International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 08-08-2020; Accepted: 23-08-2020: Published: 07-09-2020
www.botanyjournals.com
Volume 5; Issue 5; 2020; Page No. 59-63

Evaluation of antibacterial activity of some selected plants of family asteraceae and HPTLC analysis of most effective plant *Synedrella nodiflora* (L.) Gaertn

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Abstract
An investigation was carried out with an objective to evaluate the antibacterial potential by using aqueous extracts of six different botanicals from the family Asteraceae including *Chromolaena odorata* (L.) R.M. King & H. Rob., *Helianthus annus* L., *Sphagneticola trilobata* (L.) Pruski, *Synedrella nodiflora* (L.) Gaertn, *Tagetes erecta* L. and *Tridax procumbens* (L.) L. in association with phytochemical analysis. The leaves of different plants were extracted in water and Gram-positive bacterial strain *Bacillus subtilis* were used for activity. Streptomycin was used as a standard. All plant extract showed significant antibacterial activity. The plant methanolic extract showed the highest antibacterial activity was subjected to HPTLC analysis at 254 nm and 366 nm and showed 10 and 12 different phytoconstituents.

Keywords: asteraceae, antibacterial property, bacillus subtilis

1. Introduction
Plants are the cheapest and safer alternative sources of antimicrobials. Indeed, medicinal plants with antimicrobial properties are being increasingly reported from different parts of the world. Natural products especially from higher plants open up a new source of antimicrobial agents with possible novel mechanisms of action (Kostova and Dinchev, 2005 [9]; Pretorius and Watt, 2001; Bhalodia *et al.*, 2011) [2]. The potential of antimicrobial properties of plants are related to their ability to synthesize compounds by the secondary metabolism. The use of plant extracts and phytochemicals, both with known antimicrobial properties, are of great significance to therapeutic treatments (Nagesh and Shanthamma, 2009) [11]. Screening of various bioactive compounds from plants has led to the discovery of new medicinal drug which have efficient protection and treatment roles against various diseases (Sheeja and Kuttan, 2007) [14]. *Bacillus subtilis* is considered as model organism. The genus *Bacillus* comprise Gram- positive, rod-shaped bacteria (Gordon *et al.*, 1973). Also, these bacteria have been also known to be responsible for food poisoning (Kramer and Gilbert, 1989).
The family Asteraceae, belonging to the class of eudicotyledons is the largest family of flowering plants, traditionally known for its medicinal properties (Chethan *et al*., 2012) [14]. They have been widely studied for their anti-inflammatory, antimicrobial, anticancer, antisyphilitic, antigonorrheal and insecticidal properties (Maia *et al.*, 2002) [10].
Nowadays, the control of diseases is a global problem. It is noteworthy that the biological activities have little place in the novel mechanisms of the action in the incidence of the plant diseases. The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases (Dimayuga, 1991) [5]. Due to the cost effectiveness, safety, increasing failure of chemotherapy and antibiotic resistance, search for plant resources has been increased for their potential antimicrobial activity. Phytochemicals are known to possess antibacterial, anti-diabetic, antioxidant, anti-inflammatory, antifungal, antiarthritic, and radio-protective activity and due to these properties they are largely used for medicinal purpose (Ankit *et al*., 2012).
Present work deals with the antibacterial activity of extract of six plant species from Asteraceae family including *Chromolaena odorata* (L.) R.M. King & H. Rob., *Helianthus annus* L., *Sphagenticola trilobata* (L.) Pruski, *Synedrellla nodiflora* (L.) Gaertn, *Tagetes erecta* and *Tridax procumbens* and combination of them against *Bacillus subtilis.*

2. Materials and Methods
Preparation of plant materials
The part of the selected plant species for determine the antimicrobial activity were leaves and they were collected from the campus of K.A.H.M. Unity Women's College, Manjeri, Malappuram, Kerala and Kadannamanna, Malappuram, Kerala. The collected leaf samples were first washed under running tap water and air-dried in shade at room temperature for few weeks. Using a mixer grinder, the air-dried plant leaves were ground into fine powder.

Preparation of aqueous extracts
For aqueous extract, 5 g of the fine powder of each plant was soaked in 25 ml of sterile distilled water and kept in a rotary shaker at 200 rpm for 4 hours for maximum extraction. After 24 hours, the extracts was filtered using Whatmann filter paper (No.1). The stalk solution was then diluted to concentrations of 10%, 20%, 30%,40%,60% and 80% of extract.

Selection of Microorganism
Soil samples were collected from K.A.H.M. Unity Women's College, Manjeri, Malappuram. For isolation purpose 10 g of the sample was suspended in100 ml sterile distilled water and shaken vigorously for 2-3 min. All contamination sensitive procedures were performed inside a laminar flow hood. The supernatant was serially diluted up to 10^{-3}

dilution. After that each of serial dilution is transferred into nutrient agar plate by using spread plate method. To avoid desiccation, plates were incubated inside polyethylene bags. The inoculated plates were incubated at 26±ºC for 24 hours and examined for the appearance of colonies. After incubation, the colonies that exhibited cultural characteristics typical of *Bacillus* species were isolated and made pure cultures. By this slimy cream-coloured colonies were obtained. Then colonies were isolated, and subjected to identification and used for antimicrobial activity screening.

Sterilization

In order to avoid any type of contamination and cross contamination by the test organisms. Antimicrobial screening was done in laminar hood and all types of precautions were highly maintained. Petri dishes and other glassware were sterilized by autoclaving at a temperature of 121°C and a pressure of 15-lbs/sq. inch for 20 minutes. Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Inoculation

Antibacterial activity of extracts carried out by the disc diffusion method. Nutrient agar plates were prepared for the growth of bacterial cultures. All the steps were performed in sterile environment in order to prevent contamination. After solidification fresh culture of *Bacillus subtilis* were swabbed on the respective plates using L-shaped sterile glass rod. Using sterile forceps four discs were placed on the surface of the inoculated plate. Distilled water and streptomycin antibiotic discs were used as control and positive controls respectively. The different percentage solution of each plant extracts were impregnated at 2 ml each into sterile, blank discs having 6 mm in diameter. The plates were then inverted and kept in an incubator at 28±ºC for 24 hrs. Antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf extract. Hence, zone of inhibition was used to determine the effectiveness of the plant extract accurately.

HPTLC (High Performance Thin Layer Liquid Chromatography) analysis

HPTLC analysis was carried out for methanolic leaf extract of *synedrella nodiflora* at Centre for Medicinal Plant Research (CMPR), Arya Vaidya Sala, Kottakkal, Malappuram.

Estimation of alkaloids

Total alkaloid content determination by using Harborne (1973) method.

3. Results

Antibacterial activity

Antibacterial activity investigation of selected plants revealed that all extracts showed significant zone of inhibition. The results indicate that aqueous extract was found to be effective, it exhibits zones of inhibition with the tested strain.

Among all the tested plant materials the largest zone of inhibition were induced by *Synedrella nodiflora* extract. They had the best antibacterial activity even at 10%. *Tridax procumbens* were found to have the least effect on the tested bacteria. The scale of potency of the aqueous extracts of tested plants inhibiting the *Bacillus subtilis* growth is as follows: *Synedrella nodiflora* > *Chromolaena odorata* > *Helianthus annuus*> *Tagetes erecta* > *Sphagneticola trilobata* > *Tridax procumbens* respectively.Experimental evaluation showed that the leaves of all selected plants also possess slight antimicrobial property.

Zone of inhibition significantly increased when compared with control,which used were sterile water (no significant activity) and also reduced when compared with positive control, which used were streptomycin disc (30mm diameter). The diameter of zone of inhibition ranged from 7.1 mm –16.4 mm. In higher concentrations, 40%,60 and 80% of extracts showed significant antibacterial property. All leaf extracts showed increasing zone of inhibition with increasing the concentration of extracts.

Absorbance of the selected plants

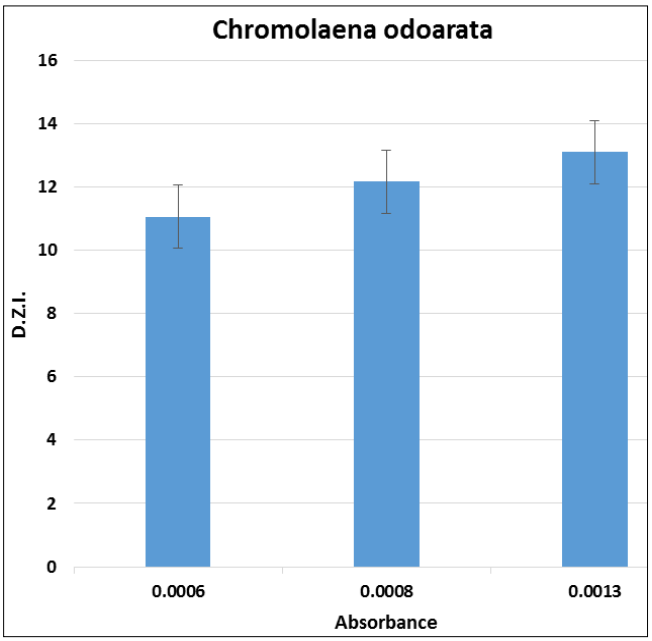
Optical Density values of 40%,60% and 80% concentration of leaf extracts of the 6 selected Asteraceae members is given in the table 2. Absorbance value of leaf extract of *Chromolaena odorata* is 0.0006, 0.0008 and 0.0013 average diameter of zone of inhibition is 11.06, 12.16 and 13.13 mm 40%, 60% and 80% concentration of leaf extracts respectively. *Helianthus annuus* showed absorbance value 0.0006,0.0011 and 0.0012 average D.Z. I. is 8.9, 9.7 and 11.23 mm. Absorbance value of leaf extract *Sphagneticola trilobata* 0.005, 0.0006 and 0.0012 average D.Z.I. is 7.9, 9.3, 11.06mm. *Synedrella nodiflora* showed absorbance value 0.0012, 0. 0015 and 0.0016 average D.Z.I. is 12.4, 14. 03, 16.4mm. *Tagetes erecta* 0.0010, 0. 0011, and 0.0013 average D.Z.I. is 8.06, 9. 23. 11. 13 mm (Figure 5). *Tridax procumbens* 0.0007, 0.0009 and 0.0011 its average D.Z.I. is 7.1, 8. 12, 9. 2mm respectively.

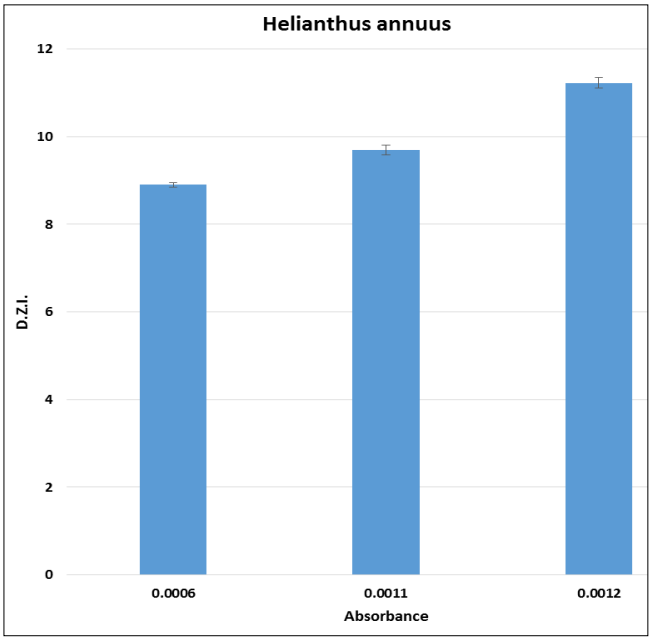
Table 1: Average diameter zone of inhibition and absorbance.

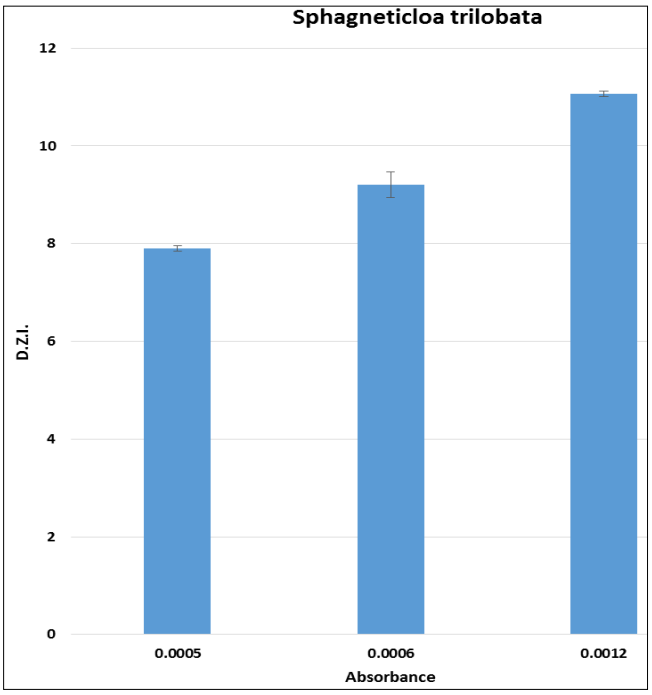
PLANT NAME	Conc	P1(mm)	P2 (mm)	P3 (mm)	Average D.Z.I (mm)	Standard Deviation	Abso Rbance
Chromolaena odorata (L.) R. M. King & H. Rob	40%	11.1	11.1	11	11.06	0.577350	0.0006
	60%	12.2	12	12.3	12.16	0.152752	0.0008
	80%	13.2	13.2	13	13.13	0.115470	0.0013
Helianthus annus L.	40%	9.8	9.8	9.6	8.9	0.577350	0.0006
	60%	11.3	11.1	11.3	9.7	0.115470	0.0011
	80%	9.8	9.8	9.6	11.23	0.115470	0.0012
Sphagneticola trilobata (L.) Pruski	40%	7.9	7.9	8	7.9	0.577350	0.0005
	60%	9.3	9.4	9.3	9.3	0.264575	0.0006
	80%	11.1	11	11.1	11.06	0.577350	0.0012
Synedrella nodiflora (L.) Gaertn	40%	12.4	12.3	12.5	12.4	0.1	0.0012
	60%	14.1	14	14	14.03	0.577350	0.0015

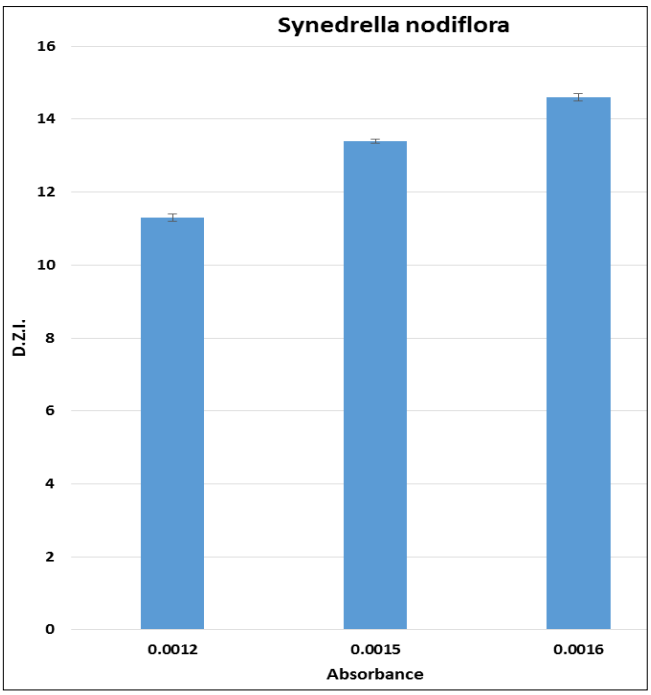
	80%	16.5	16.3	16.4	16.4	0.1	0.0016
Tagetes erecta L.	40%	8.1	8	8	8.06	0.0.57735	0.0010
	60%	9.3	9.2	9.2	9.23	0.577350	0.0011
	80%	11.1	11.1	11.2	11.13	0.577350	0.0013
Tridax procumbens (L.) L.	40%	7.1	7	7.1	7.1	0.577350	0.0007
	60%	8.12	8.33	8.2	8.12	0.105984	0.0009
	80%	9.2	9.3	9.3	9.2	0.085440	0.0011

Graphs plotted with average zone of inhibition against its OD values.









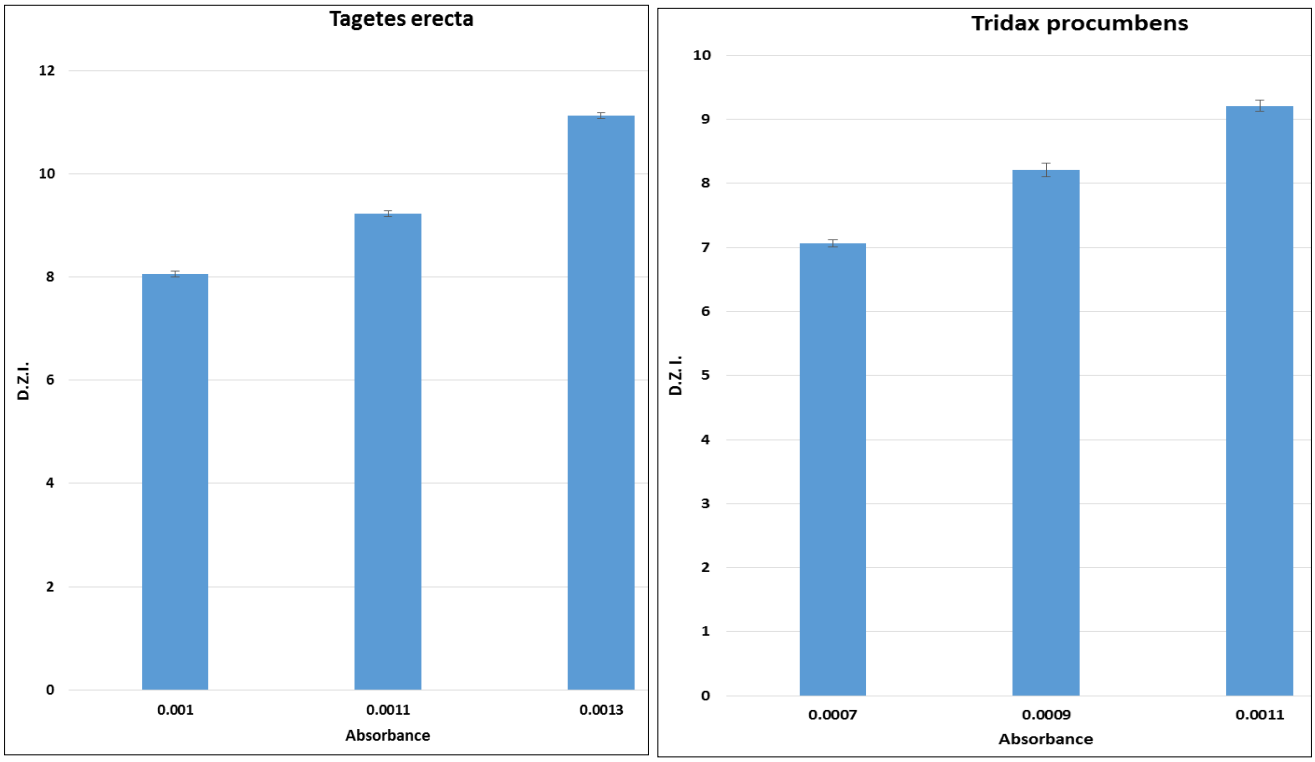


Fig 1

HPTLC analysis

HPTLC of methanolic extract of Synedrella *nodiflora* Were scanned at wave length 254 nm and 366 nm it showed 12 and 10 different phytoconstituents respectively.

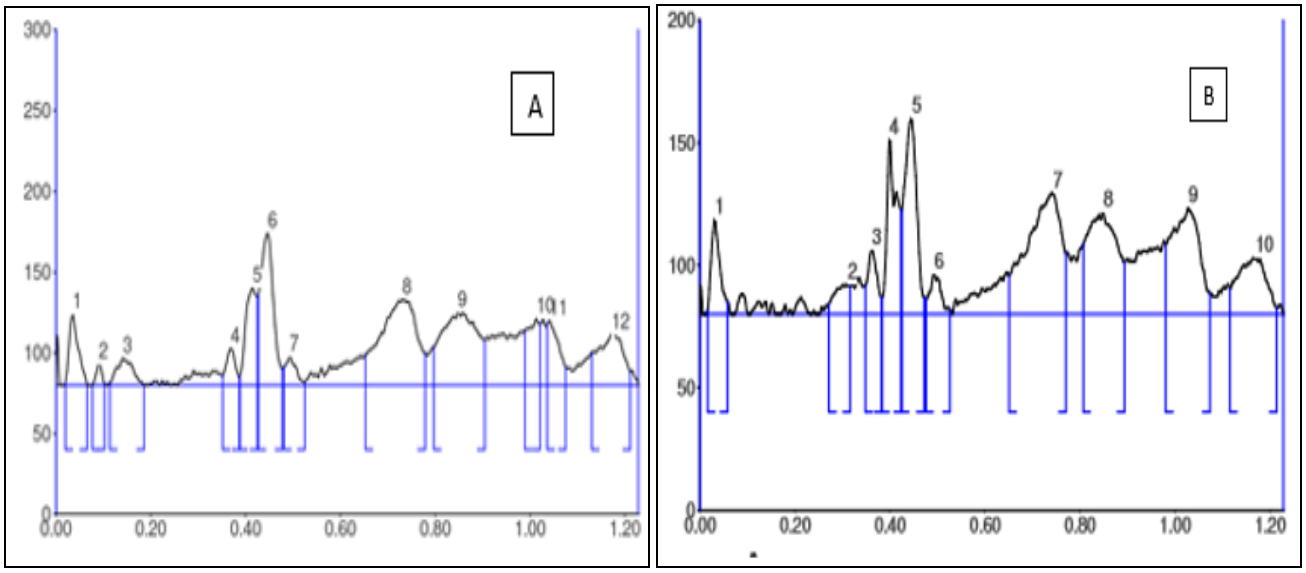


Fig 2

Densitogram display scanned at (A): 254 nm and at (B): 366 nm.

Estimation of alkaloids

In this study presence of alkaloid is identified, *Synedrella nodiflora*. The alkaloid content present in the extract is 1.16 ± 0.20.

4. Discussion

In this work, antibacterial activity of leaf extract of six selected plants in Family Asteraceae demonstrated against *Bacillus subtilis.* The highest antibacterial activity being observed in *Synedrella nodiflora* against tested bacteria. There were so many studies done by different scientists on the antibacterial activities of selected plant species in this study. There are many documented reports on plant extracts against different bacteria. However, difficulties arise in comparing the results due to different methodologies used including solvents, concentrations, microbial strains, and antimicrobial test methods.

The results obtained in this study are in agreement with several authors who demonstrated that selected plants of this study possess good antibacterial activity. Bhogaonkar *et al*. (2011) [3]. tested. Antimicrobial activity of *Synedrella nodiflora against E. coli. Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*. Leaves were extracted with petroleum ether, chloroform, acetone and methanol.

Petroleum ether extract showed maximum activity against all the bacteria tested except *E. coli.* It is thus concluded that Cinderella *nodiflora* possess good antibacterial activity. It can be exploited for preparation of drug.

This study is in agreement with the work on the seed of *Helianthus annuus* and the only variant from the report is the difference in the zone of inhibition of the two studies may be due to difference in the vegetative part of the plant used (Evans, 2009) [6]. and the antibiotic resistant pattern of the bacterial used in the study.

The antibacterial properties observed in this study could be attributed to the bioactive compounds present in the plant such as the alkaloids, flavonoids, and essential oils. According to Batista *et al*. (2011) [1]. and Nurtjahja *et al.* (2013) [12]. Plant products exhibit a stronger antimicrobial activity against Gram-positive bacteria, and the antimicrobial activity of the extracts may be attributed to the presence of secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolic compounds. This investigation has opened up the possibility of the use of this plant in drug development for human consumption for the treatment of food poison and various diseases caused by the tested bacteria.

5. Conclusion

The present study revealed that the aqueous leaf extracts of selected plants of Asteraceae was rich in phytochemical constituents and showed the antibacterial properties against *Bacillus subtilis.* All the tested plants showed antibacterial property but their effectiveness varied. Among all, *Synedrella nodiflora* was found to be most important at its antibacterial activity. Finally the present investigation concluded that plants studied here can be seen as a potential source of useful drugs. These plant extract used as antibacterial agent for destroying the pathogenic organisms and also used curing number of diseases. Further work needs to be done to isolate the specific chemicals that have antibacterial activity. Hence, isolation of the bioactive components through high performance liquid chromatography would be of interest for further studies. These plants can be used to discover bioactive natural products that serve as lead for the development of new phytopharmaceuticals.

6. References

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