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## Documentation of floristic diversity in Ponparakunnu hills, Kozhikode District, Kerala and biological activities of *Eclipta prostrata* L. and *Eupatorium triplinerve* Vahl (Asteraceae)

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### Abstract

The present study was carried out to document the diversity of medicinal plants in the Ponparakunnu hills, Kozhikode district, Kerala and to elucidate the biological activities of *Eclipta prostrata* and *Eupatorium triplinerve*. The repeated field survey was conducted and totally 30 species belonging to 18 families were documented. From the documentation study the family Asteraceae was found to be dominant in the study area. The phytochemical analysis in ethyl acetate and ethanolic leaf extracts of *Eclipta prostrata* and *Eupatorium triplinerve* revealed the presence of carbohydrate, protein, phenol, tannin, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids. The DPPH *in vivo* antioxidant activity depicted that ethanolic and ethyl acetate leaf extract of *Eupatorium triplinerve* ( $32.2 \pm 1.27$ ) and *Eclipta prostrata* ( $30.2 \pm 1.26$ ) able to scavenge the stable DPPH radical. The appreciable antibacterial activity was recorded in ethyl acetate leaf extract of *Eupatorium triplinerve* with the maximum zone of inhibition (0.6 mm at 10  $\mu$ L/mL) when compared with the standard Chloramphenicol. Hence biological activity in the selected plants *Eclipta prostrata* and *Eupatorium triplinerve*, can be widely used in the pharmaceutical industry for the development of new drugs.

**Keywords:** Ethnobotany, phytochemical constituents, antibacterial activity, antioxidant activity

### Introduction

India is rich in biodiversity with variety of life forms on earth. Ethnobotany is the study of a region's plants and their practical uses through the traditional knowledge of local culture, people and are considered as the backbone of the assessment of biodiversity (Saranya Ravi., 2016) [9]. Plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1999) [13] and are the almost exclusive source of drugs for the majority of the world's population (Hamburger and Hostettmann, 1991) [5]. Medicinal plants contain important phytochemicals such as alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins, lignin, vitamin C, vitamin E, and carotenoid are utilized both by humans and animals as important components of diets (Okwu, 2005) [8] that are responsible for numerous activities such as anti-inflammatory, anti-cancerous, anti-fungal, anti-bacterial activity (Sharma *et al.*, 2015) [10]. Among different plant derivatives, secondary metabolites have been proven to be the most important group of compounds, with a wide range of antimicrobial activities (Raman *et al.*, 1999 and Ahmad *et al.*, 2002) [14, 15].

*Eclipta prostrata* belonging to the family Asteraceae is used as an astringent, depurative, emetic, febrifuge, ophthalmic, purgative, styptic and tonic. It is also used internally in the treatment of dropsy and liver complaints, anaemia, diphtheria, tinnitus, tooth loss and premature graying of the hair (Saraf *et al.*, 1991; Chopra *et al.*, 1992; Singh *et al.*, 1993; Wagner *et al.*, 1996) [16-19]. Previous phytochemical investigations of *E. prostrata* revealed the occurrence of thiophene derivatives, glycosides (Yahara *et al.*, 1994; Rahman *et al.*, 2006) [20, 21], alkaloids (Abdel, 1998) [22], and triterpenes.

*Eupatorium triplinerve* belonging to the family Asteraceae, are widely used in folk medicine and its analgesic, anticoagulant, antianorexic, antiparasitic, anthelmintic, sedative, antifungal, and antibacterial properties have been reported (Kokate *et al.*, 1971; Chaurasia and Kher, 1978; Verpoorte and Dihal, 1987; Yadav and Saini, 1990; Garg and Nakhare, 1993; Jelager *et al.*, 1998; Gupta *et al.*, 2002;) [7, 2, 11, 12, 3, 6, 4]. In addition to that the plant extract is used as an antiseptic to treat ulcers, hemorrhages (Ghani, 1998) [23] also as sedative, anxiolytic and antidepressive (Melo *et al.*, 2013) [24]. The oil of the plant has been found to possess antimicrobial activity (Yadav and Saini, 1990) [12].

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*E. triplinerve* has also been reported as a stimulant and tonic in small doses (Anonymous, 1952).

The present study was carried out to document the diversity of plants in the Ponparakunnu hills, Kozhikode district, Kerala and to evaluate the floristic survey, conservation, management of diversity, prevention and destruction of the habitats of the native endangered species in a particular locality that can be used for the drug development by pharmaceutical industries.

## Materials and Methods

### Study Area

The present study was conducted in Ponparakunnu Hills, Kozhikode district, Kerala with a latitude of 11.2661° N, longitude of 75.9130° E, covers a total area of 128 square kilometers (km). The climate is tropical, with a cool climate with a temperature between 10-30 °C. There is significant rainfall during most of the month in year. Floristically the area has a moderate vegetation including trees, shrubs, herbs and climbers.

### Data Collection

Extensive and repeated field survey was carried out during the month of October- December 2022. The Information about medicinal plants were collected based on the personal observation and with the help of local people residing in the locality. After surveying the preliminary information like binomial name, author citation, family name, habit, plant part used and its medicinal uses were recorded. The documented plants were classified into different types such as trees, shrubs, herbs and climbers. The plant parts such as leaf, stem, root, bark and whole plant were used for decoction, infusion, paste, powder and extracts crushed from the plant parts are used for curing various diseases for human ailments.

### Plant collection and preparation of the plant material

The fresh plant material of *Eclipta prostrata* and *Eupatorium triplinerve* were collected and were taxonomically identified. The leaves of *Eclipta prostrata* and *Eupatorium triplinerve* were washed thoroughly under running tap water and then rinsed well with distilled water. It was allowed to air dried under shade for one week. Dried leaves were powdered using a mechanical blender and the fine powder obtained by sieving was transferred into air tight containers with proper labeling for further studies. Then the powder was subjected to extraction.

### Plant extract preparation

25 grams of the powder were weighed and it was suspended in 100mL of ethanolic and ethyl acetate solvent in a separate conical flask. Then the conical flasks were placed in an orbital shaker and was set at 120 rpm for 14 hours. The ethyl acetate and ethanolic extract of *Eclipta prostrata* and *Eupatorium triplinerve* were filtered through Whatman filter paper (Grade 1) and the extracts were stored in refrigerator at 4 °C for further studies.

### Phytochemical Screening

Preliminary Phytochemical screening of ethyl acetate and ethanolic leaf extracts of *Eclipta prostrata* and *Eupatorium triplinerve* were determined by using standard protocol (Karpagam *et al.*, 2008) [25].

**Test Organisms:** All the bacterial strains were obtained from microbiology Bioline laboratory, R.S Puram Coimbatore

district. Two gram positive strains and two negative strains such as *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumoniae* bacterial strains were used to assess the antibacterial properties of the *Eclipta prostrata* and *Eupatorium triplinerve*. The antibacterial activity on selected bacterial strains were assayed by well diffusion method.

### Agar Well Diffusion Method

Using a sterile Pasteur pipette, the wells were made on the surface of the agar that had already been seeded with the indicator strain. Using agar well diffusion techniques, ethyl acetate and ethanol extracts of *Eclipta prostrata* and *Eupatorium triplinerve* (10 µL, 20 µL and 25 µL) were tested for their antibacterial activities. Bacterial cultures of *E.coli*, *S. aureus* and *K. pneumonia*, was employed and after 24 hours of incubation at 29 °C, the diameters of the zone of inhibition were measured with a calliper. Growth inhibition was evaluated after 24 hours and compared with the results obtained from the control chloramphenicol and the zone of inhibition for each micro-organisms were noted.

### In vitro Antioxidant Activity

#### DPPH radical scavenging activity

The antioxidant activity of *Eclipta prostrata* and *Eupatorium triplinerve* in the ethyl acetate and ethanolic leaf extracts were evaluated by determining the hydrogen or electron donating capacity of the sample to the stable radical DPPH ensuring in the decolorization of the dark purple DPPH solution. The sample extracts of various concentrations were added to 3mL of 0.1mM DPPH solution and incubated for 30 minutes. The methanol was considered to be blank and the reaction mixture without the sample was served as control. The absorbance of the sample was recorded at 517nm.

## Results and Discussion

### Documentation of Medicinal Plants

The present study was carried out to document the medicinal plant in Ponpara kunnu hills, Kozhikode district, Kerala. The results of the documentation of 30 medicinal plants revealed that 30 species belonging to 18 families among them Asteraceae is the dominant family (table 1). Similar studies were observed in ethnobotanical survey based on indigenous knowledge of the Chengottumala hills, Kozhikode district, conducted by Rekka Raja and Aswani (2023). They provided information about 75 species of medicinal plants which is used to treat various diseases.

### Phytochemical Analysis

The qualitative preliminary phytochemical analysis of the ethyl acetate and ethanolic extracts of *E. prostrata* and *E. triplinerve* confirmed the presence of the secondary metabolites in a considerable amount. The ethyl acetate and ethanolic leaf extracts of *Eupatorium triplinerve* and *Eclipta prostrata* indicated the presence of carbohydrate, protein, phenol, tannin, flavonoids, saponins, glycosides, steroids, terpenoids and alkaloids both in ethanol and ethyl acetate extract. Similarly Sugumar *et al.*, (2015) [26] conducted a preliminary phytochemical analysis *E. triplinerve* and the study revealed the presence of broad spectrum of secondary metabolites (Alkaloids, flavanoids, tannin, saponin, phenol, triterpenoids, steroids). Reported the presence of mainly steroids, coumarins, alkaloids, saponins, tannins and polysaccharides and flavanoids were reported to be absent. Shilpam Sinha reported the maximum phenolic and flavanoid content in the extracts of *E. prostrate* reported the presence of

steroids, tannins, saponins, flavonoids, diterpenes and triterpenes and also about 19 active principles and a wide range of alcohols, fatty acids, esters and many other acyclic alkanes in *E. prostrata*.

### DPPH *In vitro* Antioxidant Activity

The DPPH radical scavenging activity of the ethanolic and ethyl acetate leaf extract of *Eclipta prostrata* and *Eupatorium triplinerve* showed IC<sub>50</sub> values indicated the highest free radical scavenging activity. This indicated that the ethyl acetate and ethanol extract possess highest DPPH Radical Scavenging activity. Similarly, Shirona *et al.*, (2014) [28] reported that *E. triplinerve* shows antioxidant activity. Shilpam Sinha and Richa Raghuvanshi., (2016) [29] analysed the antioxidant potential of *E. prostrata* in different solvents and the methanolic extract of plant showed most potent antioxidant activity and concluded *E. prostrata* as an important source of antioxidants. Study reported by Mohamed Saleem Gani and Nalini Devi (2015) [30] confirmed that *E. prostrata* shows very good antioxidant property having the DPPH radical scavenging activity which is suitable for the prevention of many human affecting diseases. This study concluded that free radicals to reduce oxidative stress that are involved in various disorders.

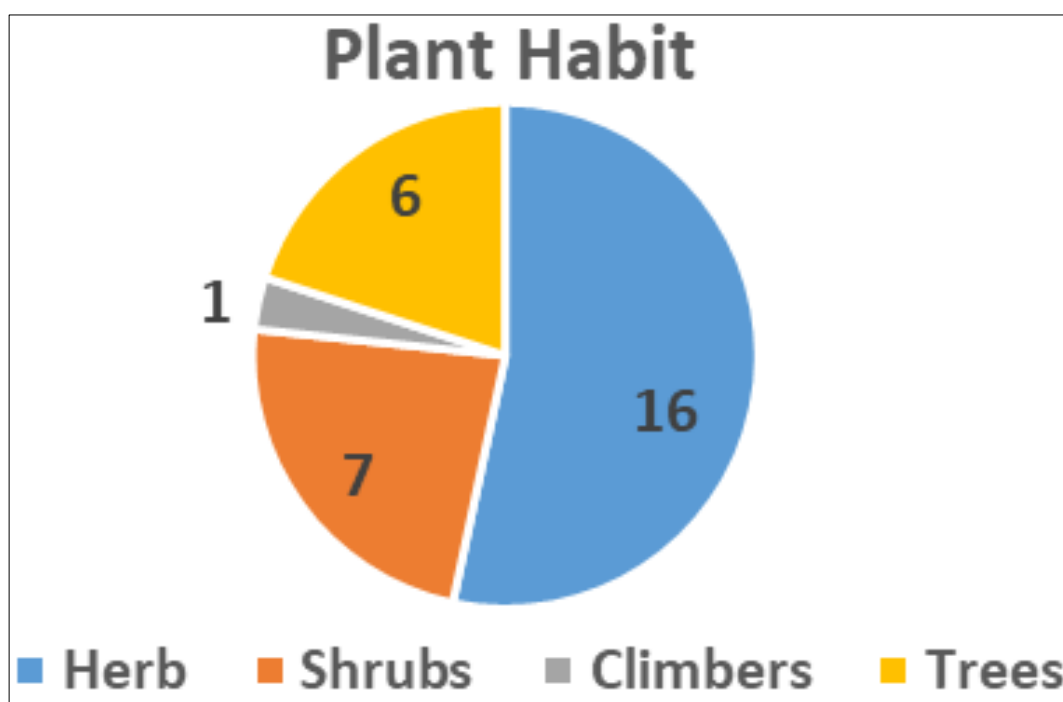
### *In vitro* Antibacterial Activity

The ethanolic and ethyl acetate leaf extract of *Eclipta prostrata* and *Eupatorium triplinerve* showed antibacterial activity against gram positive bacterial strains namely *Bacillus subtilis*, *Staphylococcus aureus* and Gram negative strains *Escherichia coli* and *Klebsiella pneumoniae*. The ethanolic leaf extract exhibited good activity against *Staphylococcus aureus* and the ethyl acetate leaf extract exhibited superior activity when compared with the standard Chloramphenicol. Similarly Prakashkumar Unnikrishnan *et al.*, (2014) [27] reported that the essential oil extracted from the leaves and stem of *E. triplinerve* is the source of thymohydroquinone dimethyl ether which is found to have significant antimicrobial effect. Sona Dev *et al.*, (2018) [31] reported that Silver nanoparticles (AgNPs) which are bio synthesized from the leaf extracts of *E. triplinerve* showed excellent antibacterial activity. Akhtar Nahid *et al.*, (2017) [32] interpreted the antimicrobial activity of *E. prostrata* as the plant extract showed the ability to inhibit the growth of both gram positive and gram negative bacterial strains. Karthikumar *et al.*, (2007) [33] reported that the ethanol and ethyl acetate extracts of *E. prostrata* showed the highest antibacterial activities against all the tested bacterial strains at high concentration. Hence the present study indicates that the plants *E. prostrata* and *E. triplinerve* have high medicinal values and can be used to treat diseases caused by the micro-organisms and can be used in the pharmaceutical industries to produce new drugs.

**Table 1:** List of Medicinal plants with its uses documented in Ponpara kunnu hills, Kozhikode district, Kerala.

Sl. No	Binomial name	Family	Habit	Useful part	Medicinal uses
1	<i>Achyranthes aspera</i> L.	Amaranthaceae	Herb	Whole plant	The whole plant mixed with honey helps to improve digestion. The juice of the leaves helps in wound healing.
2	<i>Adhatoda vasica</i> L.	Acanthaceae	Shrub	Leaves	Decoction of leaves helps in the reduction of inflammation, cough, cold etc. Intake of the juice of leaves helps in reducing the acid formation in stomach.
3.	<i>Allamanda cathartica</i> L.	Apocynaceae	Shrub	Whole plant	Intake of leaf decoction in small doses is used as antidote in poisoning. Used in the treatment of liver tumor, jaundice and malaria.
4	<i>Albizia lebbek</i> (L.) Benth	Fabaceae	Tree	Bark, Leaf	Leaves used in the treatment of eye problems.
5	<i>Alternanthera sessilis</i> (L.) R.Br. ex DC.	Amaranthaceae	Herb	Leaf	Used as a remedy against intestinal cramps, fever, diarrhoea, dysentery. Juice of the plant is used to treat white discharge of urine and also used in the treatment of menstrual disorders.
6.	<i>Amaranthus spinosus</i> L.	Amaranthaceae	Herb	Leaves Stem Root	Root juice used in the treatment of fever, diarrhea and dysentery. Sap is used in the treatment of ophthalmic disease.
7.	<i>Anacardium occidentale</i> L.	Anacardiaceae	Tree	Leaf Bark Fruits	Bark is having the ability to reduce blood sugar level. Bark is used as an antidote in snake bites.
8	<i>Asystasia gangetica</i> (L.) T. Anderson	Acanthaceae	Herb	Leaves Roots	Leaf decoction used in the treatment of fever, epilepsy, stomach pain etc. Root powder is used as a remedy for stomach pain. Root decoction is used as an antidote for snake bite. Juice of the whole plant used for the remedy for sore throat, cough and cold.
9.	<i>Azadirachta indica</i> A. Juss.	Meliaceae	Tree	Leaves Bark	Bark is used for the treatment of malarial fever, blood purification. The leaf decoction is used for eye disorders, skin diseases, stomach problems.
10.	<i>Biophytum sensitivum</i> (L.) DC	Oxalidaceae	Herb	Whole plant	Whole plant is crushed and is mixed with honey to cure cough. Leaf decoction is used as an expectorant to cure cough and sore throat. Root decoction is used to cure fever, gonorrhoea.
11.	<i>Boerhavia diffusa</i> L.	Nyctaginaceae	Herb	Whole plant	Leaf decoction is used to treat jaundice. Root decoction used for the treatment of dysentery. Also used in the treatment of gastric problems, internal inflammation and anaemia.
12	<i>Cardiospermum halicacabum</i> L.	Sapindaceae	Herb	Whole plant	The leaf decoction is used in the treatment of rheumatism and used as poultice on swelling. Leaf juice is used in the treatment of ear ache.
13	<i>Cassia fistula</i> L.	Fabaceae	Tree	Leaves Bark	Root decoction is used in the treatment of wounds and ulcers. Leaf juice is used as a remedy for malaria, diabetes and dysentery.

				Root	
14	<i>Cleome rutidosperma</i> DC	Cleomaceae	Herb	Leaves	Leaf extract is used to treat diarrhoea, skin irritation, inflammation etc.
15	<i>Clitoria ternatea</i> L.	Fabaceae	Climber	Whole plant	Flowers are boiled in water and is used as a remedy for menstrual problems. Roots used for diuretic and also used as an antidote in snake bites.
16	<i>Commelina benghalensis</i> L.	Commelinaceae	Herb	Whole plant	Leaves of the plants are boiled and the solution is taken orally to treat diarrhoea. Root decoction is used to treat stomach disorders.
17	<i>Cyanthillium cinereum</i> (L.) H. Rob	Asteraceae	Herb	Whole plant	Decoction of whole plant is used to treat fever. Root decoction is used in the treatment of dysentery and stomach disorders. Leaf juice is used to treat piles.
18	<i>Cynodon dactylon</i> L.	Poaceae	Shrub	Leaves	Whole plant decoction used to treat fever, cough, dysentery and epilepsy.
19	<i>Eclipta prostrata</i> (L.)	Asteraceae	Herb	Leaves	The oil extracted from the plant is used to treat hair loss. Leaf decoction is used to treat cancer, cuts, pimples, rashes and skin irritation. The stem and root is used to treat anaemia and dysentery.
20	<i>Eleusine indica</i> (L.)	Poaceae	Herb	Leaves Root	Plant infusion is used in the treatment of skin rashes. Fresh leaf juice is used as anthelmintic Root decoction is used to treat fever, asthma etc.
21	<i>Eupatorium triplinerve</i> Vahl.	Asteraceae	Shrub	Whole plant	Whole parts of plant including stem, roots and leaves can be made into decoction to cure malarial fever. Paste of leaves can be used to cure insect bites, wounds and bleeding; both internal and external
22	<i>Euphorbia hirta</i> L.	Euphorbiaceae	Herb	Leaves Stem	Whole plant decoction is used to treat dysentery and skin diseases. Stem juice is used for wound healing, rashes, fever, cough, cold etc.
23	<i>Hamelia patens</i> Jacq	Rubiaceae	Shrub	Leaves	Extract of leaves is used to treat skin diseases, cramps, headache, fever, dysentery.
24	<i>Myristica fragrans</i> Houtt.	Myristicaceae	Tree	Seeds	Seed is taken internally in the treatment of dysentery, diarrhoea, vomiting, stomach problems etc
25	<i>Ocimum sanctum</i> L.	Lamiaceae	Herb	Whole plant	Helps to cure fever, headache, cough, cold etc. Used in the treatment of infections and any other skin diseases.
26	<i>Phyllanthus niruri</i> L.	Phyllanthaceae	Herb	Whole plant	In Traditional medicine it is used for the treatment of jaundice, cough, cold, dysentery. It is also used for skin ailments, urinary tract infections, swellings etc.
27	<i>Psidium guajava</i> L.	Myrtaceae	Tree	Whole plant	Guava leaf extract reduces menstrual pain. The intake of fruits helps in lowering blood pressure.
28	<i>Rauvolfia serpentina</i> (L.) Benth. Ex Kurz	Apocynaceae	Shrub	Leaves Roots	Leaf juice is used to cure eye diseases, also treat wounds and skin itchininess. The bark, stem and roots are used as antidote for snake bites and poisoning.
29	<i>Sida acuta</i> Burm. F.	Malvaceae	Shrub	Leaves Roots	A decoction of the plant is used for the treatment of fever. Juice of plant is used for indigestion. The root is chewed to relive toothache.
30	<i>Synedrella nodiflora</i> (L.) Gaertn.	Asteraceae	Herb	Leaves	Juice of crushed leaves used for rheumatism treatments. Young leaf infusion is used as laxative.



**Fig 1:** Representation of list of plant habit in Ponparakunnu hills, Kozhikode District, Kerala



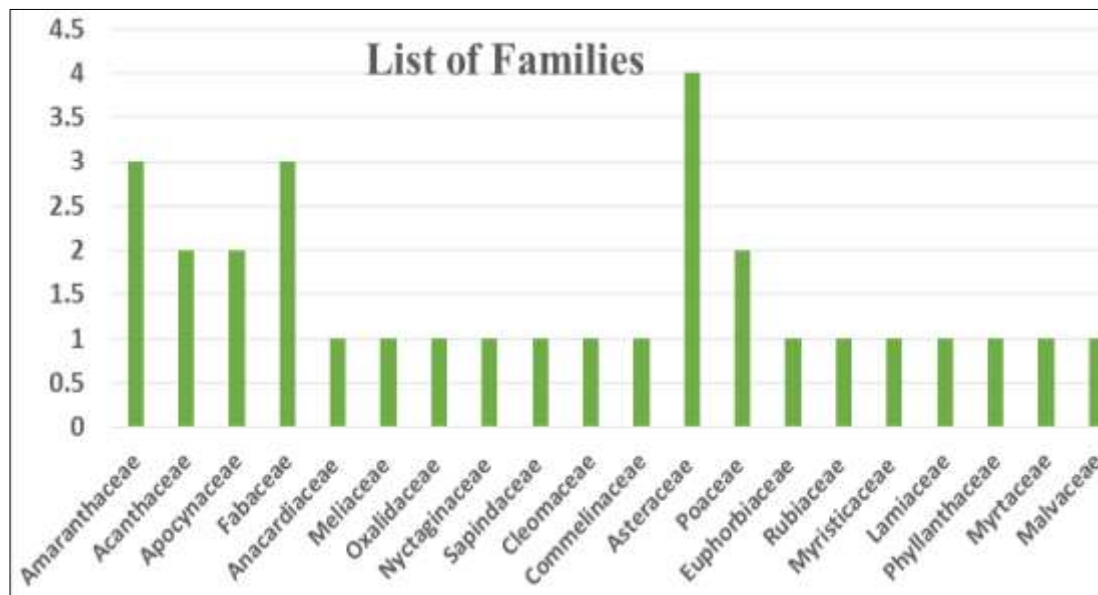


Fig 2: List of family of medicinal plants documented in Ponpara kunnu, hills, Kozhikode district, Kerala.

**Qualitative Phytochemical Screening**

Table 2: Showing the Phytochemical constituents of ethyl acetate and ethanolic leaf extracts of *Eclipta prostrata* and *Eupatorium triplinerve*.

Sl. No	Phytochemical Constituents	<i>Eclipta prostrata</i>		<i>Eupatorium triplinerve</i>	
		Ethyl Acetate extract	Ethanol extract	Ethyl Acetate extract	Ethanol extract
1.	Carbohydrates	+++	+++	+++	+++
2.	Protein	+	+++	++	+++
3.	Phenol and Tannin	-	+++	-	+++
4.	Flavonoids	+	+	+	+
5.	Saponins	-	-	-	+
6.	Glycosides	+	+	+++	++
7.	Steroid	++	++	+++	+++
8.	Terpenoid	+	+	+	+
9.	Alkaloid	-	-	+	+

(+++): Highly Present, (++): moderately present, (+): Low; (-) - Absent

**In vitro Antioxidant Activity**

**DPPH Radical Scavenging Activity**

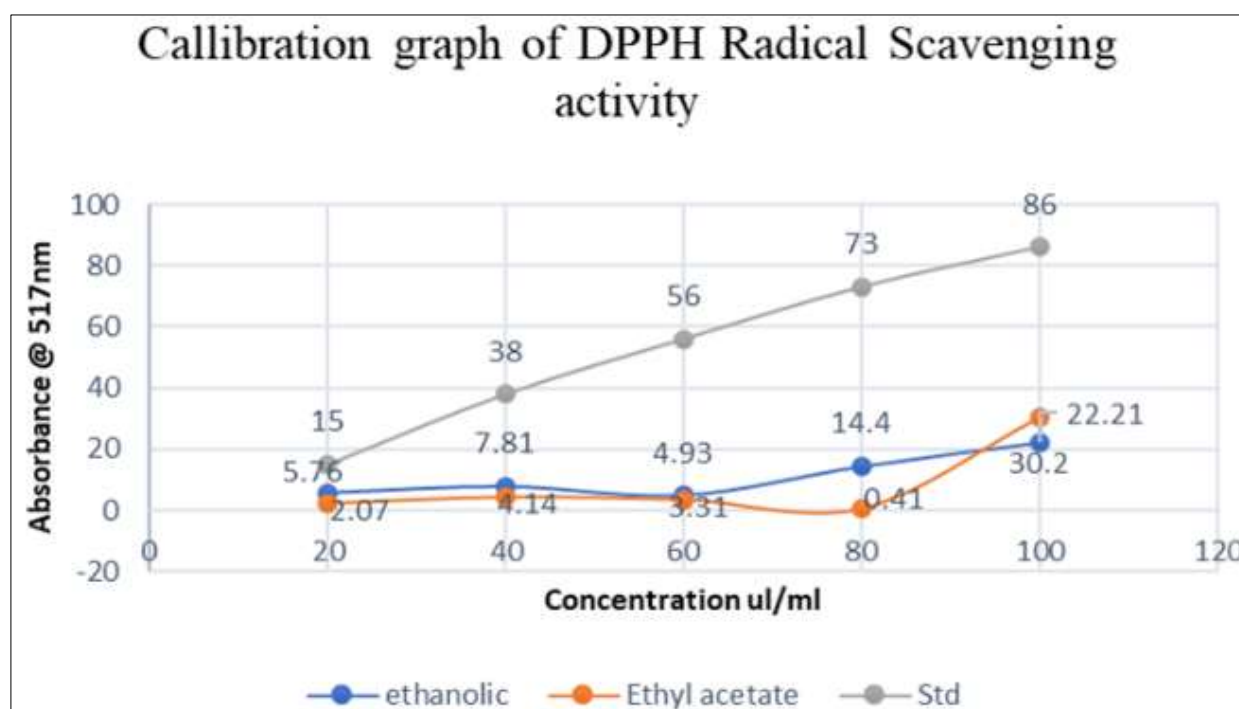
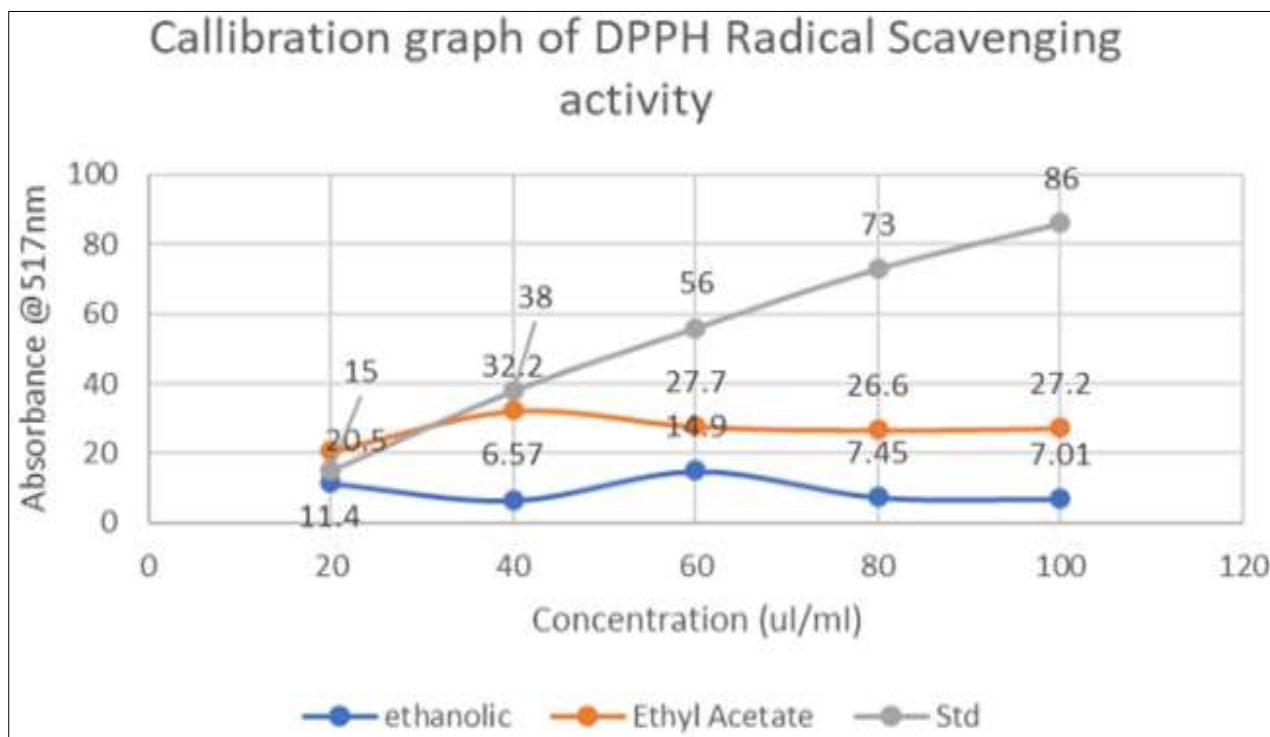


Fig 3: Comparison of % DPPH Radical scavenging activity of ethyl acetate and ethanolic leaf extract of *Eclipta prostrata* and standard BHT



**Fig 4:** Comparison of % DPPH Radical scavenging activity of ethyl acetate and ethanolic leaf extract of *Eupatorium triplinerve* and standard BHT

### Antibacterial Activity

**Table 3:** Antibacterial activity of ethyl acetate and ethanolic leaf extract of *Eclipta prostrata*.

Volume (ul/ml)	Extract	Zone of Inhibition (mm)			
		Gram positive		Gram negative	
		<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
10	Ethanol	0.9 ± 1.5 <sup>a</sup>	0.5 ± 1.6 <sup>a</sup>	0.2 ± 1.5 <sup>b</sup>	0.7 ± 1.2 <sup>a</sup>
10	Ethyl acetate	0.5 ± 0.5 <sup>c</sup>	0.6 ± 1.3 <sup>c</sup>	0.5 ± 0.5 <sup>c</sup>	0.4 ± 1.0 <sup>b</sup>
15	Standard (Chloramphenicol)	1.2 ± 1.0 <sup>b</sup>	0.8 ± 1.5 <sup>b</sup>	0.9 ± 2.0 <sup>a</sup>	1.2 ± 0.53 <sup>c</sup>

All values are Mean of triplicate determination (n=3) ± Standard deviation, and represented using statistically significance at P<0.005 <sup>a></sup> <sup>b></sup> <sup>c></sup> in each column

**Table 4:** Antibacterial activity of ethyl acetate and ethanolic leaf extract of *Eupatorium triplinerve*.

Volume (ul/ml)	Extract	Zone of Inhibition (mm)			
		Gram positive		Gram negative	
		<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>
10	Ethanol	0.2 ± 1.5 <sup>a</sup>	0.3 ± 0.5 <sup>c</sup>	0.5 ± 1.8 <sup>b</sup>	0.6 ± 1.2 <sup>b</sup>
10	Ethyl acetate	0.3 ± 1.0 <sup>c</sup>	0.6 ± 1.7 <sup>a</sup>	0.2 ± 1.5 <sup>c</sup>	0.6 ± 0.5 <sup>c</sup>
15	Standard (Chloramphenicol)	0.5 ± 1.3 <sup>b</sup>	0.7 ± 1.6 <sup>b</sup>	0.5 ± 2.0 <sup>a</sup>	0.8 ± 1.6 <sup>a</sup>

All values are Mean of triplicate determination (n=3) ± Standard deviation, and represented using statistically significance at P<0.005 <sup>a></sup> <sup>b></sup> <sup>c></sup> in each column.

### Conclusion

From the present study it can be concluded that Ponpara Kunnu hills, Kozhikode district, Kerala has wide range of medicinal plants and the selected plant *Eclipta prostrata* and *Eupatorium triplinerve* have prominent antioxidant and antibacterial activity and studied viewed that can be used in traditional medicines to treat various ailments.

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