Truong *et al.*, 2019). Though being a polar solvent, least number of

Table 2. Phytoconstituents present in plant extracts of *S. trilobata* prepared in different solvents

Plant extracts	Phenols	Flavonoids	Alkaloids	Saponins	Glycosides	Steroids	Tannins	Terpenoids
ME	+	+	+	-	+	+	+	+
CE	+	+	-	-	+	+	+	+
PEE	+	+	-	+	+	+	+	-
AE	+	+	+	-	+	+	-	-

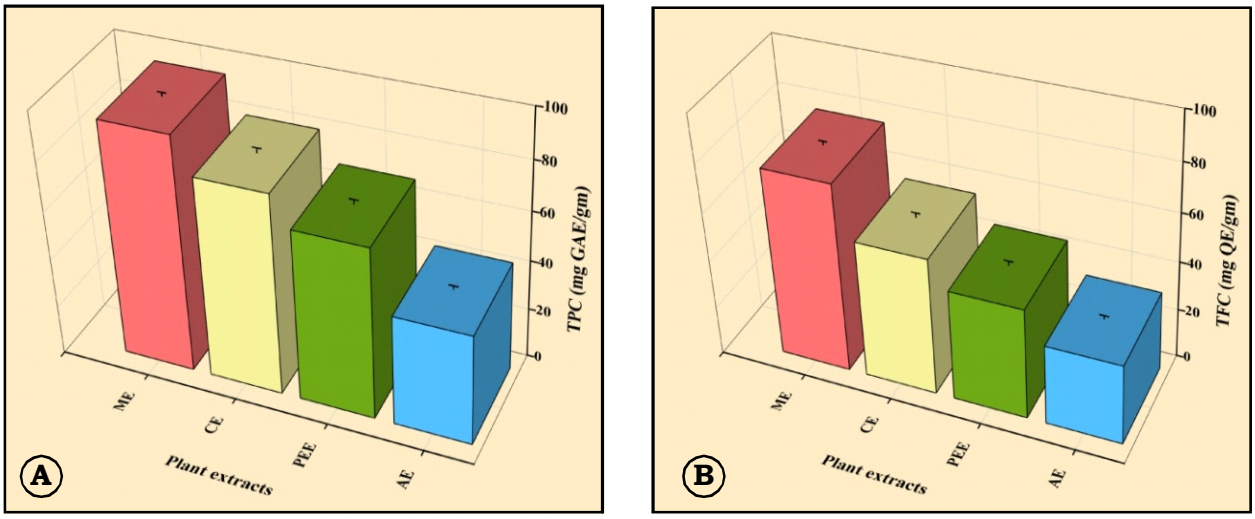
(+) indicates presence of phytochemicals and (-) indicates absence of phytochemicals.
ME: Methanol extract, CE: Chloroform extract, PEE: Petroleum ether extract and AE: Aqueous extract.

phytoconstituents were observed in aqueous extract. This was probably because of poor solubility of phytochemicals in water. Similar observations were also reported in *Cassia fistula*, *Manilkara zapota* and other medicinal plants (Archana and Bose, 2022).

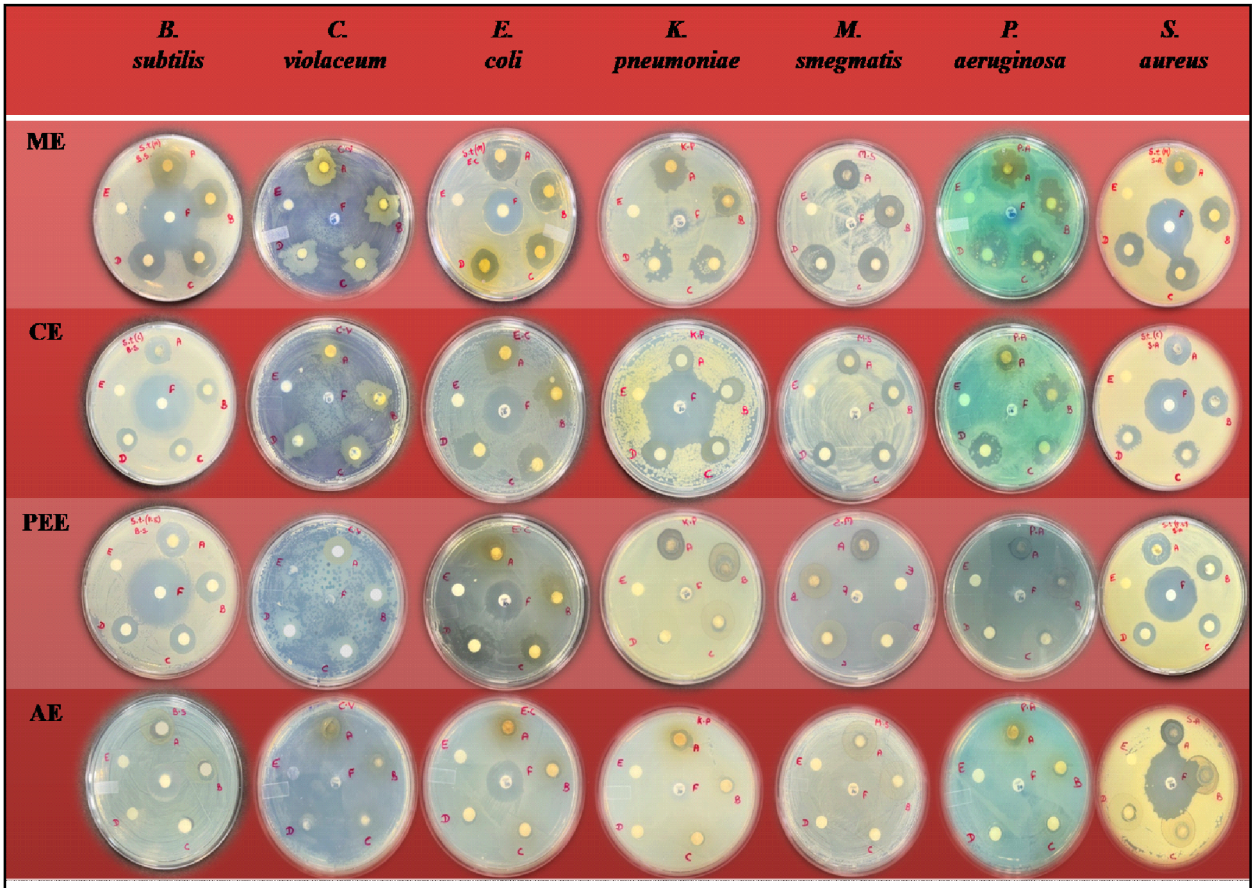
Plant extracts were examined for their TPC and TFC and for this firstly standard curve (gallic acid for TPC; quercetin for TFC) was prepared with an equation. Methanol extract displayed highest amount of TPC (95.87 ± 0.51) followed by chloroform extract (81.29 ± 0.88). Petroleum ether and aqueous extract showed low value of TPC as compared to ME and CE i.e. 69.18 ± 0.78 and 44.36 ± 0.33, respectively (Fig. 1). Similar to the results obtained from TPC, TFC was also found to be maximum in methanol extract among all the four extracts (77.83 ± 0.53) followed by chloroform extract (56.32 ± 0.29). Like TPC, least TFC was also observed in aqueous extract (32.55 ± 0.45).

Both TPC and TFC were highest in the methanol extract, whereas least amount of TPC and TFC was seen in the case of aqueous extract. These observations, to some extent, can be explained taking into account the percentage yield obtained for these plant extracts. Similar results with maximum TPC and TFC in methanol extract were also obtained by Afzal and Rajesh (2021) for the same plant. Workers have also reported maximum amount of TPC and TFC in methanol extract from other plant species like *Calophyllum inophyllum*, *Salvia macrosiphon* and *Phyllanthus* species (Zain *et al.*, 2018; Abbas *et al.*, 2021; Hapsari *et al.*, 2022).

Extracts prepared from *Sphagneticola trilobata* were assessed against seven bacterial strains using four different concentrations for each plant extract (100, 50, 25 and 12.5 mg/ml). The results obtained clearly showed the splendid efficacy of plant towards heterogeneous bacterial strains (Figs. 2 and 3). Antibacterial efficacy of methanol extract led all plant extracts against the bacterial strains used in the study. Highest inhibition zone (23 mm) was obtained from methanol extract towards *K. pneumoniae*. Similar zone of inhibition (22 mm) was obtained against *B. subtilis*, *E. coli*, *M. smegmatis* and *S. aureus* with methanol extract. In case of chloroform extract, highest inhibition zone (22 mm) was observed towards



ME: Methanol extract, CE: Chloroform extract, PEE: Petroleum ether extract and AE: Aqueous extract.
Fig. 1. (A) TPC and (B) TFC of *S. trilobata* plant extracts prepared in four solvents.



A-PE (100 mg/ml), B-PE (50 mg/ml), C-PE (25 mg/ml), D-PE (12.5 mg/ml), E-Negative control (DMSO), F-Positive control (0.1 mg/ml) Ampicilliu), ME-Methanol Extract, CE-Chloroform Extract, PEE-Petroleum Ether Extract and AE-Aqueous Extract.

Fig. 2. ZOI obtained with different plant extracts of *S. trilobata* against seven bacterial strains.

S. aureus, whereas least (14 mm) was recorded against *K. pneumonia.* Aqueous extract exhibited less inhibition zone against tested strains in comparison to other three plant extracts. Lowest zone of inhibition zone (8 mm) was noted against *M. smegmatis* in aqueous extract.

Highest inhibition zone was obtained with positive control Ampicillin i.e. 34 mm against *K. pneumoniae* and encompassing this value 33 mm and 31 mm was obtained with positive control against *B. subtilis* and *S. aureus,* respectively. In this study, ampicillin did not show any inhibitory activity against two bacterial strains i.e. *M. smegmatis* and *P. aeruginosa.* Interestingly, the all plant extracts showed significant inhibitory activity against these two bacterial strains. This shows the vast antibacterial potential of the plant especially against ampicillin resistant bacterial strains. Remya and Sindu (2021) reported remarkable antibacterial activity in different extracts of *S. trilobata* including whole plant, stem and leaves. Martínez *et al.* (2021) reported maximum antibacterial activity in *S. trilobata* when compared with *Lippia graveolens* and *Gliricidia sepium* against four different pathogenic strains. Significant antimicrobial activity from leaf extract of this plant was reported by Leite *et al.* (2019) and Mardina *et al.* (2021). Baisaenga *et al.* (2017) reported significant antibacterial activity of *S. trilobata* essential oil extracted from leaves against gram positive bacteria i.e. *Propionibacterium granulosum.* Biological activities exhibited by *S. trilobata* might be due to the presence of compounds like luteolin, diterpenes and eudesmanolide lactones (Al-Foyjul Islam *et al.*, 2019).

Microbroth dilution assay was performed to find out the minimum concentration of the plant extract required to hamper the growth of a particular bacterium (Table 3). MIC values obtained from different plant extracts

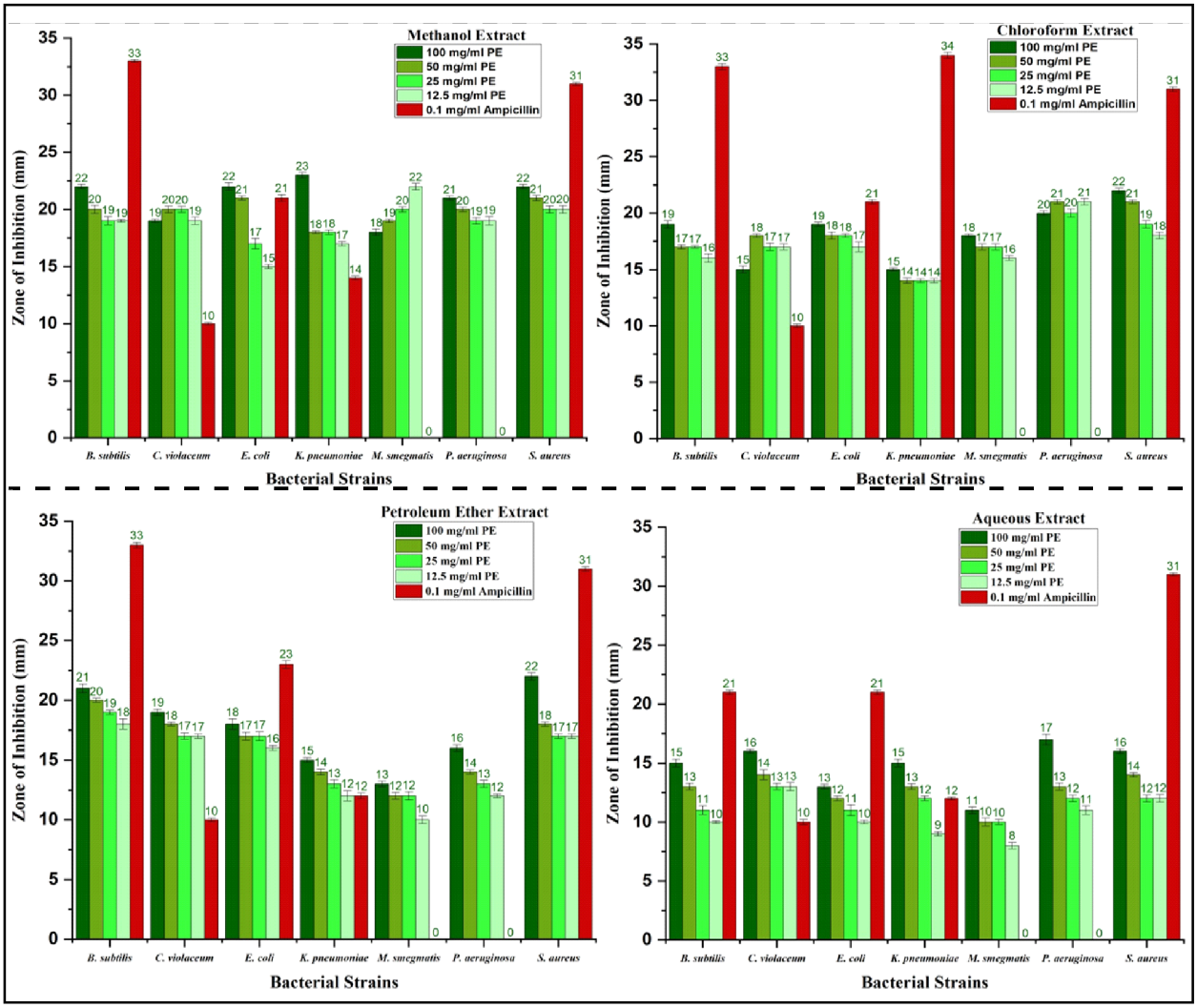


Fig. 3. ZOI obtained with different plant extracts of *S. trilobata* against seven bacterial strains.

Table 3. MIC (Minimum inhibitory concentration) values of *S. trilobata* extracts and positive control against seven bacterial strains

PE/positive control	*B. subtilis*	*C. violaceum*	*E. coli*	*K. pneumoniae*	*M. smegmatis*	*P. aeruginosa*	*S. aureus*
ME	0.781	0.781	0.390	1.562	1.562	0.781	0.390
CE	3.125	1.562	0.390	6.25	6.25	1.562	3.125
PEE	0.781	6.25	3.125	12.5	0.0	6.25	0.781
AE	0.0	12.5	12.5	0.0	0.0	12.5	12.5
Ampicillin	0.006	0.0	0.025	0.1	0.0	0.0	0.003

ME–Methanol extract, CE–Chloroform extract, PEE–Petroleum ether extract, AE–Aqueous extract and Positive control–Ampicillin. MIC values are expressed in mg/ml; here 0.0 denotes no activity.

of *S. trilobata* ranged between 0.390 to 12.5 mg/ml. For methanol extract, MIC values ranged between 0.390 to 1.562 mg/ml and least value was observed against *E. coli* and *S. aureus* i.e. 0.390 mg/ml. Chloroform extract was also found equally effective opposing *E. coli* with low MIC value i.e. 0.390 mg/ml. Maximal MIC value i.e. 12.5 mg/ml was observed against *K. pneumoniae* for petroleum ether extract as well as for aqueous extract against *C. violaceum*, *E. coli*, *P. aeruginosa* and *S. aureus*. Petroleum ether extract did not possess any inhibitory action against *M. smegmatis* similarly aqueous

extract against *B. subtilis*, *K. pneumoniae* and *M. smegmatis*.

Positive control i.e. ampicillin exhibited comparatively lower MIC than the PEs and ranged between 0.003 to 0.1 mg/ml. Lowermost MIC of ampicillin was recorded against *S. aureus* i.e. 0.003 mg/ml, whereas ampicillin didn't show any activity against *C. violaceum*, *M. smegmatis* and *P. aeruginosa*.

CONCLUSION

Vegetative aerial parts of *S. trilobata* possess high TPC, TFC and significant antibacterial potential. Methanol solvent turned out to be the best solvent for extraction purpose as highest yield was witnessed therein and maximum number of phytoconstituents were also detected in methanol extract along with highest TPC and TFC. Maximum zone of inhibition and lowest minimum inhibitory concentrations were noticed in the methanol extract. Aqueous extract showed least antibacterial activity and this could be attributed to poor yield and least value of TPC and TFC. Interestingly, ampicillin was not found effective against two bacterial strains, namely, *M. smegmatis* and *P. aeruginosa,* whereas splendid antibacterial efficacy was noticed with plant extract. This clearly depicts the immense antibacterial potential of this plant against ampicillin resistant strains. Phenols are known for numerous effective actions like antibacterial, antioxidant, anti-inflammatory, anti-mutagenic and anti-viral in accordance to which the existence of phenols in every extract clearly justifies the significant medicinal potential of the plant. It can be concluded that the phytoconstituents are directly responsible for the distinctive property of the plant and this plant has considerable medicinal value due to the existence of specific bio-constituents which are not yet explored to the full potential. However, the present study needs further scrutiny and attention in pharmaceutical fields that conceivably be worthwhile for isolation and characterization of bioactive compounds possessing antibacterial properties. In addition, more bacterial strains especially MDR strains are to be screened further with the plant extract/isolated compounds.

REFERENCES

Abbas, A., Naqvi, S. A. R., Anjum, F., Noureen, A. and Rasool, N. (2021). Antioxidant, antibacterial and antifungal potential study of *Salvia macrosiphon* Boiss. stem extracts. *Pak. J. Pharm. Sci.* **34**: 1903-1907.

Afzal, S. and Rajesh, B. R. (2021). Phytochemical screening and pharmacological evaluation of leaf extract of *Sphagneticola trilobata*. *World J. Pharm. Res.* **10**: 1143-1154.

Akinmoladun, A. C., Falaiye, O. E., Ojo, O. B., Adeoti, A., Amoo, Z. A. and Olaleye, M. T. (2022). Effect of extraction technique, solvent polarity and plant matrix on the antioxidant properties of Chrysophyllum albidum G. Don (African Star Apple). *Bull. Natl. Res. Cent.* **46**: 01-09.

Al Foyjul Islam, M., Barua, A., Binoy, M. H., Jahir Alam, M., Ershad, M., Choudhuri, M. S. K. and Masuma, R. (2019). Pre-clinical investigation of anti-diarrheal and CNS depressant effect of *Wedelia trilobata* in Swiss albino mice. *Orient Pharm. Exp. Med.* **19**: 435-444.

Archana, H. and Bose, V. G. (2022). Evaluation of phytoconstituents from selected medicinal plant and its synergistic antimicrobial activity. *Chemosphere* **287**: 132276.

Awuchi, C. G. (2019). Medicinal plants: The medical, food and nutritional biochemistry and uses. *Int. J. Adv. Acad. Res.* **5**: 220-241.

Baisaenga, N., Kawareeb, R., Boonmaa, A., Sadnena, J., Khamkaewa, S. and Klayraungb, S. (2017). Antibacterial activity of essential oil from *Wedelia trilobata* leaves against *Propionibacterium granulosum*. *Sci. Asia.* **43**: 275-280.

Borghi, S. M., Mizokami, S. S., Carvalho, T. T., Rasquel-Oliveira, F. S., Ferraz, C. R., Fattori, V., Hayashida, T. H., Peron, J. P. S., Gomilios-Neto, D., Ambrosio, S. R., Arakawa, N. S., Casagrande, R. and Verri Jr, W. A. (2021). The diterpene from *Sphagneticola trilobata* (L.) Pruski, kaurenoic acid, reduces lipopolysaccharide-induced peritonitis and pain in mice. *J. Ethnopharmacol.* **273**: 113980.

Cheek, M., Nic Lughadha, E., Kirk, P., Lindon, H., Carretero, J., Looney, B., Douglas, B., Haelewaters, D., Gaya, E., Llewellyn, T., Ainsworth, A. M., Gofforov, Y., Hyde, K.,

Crous, P., Hughes, M., Walker, B. E., Forzza, R. C., Wong, K. M. and Niskanen, T. (2020). New scientific discoveries: Plants and fungi. *Plants People Planet*. **2**: 371-388.

Dhawan, D. and Gupta, J. (2017). Research article comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. *Int. J. Biol. Chem*. **11**: 17-22.

Fajarningsih, N. D., Munifah, I. and Zilda, D. S. (2018). Evaluation of antibacterial assays for screening of marine invertebrate extracts. *Squalen Bull. Mar. Fish. Postharvest Biotechnol.* **13**: 01-08.

Gowtham, H. G., Ahmed, F., Anandan, S., Shivakumara, C. S., Bilagi, A., Pradeep, S., Shivamallu, C., Shati, A. A., Alfaifi, M. Y., Elbehairi, S. E. L., Achar, R. R., Silina, E., Stupin, V., Murali, M. and Kollur, S. P. (2023). *In silico* computational studies of bioactive secondary metabolites from *Wedelia trilobata* against anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein associated with cancer cell survival and resistance. *Molecules* **28**: 1588.

Hapsari, S., Yohed, I., Kristianita, R. A., Jadid, N., Aparamarta, H. W. and Gunawan, S. (2022). Phenolic and flavonoid compounds extraction from *Calophyllum inophyllum* leaves. *Arabian J. Chem*. **15**: 103666.

Husain, N. and Kumar, A. N. I. L. (2017). Characterisation of antioxidant property of root extract of *Sphagneticola trilobata* in recovery of oxidative stress. *Indian J. Sci. Res*. **12**: 116-210.

Iqbal, J., Abbasi, B. A., Mahmood, T., Kanwal, S., Ali, B., Shah, S. A. and Khalil, A. T. (2017). Plant-derived anticancer agents: A green anticancer approach. *Asian Pac. J. Trop. Biomed*. **7**: 1129-1150.

Karthika, C. and Manivannan, S. (2018). Pharmacognostic, physico-chemical analysis and phytochemical screening of the leaves of *W. trilobata*. L. *Int. J. Chemtech. Res*. **2**: 124-131.

Kashyap, D., Tuli, H. S., Yerer, M. B., Sharma, A., Sak, K., Srivastava, S., Pandey A., Garg V. K., Sethi G. and Bishayee, A. (2021). Natural product-based nanoformulations for cancer therapy: Opportunities and challenges. *Semin. Cancer Biol.* **69**: 05-23.

Kaur, S., Gupta, S. and Gautam, P. B. (2019). Phytochemical analysis of Eucalyptus leaves extract. *J. Pharmacogn. Phytochem*. **8**: 2442-2446.

Kumari, P., Khatkar, B. S. and Duhan, A. (2019). Aonla phytochemicals: Extraction, identification and quantification. *J. Food Sci. Technol.* **56**: 2278-2286.

Leite, A. G. B., Farias, E. T. N., Oliveira, A. P. D., Abreu, R. E. F. D., Costa, M. M. D., Almeida, J. R. G. daS., Estevão, L. R. de M. and Evêncio-Neto, J. (2019). Phytochemical screening and antimicrobial activity testing of crude hydroalcoholic extract from leaves of *Sphagneticola trilobata* (Asteraceae). *Ciência Rural*. **49**: 04.

Luyen, N. T., Binh, P. T., Tham, P. T., Hung, T. M., Dang, N. H., Dat, N. T. and Thao, N. P. (2019). Wedtrilosides A and B, two new diterpenoid glycosides from the leaves of *Wedelia trilobata* (L.) Hitchc. with α-amylase and α-glucosidase inhibitory activities. *Bioorg. Chem*. **85**: 319-324.

Mardina, V., Ilyas, S., Halimatussakdiah, H., Harmawan, T., Tanjung, M. and Yusof, F. (2021). Anticancer, antioxidant and antibacterial activities of the methanolic extract from *Sphagneticola trilobata* (L.) J. F. Pruski leaves. *J. Adv. Pharm. Technol. Res*. **12**: 222.

Muhamad, S. H. A., On, S., Sanusi, S. N., Hashim, A. A. and Addinna Zai, M. H. (2019). Antioxidant activity of Camphor leaves extract based on variation solvent. *J. Phys*. **1349**: 012102.

Pico, J., Pismag, R. Y., Laudouze, M. and Martinez, M. M. (2020). Systematic evaluation of the Folin-Ciocalteu and Fast Blue BB reactions during the analysis of total phenolics in legumes, nuts and plant seeds. *Food Function* **11**: 9868-9880.

Prasanna, K. S., Reddy, G. J., Kiran, M. and Raju, K. T. (2019). Biological activities and phytochemical constituents of trailing Daisy trilobata: A review. *J. Drug Deliv. Ther*. **9**: 888-892.

Remya, G. N. and Sindu, N. (2021). Comparative evaluation of phytochemical and antibacterial activity of different parts of *Sphagneticola trilobata* (L.) Pruski. *Plant Genet. Resour. Util: An Appraisal*. pp. 143-148.

RV, S. R. (2019). Primary phytochemical investigation of *Sphagneticola trilobata* (L.) *Pruski. J. Pharmacogn. Phytochem*. **8**: 968-971.

Saedi, S., Shokri, M. and Rhim, J. W. (2020). Antimicrobial activity of sulfur

nanoparticles: Effect of preparation methods. *Arab. J. Chem.* **13**: 6580-6588.

Salmerón-Manzano, E., Garrido-Cardenas, J. A. and Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *Int. J. Env. Res. Pub. He.* **17**: 3376.

Suchantabud, A., Katisart, T. and Talubmook, C. (2017). Chronic toxicity of leaf extract from *Sphagneticola trilobata* (L.) Pruski. *Pharmacogn. J.* **9**: 323-328.

Sujatha, R., Siva, D. and Nawas, P. M. A. (2019). Screening of phytochemical profile and antibacterial activity of various solvent extracts of marine algae *Sargassum swartzii*. *World Scientific News* **115**: 27-40.

Tristantini, D. and Amalia, R. (2019). Quercetin concentration and total flavonoid content of anti-atherosclerotic herbs using aluminum chloride colorimetric assay. *AIP Conf. Proc.* 2193. **030012**: 1-7.

Truong, D. H., Nguyen, D. H., Ta, N. T. A., Bui, A. V., Do, T. H. and Nguyen, H. C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* anti-inflammatory activities of *Severinia buxifolia*. *J. Food Qual.* **2019**: 1-9.

Verma, A. K. and Singh, S. (2020). Phytochemical analysis and *in vitro* cytostatic potential of ethnopharmacological important medicinal plants. *Toxicology Reports* **7**: 443-452.

Zain, S. N. D. M. and Omar, W. A. W. (2018). Antioxidant activity, total phenolic content and total flavonoid content of water and methanol extracts of Phyllanthus species from Malaysia. *Pharmacogn. J.* **10**: 677-681.