Research Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2014, Mar., Vol. 5 (1)

ISSN: 0976-9390
IJCPS

Antibacterial and antioxidant properties of two medicinal plants from Kerala, India

ShironaTK, Sruthy KB and Rajendran N.

School of Biosciences and Technology, VIT University,Vellore, Tamilnadu, India.

*Corresponding Author: E-Mail: Sruthykb123@gmail.com

ABSTRACT

Traditional or folk medical practices are based on the use of plants and plant extracts, as they are playing an important role in protecting health to a large section of people all over the world. This study was conducted in order to investigate the antibacterial and antioxidant properties of two medicinal plants *Hydnocarpus pentandra* and *Eupatorium triplinerve* from Kerala. Antibacterial activity of the solvent extracts was carried out by disk diffusion method. Antioxidant activities of the leaf extracts were assessed on the basis of the stable 1, 1-diphenyl 2-picrylhydrazyl free radical activity method. The phytochemical investigation was done by detecting the presence of glycosides, carbohydrates, proteins, saponins and phenols. Using Fourier Transform Infrared Spectroscopy (FT-IR), functional groups present in the leaf sample were analyzed. From these results of these analysis, it could be concluded that these two plants, *H.pentandra* and *E.triplinerve*are potent sources of natural antioxidants with a free radical scavenging activity of 21.3 and 9.2 respectively and the methanolic extract of the two plants inhibited the growth of *Escherichia coli* and *Bacillussubtilis* indicating that these plants have good antibacterial property. Further studies on these two plants would provide more information about the bioactive compounds that have specific medicinal properties.

Keywords: DPPH(1,1-diphenyl-2-picryl hydrazylfree radical, FT-IR(Fourier Transform Infrared Spectroscopy), Chaalmougric acid, Antioxidants, Antibacterial effect, Disc diffusion.

1. INTRODUCTION

Medicinal plants have been identified and used throughout the human history to treat various problems. There are so many medicinal plants used as treatments for different diseases in local areas also. Angiosperms are the original source of most plant medicines. India has a unique distinction of having six recognized systems of medicines to treat the patients suffering from various diseases. They are ayurveda, sidha, unani, yoga and neuropathy [1]. *Hydnocarpus pentandra*is a medicinal plant belonging to the family *Flacourtiaceae*. In India it is endemic to western Ghats of Maharashtra, Goa, Karnataka, Kerala and Tamilnadu. *H. pentandra*is a medicinal herb and its seed oil is used as anti inflammatory agent and is used as local application in rheumatism, sprains and in chest infections. This plant is reported to contain cyclopentyl mono-carboxylic acid, chaulmoogric acid, hydnocarpic and garlic acid [2]. It also consists of lower homologues of chaulmoogric acid, olic and palmitic acid. The seeds of the plant yields chalmoogra oil that has been applied to treat in leprosy. Seed and seed oil are also used in treating leucoderma, worm infections and several other skin infections. *Eupatorium triplinerve* is another herb belonging to the family *Asteraceae*. The plant leaves are used to make stimulatory medicines for cardiac diseases. This plant exerts mild sedative, anxiolytic and antidepressive effects on the central nervous system. The leaves and stem of this plant are used in treating stomach pain, edema etc. This is a medicinal herb that has also anthelmintic activity [3].

This present study was aimed at determining the antioxidant and antibacterial activity of these plants. The stable DPPH radical model has been a widely used quick simple method for the evaluation of free radical scavenging activity at a faster rate. DPPH can trap other free radicals and therefore rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of radical nature of that reaction. Antibacterial compounds present in plants are known to suppress antibacterial activity. Antibacterial studies has been conducted using the extract of medicinal plants is to understand

the potential of these plants for identifying the bioactive compounds for developing antibacterial drugs. The phytochemical components of the plants were analyzed by means of various chemical methods while the functional components were determined by FT-IR. These results have been presented here to explain about antibacterial and antioxidant activities of these two plants.

2. MATERIALS AND METHODS

2.1. Preparation of plant extracts

Fresh leaves of *H. pentandra* and *E. triplinerve* were collected from the central and northern regions of Kerala, respectively. The collected leaves were cleaned and dried for two weeks under sunlight. The dried leaves were ground into powder using mixer grinder and stored at room temperature. Methanol was used as solvent. 20g of leaf powder and 200ml of methanol wereadded into a Soxhlet apparatus and the solvent extraction was carried out.The extract was concentrated by evaporation method.

2.2. Phytochemical Analysis

Different qualitative chemical tests were performed for establishing the phytochemical profile of leaf extract for its chemical composition.

2.3. Detection of carbohydrates

2.3.1. Fehling's test

5mg of extract was dissolved in 2.5ml of distilled water, filtered and subjected to Fehling's test.1 ml of filtrate was boiled in a water bath with equal amounts of Fehling's reagent 1 and 2. Formation of red colour indicated positive reaction.

2.3.2. Detection of saponins

3mg of extract was diluted with distilled water and made up to 5ml. The suspension was shaken using rotary shaker for 15 min. Appearance of a layer of foam indicated positive result.

2.4. Detection of phenolics

2.4.1. Ferric chloride test

The extract was dissolved in 3ml distilled water. Few drops of neutral 5% ferric chloride solution was added. A bulky white precipitate indicated the presence of phenolics.

2.4.2. Lead acetate test

The extract was dissolved in 5ml distilled water and then, 3ml of 10% lead acetate was added. The presence of phenolics was understood from the formation of bulky white precipitate.

2.5. Detection of proteins

2.5.1. Biuret's test

An aliquot of diluted extract was treated with one drop of 2% copper sulphate solution. To this 1ml of 95%ethanol was added followed by the addition of potassium hydroxide. Formation of purple colour indicated presence of proteins.

2.6. Detection of glycosides

2.6.1. Borntranger's test

After a few milligrams of extract was hydrolysed with concentrated hydrochloric acid for 2 hours on water bath, it was filtered and then subjected to Borntranger's test. To 2ml of filtrate hydrolysate, 3ml of chloroform was added and shaken. Chloroform layer was separated and 10% ammonia solution was added to it.Formation of pink colour indicated the test as positive.

2.7. Antioxidant activity

The antioxidant of the plant extract and the standard were assessed on the basis of the free radical scavenging effect on the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH)free radical activity method. Different percentage solutions (1%, 10%, 25%, 50%, 100%) of the test extracts were prepared in methanol. 1M ascorbic acid was used as standard. 0.1mM DPPH was prepared and mixed with different percentage solutions of the extract. The standard and the solution mixtures were kept in dark for 30 min and then the O.D. was measured at 517 nm using UV spectrophotometer. Methanol was used as blank. OD was measured and the percentage of inhibition was calculated using the formula given as follows;

Percentage of inhibition of DPPH activity = A-B/A x 100

where, A is the O.D. of the standard and B is the O.D. of the sample.

2.8. Antibacterial activity of leaf extract of *H. pentandra* and *E. triplinerve*

2.8.1. Determination of antibacterial activity

Two bacterial strains *Escherichia coli* and *Bacillus subtilis* were used as test organisms. Both the strains were obtained from the microbiology laboratory, VIT University, Vellore.

2.8.2. Disc diffusion method

Antibacterial activity of methanolic extract was tested by disc diffusion method. Nutrient agar was prepared as culture medium. After the media was solidified bacterial culture was inoculated onto the plates by swabbing technique. Disk with 8mm diameter was dipped in plant extract of different concentrations and placed on the surface of the culture medium. A single disk without plant extract was also kept as

control on all the petriplates. The petridishes were incubated for 24h at 37^{0}C. After 24h, zone of inhibition was observed around the disc. The diameter of thezone of inhibition was measured. The studies performed for both the bacteria with two leaf extracts.

3. RESULTS

3.1. Phytochemical profiles of *H.pentandra* and *E. triplinerve*

The phytochemical analysis of *H.pentandra* revealed the presence of protein and phenolic compounds and *E.triplinerve*revealed the presence of phenolics, carbohydrates and saponins (Table 1).

Table - 1: Phytochemical profiles of *H. pentandra* and *E. triplinerve*

Compound	*H. pentandra*	*E. triplinerve*
Carbohydrates	-	+
Proteins	+	-
Phenolics	+	+
Saponins	+	+
Glycosides	-	-

3.2. FT-IR Analysis

The analysis of FT-IR provided the information about different functional compounds present and they were identified from the standard reference spectrum (Figure 1 and 2). Each functional group absorbs infrared rays of different wavelength and they were obtained as a form of spectra and these results were given in Table 2.

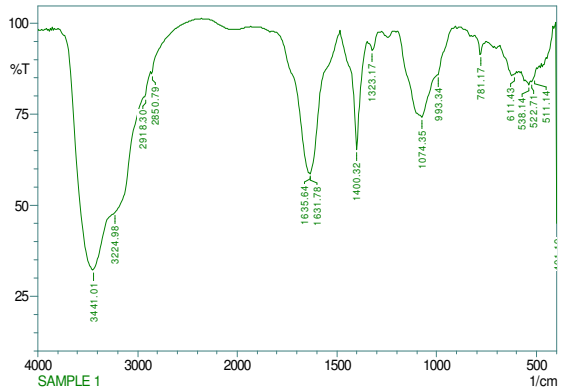

Figure - 1: FT-IR spectrum of *H. pentandra*

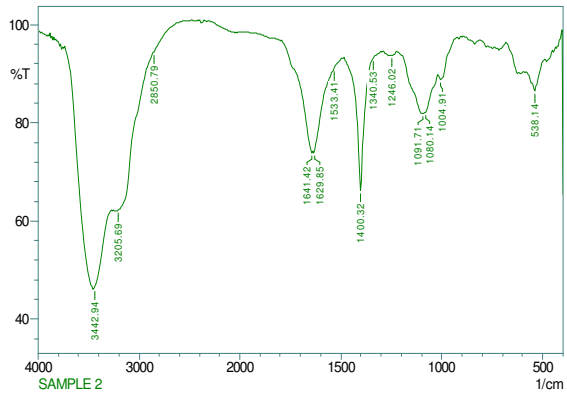

Figure - 2: FT-IR spectrumof *E. triplinerve*

Table - 2: Functional groups(bonds) present in *H. pentandra* and *E. triplinerve*

Wave number	Functional groups present	*H. pentandra*	*E. triplinerve*
3441.01	N-H stretch, amines	+	+
3224.98	O-H stretch, carboxylic acid	+	+
2918.30	C-H stretch, alkanes	+	+
2850.79	C-H stretch, alkanes	+	+
1635.64	C=C stretch conjugated, alkenes	+	+
1631.78	C=C stretch conjugated, alkenes	+	+
1400.32	C-F stretch, alkyl halides	+	+
1323.17	S=o stretch, sulfonates	+	+
1246.02	C-C (O)-C stretch acetates, esters	-	+
1074.35	PH bends, phosphines	+	-
993.34	C-O stretch, anhydrides	+	-
781.17	C-Cl stretch, alkyl halides	+	-
611.43	C-Br stretch, alkyl halides	+	-
538.14	C-Br stretch, alkyl halides	+	-
522.71	C-Br stretch, alkyl halides	+	-
511.41	C-Br stretch, alkyl halides	+	-

3.3. Antioxidant activity (DPPH free radical scavenging activity) of methanolic leaf extracts

The stable DPPH radical model is a widely used quick simple method for the evaluation of free radical scavenging activity. DPPH can trap other free radicals and therefore rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of radical nature of that reaction.

From the results of DPPH radical scavenging activity of both plant extracts, it was observed that the leaf extract of *H. pentandra* had higher free radical scavenging activity. Mean value of 21.3% ± 13.5 for *H. pentandra* and 9.2% ± 8.5 for *E. triplinerve*were obtained in this study. These values indicated that *H. pentandra* has comparatively higher antioxidant activity than that was observed in *E. triplinerve* (Figure 3).

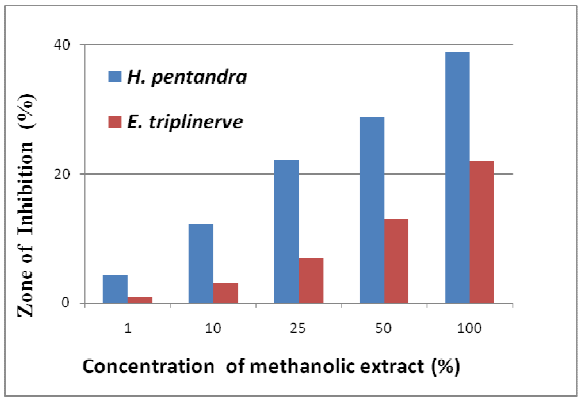

Figure - 3: Free radical scavenging activity of *H. pentandra* and *E.triplinerve*

3.4. Antibacterial activity of plant extracts

The antibacterial activity of methanolic extract of leaves of the two test plants were carried out against *B.Subtilis* and *E. coli*. The zone of inhibition was observed as shown in the figure 4 and 5 for different quantities of plant extracts (10, 15, 25 µl) for *H.pentandra* and *E. triplinerve*.

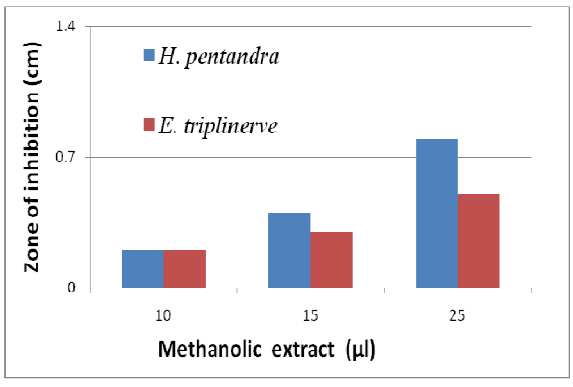

Figure - 4: Antibacterial effect of methanolic extract of *H. pentandra* and *E. triplinerve* on *E.coli*

Results indicated that the extract of *H.pentandra*had higher antibacterial activity than *E.triplinerve.* Two bacterial strains tested in this study but *E. coli* (Figure 6) was found to be susceptible to the extract of *H. pentandra.* Similar trend was also observed for the other plant extract (Figure 7). Both the plant extracts showed an increase in antibacterial activity with an increase in concentration of the extract.

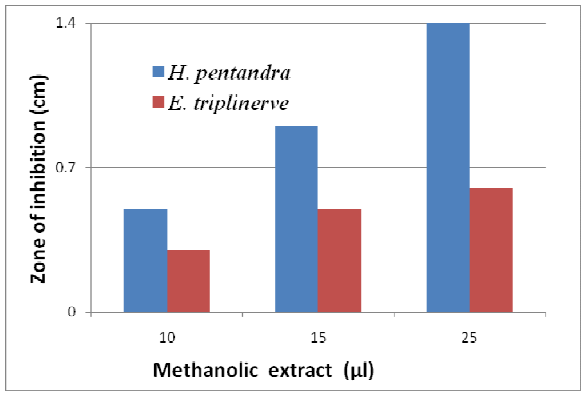

Figure - 5: Antibacterial effect of methanolic extract of *H. pentandra* and *E. triplinerve* on *B. subtilis.*

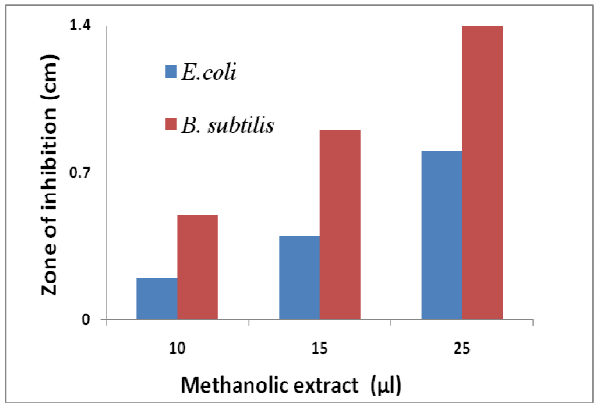

Figure - 6: Antibacterial effect of methanolic extract of *H.pentandra*on *E. coli* and *B. subtilis*

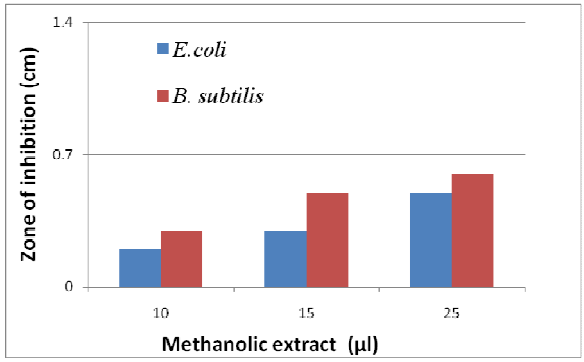

Figure – 7: Antibacterial effect of methanolic extract of *E.triplinerve* on *E. coli* and *B. subtilis*

4. DISCUSSION

The study was performed to investigate the phytochemical properties, antioxidant and

antibacterial activities of medicinal plant extracts of *H.pentandra*and *E.triplinerve*. The plant extracts are used as medicines in local areas in India and Bangladesh to treat infections since the plants are known to produce a number of secondary metabolites to protect themselves naturallyfrom microbes and insects. Of these secondary metabolites, some of them are toxic to humans and some of them are non-toxic.

Phytochemical analysis of *H.pentandra* revealed the presence of phenolics, saponins and proteins where as the analysis of *E.triplinerve* showed the presence of carbohydrates, phenolics and saponins. It is known that phytochemical compounds such as glycosides, saponins, tannin, flavonoids, phenolicsetc. known to cause antioxidant and antibacterial activities [4].

FT-IR results showed the presence of amines, alkyl halides, sulfonates,carboxylic acids, alkanes, alkenes which are common to both the plants *H.pentandra* and *E.triplinerve*.They showed differences in the presence of phosphines and anhydrides in *H. pentandra* and esters in *E. triplinerve*. Due to difference in their components, they might have showed variations in antioxidant and antibacterial activities.

In case of antioxidant activity, both the plants *H.pentandra* and *E.triplinerve* showed free radical scavenging activity. *H.pentandra* has 21.3% ± 13.5and *E.triplinerve*had 9.21% ± 8.5radical scavenging activity. By comparing both these plants, *H. pentandra* was found to have higher antioxidant activity than *E. triplinerve.* These plants have free radicals to reduce oxidative stress that are involved in various disorders. Due to the scavenging activity of antioxidants, they are used for treating those disorders. The results of the present study showed that high quantity of scavenging substances might be present in *H. pentandra.*

In the case of antibacterial activity, *H.pentantra* showed higher activity than *E.triplinerve*. The essential oils present in *E.triplinerve*are reported to have antimicrobial activities [5]. In the present study *H. pentandra* showed higher antibacterial activity than *E. triplinerve*on the two bacteria tested. The results of this study revealed that these bacterial strains Gram negative (*E. coli*) and Gram positive *(B. subtilis*) are sensitive to both the plant extracts.

From these results of the present study, it was understood that *H. pentandra* has higher antioxidant and antibacterial activities than the other medicinal plant. Since both of these plants are known to be used to treat various diseases, it is common to have the above activities. But there was a difference in activities of these two plants which may be understood from the variations in the profiles of the functional groups and phytochemical of the plants. However, further study is required to elucidate more information about those processes.

5. CONCLUSION

This study indicates that both *H.pentantra*and *E.triplinerve*have possessed potential components involving in free radical scavenging activity and antibacterial activity. However further studies are needed to elucidate the proper mechanism behind these activities.

Acknowledgement

We express our deep sense of gratitude to the management of VIT University for their encouragement and to all laboratory assistants and our friends for their help and valuable suggestions.

6. REFERENCES

1. Dhanasekharan M, Kuruppusamy S, Annadurai M and Rajashekharan KM. Evaluation of phytochemical constituents of Indian Medicinal Plant *Hydnocarpusalpina* WIGHT, **International journal of pharmacological and biological research.**2013; 1: 23-28
2. Shyam Krishnan M, Dhanalakshmi P, YaminiSudhalakshmi G, Gopalakrishnan S, Manimaran A, Sindhu S, Sagadevan E and Arumugam P. Evaluation of phytochemical constituents and antioxidant activity of Indian medicinal plant *Hydnaocarpuspentandra*, **International journal of pharmacy and pharmaceutical sciences**. 2013; 5: 453-458
3. Subash KR, JaganRao, Cheriyan BV, Bharati M, Kumar KS. In vitro antimicrobial efficacy of the essential oil of *Eupatorium triplinerve* leaves, **Journal of Indian Perfumer**. 1990; 34: 61- 63
4. Shad MA, Nawaz H, Rehman T, Ikram N. Determination of some biochemicals, phytochemicals, and antioxidant properties of different parts of Cichoriumintybus : A comparative study, **Journal of Plant and Animal sciences**. 2013; 23: 1060-1066.
5. Kaimal TNB, Lakshminarayana G, Yadava RN and Saini V. Fatty acid compositions of lipids isolated from different parts of *ceibapentandra*, *sterculiafoetida* and *hydnocarpuswightiana,* **Journal of Phytochemistry**. 1970; 9: 2225-2229.