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PRELIMINARY PHYTOCHEMICAL, FT- IR AND ANTIBACTERIAL EVALUATION OF LEAF OF *EUGENIA FLOCCOSA* BEDD (MYRTACEAE)

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ABSTRACT

The present study aims to examine the phytochemical and antibacterial potential of different solvent extracts of leaf of *Eugenia floccosa*. The preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, terpenoids, steroids, saponins, phenols, tannins, coumarins, catechin and sugars in the methanol and ethanol extracts of leaf of *E. floccosa*. The FT-IR spectrum confirmed the presence of C=O, C-H, C=C, C-O and C=C. The antibacterial activity has been observed in the petroleum ether, methanol and ethanol extracts of leaf of *E. floccosa* against the tested bacteria with wide-ranging activity. The methanol extract of leaf of *E. floccosa* showed highest zone of inhibition against *Escherichia coli* (16 mm). It is hoped that, this study would direct to the establishment of some compounds that could be used to formulate new and more effective antibacterial drugs of natural origin.

KEY WORDS: *E. floccosa*, Phytochemicals, FT-IR, Antibacterial activity.

INTRODUCTION

The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as 'Medicinal plants'. According to WHO, consultative group on medicinal plants, a medicinal plant is any plant which, in one or more of its organs contains substances that can be used for therapeutic purposes or which, is a precursor for synthesis of useful drugs. The contributions of the plants are numerous in every sector of human life. It helps in growing up of human body and also protects human being

from sickness by being used as medicine. It has now been established that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils and contains minerals and vitamins possesses medicinal properties. [1, 2]

Microbial infections were one of the major causes of death among humans and animals in pre antibiotic era. After the discovery of penicillin and other antibiotics have significantly reduced the compilations and mortality rate of infectious diseases. With the wide use of antibiotics in the treatment of bacterial infections had led to the emergence and spread of resistant strains. The continuous spread of multi drug resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide.[3,4] Thus, there is a continuous need to develop newer drugs from alternate sources.

Eugenia floccosa Bedd is one of the medicinally important plants belongs to Myrtaceae family. The leaf paste of *E. floccosa* is given to treat rheumatic pain by Kanikkar tribe of Agasthiarmalai Biosphere Reserve, Tamil Nadu. The ethanol extract of *E. floccosa* has been reported for its anti-tumour activity, antidiabetic, antihyperlipidaemic and *in vitro* antioxidant activity.[5,6,7] The present study aimed at assessing the phytochemical screening, FT-IR and *in vitro* antibacterial potential of *E. floccosa* leaf against pathogenic bacteria.

MATERIALS AND METHODS

Plant Material: The leaves of *Eugenia floccosa* Bedd were freshly collected from the well grown healthy plants inhabiting the natural forests of Kothaiyar, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plant was identified and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of extracts for phytochemical screening and antimicrobial activity: Freshly collected leaf sample of *Eugenia floccosa* were dried in shade, and then coarsely powdered separately in a Wiley mill. The coarse powder (100g) was extracted successively with petroleum ether, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the extracts (petroleum ether, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures. [8,9,10] All the extracts were

concentrated in a rotary evaporator. The concentrated extracts were used for antibacterial activity.

FT-IR analysis: A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 − 400 cm $^{-1}$. [11]

Microorganisms: Bacterial strains of *Bacillus thuringiensis*(+), *Bacillus subtilis*(+),*Staphylococcus aureus*(+), *Staphylococcus aureus* (Methicillin sensitive) (+), *Enterococcus faecalis* (+), *Mycobacterium smegmatis* (+), *Salmonella paratyphi*-A & B(-), *Salmonella paratyphi* (-), *Proteus mirabilis* (-), *Escherichia coli* (-), *Escherichia coli* (ESBL) (-), *Proteus vulgaris*(-), *Klebsiella pneumoniae* (-), *Pseudomonas aeruginosa* (-) and *Pseudomonas aeruginosa* (ESBL) (-) bacterial strains were obtained from Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India. The bacteria were incubated on a nutrient agar-slant (Stationary cultures) for 48h at 37ºC, followed by inoculation in Muller Hinton Agar (MHA) medium.

Antibacterial assay: Antimicrobial study was carried out by disc diffusion method [12] against the pathogens. A loopful of bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram Positive and Gram Negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic of tetracycline (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37ºC for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone and antibacterial activity against the pathogenic bacteria were recorded. The experiments were repeated in triplicate and the results were documented.

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemicals are chemical compounds formed during the plant's normal metabolic processes. These chemicals are often referred to as 'secondary metabolites' of which there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids. The

phytochemical analysis of petroleum ether, methanol and ethanol extracts of *E. floccosa* leaf were showed in Table 1. In the present study, qualitative phytochemical investigation revealed that, the methanol and ethanol extracts contained some phytoconstituents. Alkaloid, coumarin, catechin, saponin, glycoside, steroid, flavonoid, terpenoid, tannin and phenol are found in both the extracts of *E. floccosa* leaf.

Table 1: Phytochemical screening of powdered leaf of *Eugenia floccosa*

Tests	Petroleum ether	Methanol	Ethanol
Alkaloid	+	+	+
Anthraquinone	-	+	+
Coumarin	-	+	+
Catechin	+	+	+
Glycoside	-	+	+
Flavonoid	-	+	+
Phenol	+	+	+
Quinone	+	-	-
Saponin	-	+	+
Steroid	-	+	+
Tannin	+	+	+
Terpenoid	+	+	+
Sugar	+	+	+
Xanthoprotein	+	+	+
Fixed Oil	-	-	+

Phenolics have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and antiinflammatory activities.[13,14] Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity.[15,16] Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, and piles and as an antidote. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, antiinflammatory, antiulcer and antioxidant property for possible therapeutic applications. Tannins are known to possess general antimicrobial and antioxidant activities. Recent reports show that, tannins may have potential

value as cytotoxic antineoplastic agents. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic.[17]

Alkaloids possess antiinflammatory, antiasthmatic, and antianaphylatic properties with consequences of altered immunological status *in vivo*. Furthermore, alkaloid which is one of the largest phytochemical groups in plants has amazing effect on humans and this has led to the development of powerful painkiller medicines.[18] Flavonoids, the major group of phenolic compounds are reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which highlights many of the flavonoids health promoting functions in organisms. They are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids on the other hand, are potent water soluble antioxidants and few radical scavengers, which prevent oxidative cell damage and have strong anticancer activity.[19] Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, antiinflammatory and anticancer. [19,20]

Coumarin has been used as anticoagulant drugs and to treat lymphedema. Plant steroids are known to be important for their cardio tonic activities, posses insecticidal and antimicrobial properties. [21]These observations cited on phytochemical compounds support the present findings on the usefulness of leaf of *E. floccosa* in various medicaments. It suggests that *E. floccosa* plant can be used as antimicrobial activity, antioxidant, antiallergic, antiinflammatory, antidiabetic, anticarcinogenic and anticancer agents in the future.

FT-IR Spectroscopy studies: Spectral differences are the objective reflection of componential differences. The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The functional group identification is based on the FT-IR peaks attributed to the stretching and bending liberations. By using the macroscope fingerprint characters of FTIR spectrum, we can trace the constituents in plant powders, identify the medicinal materials true or false and even evaluate the qualities of medicinal materials. So, FTIR spectrum reflecting objectively the panorama of chemical constituents in complex system is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal

medicine.[22] The outcome of FT-IR functional groups were represented in Table 2. The FT-IR spectrum profile of *E. floccosa* leaf powder was illustrated in Figure. 1.

Table 2 IR spectroscopic data of *E. floccosa* stretching frequency (cm^{-1})

S.No.	Group	Stretching frequency (cm^{-1})
1	O-H	3405.20
2	C-H stretching (alkyl group)	2920.80
3	C=C	1448.31
4	C-H stretching (Phenyl group)	1232.06
5	C=O	1617.07
6	C-O	1384.18

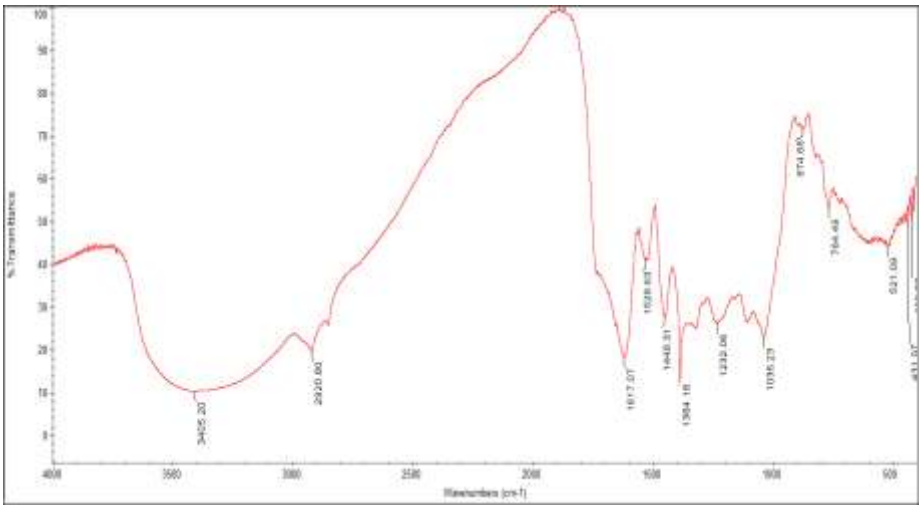

Figure 1: FT-IR Spectrum of leaf powder of *E. floccosa*

From the spectral data, presence of C=O, C-H, C=C, C-O, C-C were identified. These bonding structures are responsible for the presence of alkyl group, methyl group, alcohol group, ethers, esters, carboxylic acid and anhydrides. The FT-IR analysis revealed the presence of different functional groups ranging from O-H stretching, hydroxyl (3405.20 cm^{-1}), C-H stretching, alkyl (2920.80 cm^{-1}), C=C stretching, phenyl nucleus (1448.31 cm^{-1}), C-H stretching, aromatic ring (1232.06 cm^{-1}), C-O bending, alcohols, ethers, carboxylic acids and anhydrides (1384.18 cm^{-1}), C=O stretching, carboxylic carbonyl compounds (1617.07 cm^{-1}). Carboxylic acids present in the medicinal plant serves as main pharmaceutical product in curing ulcers, jaundice, headache, stomatitis, hemicranias, pain in liver, treatment of edema

and rheumatic joint pains. Amines, amides and amino acids are the main groups of protein synthesis and herbs serves as herb oil and hair tonic.

From the FT-IR spectra, it is clearly indicated that, although they show substantial overlap of each absorption spectrum of various components, each band represents an overall overlap of some characteristic absorption peaks of functional groups in the sample. Therefore the FTIR analysis on *E. floccosa* leaf displayed novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also to identify the medicinally important plants.

Table 3 *In vitro* antibacterial activity of different extracts of *E. floccosa* leaf

S. No	Microorganisms	Name of the extract/ Zone of Inhibition (mm)			
		Petroleum ether	Methanol	Ethanol	AB
1	*Bacillus subtilis*	8	10	9	24
2	*Bacillus thuringiensis*	7	13	8	25
3	*Enterococcus faecalis*	6	12	9	22
4	*Escherrichia coli*	-	16	13	23
5	*Escherrichia coli (ESBL)*	-	12	7	20
6	*Klebsiella pneumoniae*	-	9	7	20
7	*Mycobacterium smegmatis*	-		9	23
8	*Proteus mirabilis*	-	11	11	22
9	*Proteus vulgaris*	-	13	11	24
10	*Pseudomonas aeruginosa*	-	-	-	21
11	*Psuedomonas aeruginosa (ESBL)*	-	8	9	22
12	*Salmonella paratyphi*	-	10	8	25
13	*Salmonella paratyphi-A*	-	12	10	22
14	*Salmonella paratyphi-B*	14	10	9	20
15	*Staphylococcus aureus*	-	9	10	20
16	*Staphylococcus aureus (Methicillin sensitive)*	-	10	9	21

Antibacterial assay: The petroleum ether, methanol and ethanol extracts of *E. floccosa* leaf were examined for their antibacterial activity against the selected pathogens. The antibacterial activity has been observed in the petroleum ether, methanol and ethanol extracts of *E. floccosa* leaf against all the tested bacteria with varied activity (Table 3).

E. floccosa leaf methanol extract showed highest activity (15/16) against the bacterial pathogens followed by ethanol extract of *E. floccosa* leaf (14/16) and *E. floccosa* leaf petroleum ether extract (4/16). In the present study, we observed the antibacterial activity against sixteen pathogenic bacteria. But there is no report on *E. floccosa* leaf extracts. Thus, the present study shows the presence of antibacterial activity in *E. floccosa* leaf extracts for the first time.

Antibacterial study on 16 different pathogenic bacteria shows that, fifteen bacterial strains viz, *B. thuringiensis, B. subtilis, S. aureus, S. aureus* (methicillin sensitive), *E. faecalis, S. paratyphi* – A, *S. paratyphi* – B, *S. paratyphi, P. mirabilis, P. vulgaris, E. coli, E. coli* (ESBL) were sensitive to methanol extract of *E. floccosa* leaf. The petroleum ether extract of leaf of *E. floccosa* showed the highest zone of inhibition against *Salmonella paratyphi* – B (14 mm). The methanol extract of *E. floccosa* leaf demonstrated maximum zone of inhibition against six pathogens viz; *Escherichia coli* (16 mm), *Proteus vulgaris, Bacillus thuringiensis* (13 mm each), *Enterococcus faecalis, Salmonella paratyphi* A and *Pseudomonas aeruginosa* (ESBL) (12 mm each). The ethanol extract of *E. floccosa* leaf showed the highest zone of inhibition against *Escherichia coli* (13 mm).

In the present study, *in vitro* antibacterial efficacy of *E. floccosa* leaf of petroleum ether, methanol and ethanol extracts was quantitatively assessed on the basis of zone of inhibition. Plant part studied in the present investigation exhibited varying degree of inhibitory effect against the selected bacterial human pathogens. It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are able to exhibit inhibitory effect of both gram positive and gram negative bacteria.[23] In the present study also, petroleum ether, methanol and ethanol extracts of the selected plant showed zone of inhibition against isolated human pathogens with varied diameter. Eloff [24] reported that methanol was the most effective solvent for plant extraction than hexane and water. In the present study, petroleum ether, methanol and ethanol were used for extraction. The present study confirmed Eloff's observations with maximum activity.

The presence of antimicrobial activity in a particular part of a particular species may be due to the presence of one or more bioactive compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc. [25] Recently, a number of plants have been reported for antimicrobial properties across the world.[26,27,28,29,30] In the present investigation, methanol extract of *E. floccosa* leaf exhibited highest activity against *P. vulgaris*, *E. coli*, *B. thuringiensis*, *E. faecalis* and *S. paratyphi* – A. Among the pathogens, *P. vulgaris* is known to cause urinary tract infections and wound. *S. paratyphi* is known to cause fever and food borne illness. In general *E. coli* and *P. vulgaris* are known to cause gastroenteritis, food borne illness, urinary tract infections, neonatal meningitis, nosocomial infections, wound, septicemia, pneumonia and from mild superficial skin infections to life-threatening systemic diseases. *E. faecalis* is a frequent cause of urinary tract infection in hospitalized patients. Recent reports have suggested that the organism may frequently be acquired by cross-infection from other patients.[31] It is also responsible for urinary tract infections, bacteremia, endocarditis, meningitis and can be found in wound infections along with many other bacteria. Based on the present results, it is suggested that the *E. floccosa* plant can also be used to treat urinary tract infections and food borne illness.

CONCLUSION

Based on the present study on phytochemistry and antibacterial activities, it is concluded that the leaf of *E. floccosa* contains various compounds with high degree of antibacterial activity against various pathogens, including bacterial pathogens of urinary tract infections. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent antibacterial drugs of natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

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