The Pharma Innovation Journal 2023; 12(11): 1999-2003

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 1999-2003

www.thepharmajournal.com
Received: 30-08-2023
Accepted: 10-10-2023

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Phytofabrication and comparative antibacterial profiling of plant parts of *Eupatorium perfoliatum*

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Abstract
Because using medicinal herbs to treat illnesses has been done for years, they have assisted in doing so. When compared to single extracts, many naturally occurring phytocompounds show synergistic properties such antioxidant, antiviral, antibacterial, and anti-protozoal effects. *Eupatorium perfoliatum*, a homeopathic remedy, was chosen because of its potent antiviral and antiplasmodial properties. These medications are highly sought after for primary healthcare due to their cost-effectiveness, cultural acceptance, and low side effects. The described study aims to demonstrate the phytoconstituents involved and their possible antibacterial properties, hence claiming prospective use as antimicrobial compounds.

Keywords: Herbal medicines, *Eupatorium perfoliatum*, phytofabrication, antimicrobial, antiplasmodial

1. Introduction
The threat posed by several infectious diseases has diminished during the 20th century's antibiotic era. However, microbiological susceptibility to currently available antimicrobial drugs has decreased over time, which is what causes critical point drug resistance in hospitals (Subramaniam *et al.*, 2014) [1]. The overuse of antibiotics in recent years has undoubtedly contributed to the development of bacterial species' resistance to antibiotics and other antimicrobial treatments. Bacterial infections account for about 90% of illnesses discovered in healthcare facilities. Treatment failure appears to be primarily caused by the establishment of multidrug resistant (MDR) bacterial strains (Lacmata *et al.*, 2012) [3].
Globally, the use of herbal remedies to treat a range of medical conditions is still growing quickly. Natural therapies are experiencing a huge upsurge in popularity and public interest in both developed and developing nations. These herbal remedies can be found in food stores as well as drug stores (England *et al.*, 2023) [4]. Most of the population that lives in developing nations receives their healthcare primarily from herbal medicinal products. India boasts a sophisticated traditional medical system. Herbs are primarily used in medicinal practices such as Ayurveda, Unani, Homeopathy, Sidha, etc. The development of contemporary medications and dietary supplements for food and drink uses natural herbal products. Because traditional medicines have been used for thousands of years, there is a good degree of confidence in their safety and efficacy. The use of natural products as dietary supplements, food, and beverage ingredients, phytocosmetics, and other herbal products, as well as a source of novel chemical entities for the creation of contemporary medications, is becoming more and more popular (Chaughale and Barve, 2023) [5].
The North American medicinal herb boneset, also known as thoroughwort (*Eupatorium perfoliatum* L.), has a long history of use in treating fever and the flu. The various official monographs for *E. perfoliatum* in earlier USP editions also reflect these customary uses. Flavonoids, sesquiterpene lactones, triterpenes, and steroids are present in *E. perfoliatum*, but very little volatile oil is found in the plant. In addition, no pyrrolizidine alkaloids are found, in contrast to other Eupatorium species that have these alkaloids (Bhuiyan *et al.*, 2020) [6]. Pharmacological data point to a minor anti-inflammatory activity as well as a mild immunomodulatory effect through the stimulation of various unspecific immune system cell types, such as granulocytes. Clinical evidence is scant and does not support the sensible application of phytotherapeutics in the management of fever and flu. The limitation of only one uncontrolled clinical study is the primary cause of this (Thajuddeen and Van Heerden, 2019) [7]. Benefits from intricate homeopathic preparations containing *E. perfoliatum* point to clinical activity within the parameters that have been studied.

To sum up, *E. perfoliatum* extracts appear to be promising subjects for additional phytochemical, pharmacological, and clinical research in the hunt for potent anti-inflammatory and immunomodulatory medicinal herbs (Hensel *et al*, 2011) [8]. Therefore, the purpose of this study is to determine how different plant parts (leaf, stem, and root) of *E. perfoliatum* extracts affect isolates of both Gram-positive and Gram-negative bacteria.

Source: www.google.com

Fig 1: *Eupatorium perfoliatum* aerial parts

2. Materials and Methods

2.1 Collection of plant material

During the winter, *Eupatorium perfoliatum* saplings were grown in pots with a diameter of 15 cm that were ordered online. To avoid microbial contamination, the various components were first cleaned with tap water and then surface sterilized with a 10% sodium hypochlorite solution. After the sample was rinsed with distilled water and heated to 50 ºC, an electric blender was used to grind it into a powder.

2.2 Phytofabrication studies of *Eupatorium perfoliatum*.

2.2.1 Extraction of the plant materials

The plant parts were passed through a mesh sieve after being dried in the shade, separated, and ground into a fine powder using a mechanical grinder. The powdered materials were successively extracted with Ethyl acetate, Chloroform and Methanol in the ratio 3:2:1 (Jaiswal *et al*. 2012) [9]. After filtration through Whatman filter paper, the filtrates were evaporated to dryness in vacuum at 35 ºC to 40 ºC. The extracts were stored in screw cap vial at 4 ºC until further use.

A B C

Fig 2: Parts of *E. perfoliatum* (a) Leaves, (b) stem and (c) root

Phytochemical Screening

Various chemical tests, including those for different phytoconstituents in different solvents (hexane, chloroform, ethyl acetate, and methanol), were carried out to establish the profile of the extract for its chemical composition. (Harborne, 1998) [10].

Test for Alkaloid- Dragendroff's Test

After adding a few drops of Dragendroff's reagent to a test tube holding one ml of extract, the color appeared was observed. The presence of alkaloids was indicated by an orange appearance.

Test for Flavonoids-Ferric chloride test
Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Test for Saponin
To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

Test for Tannins
To the extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for Phenols-Ferric chloride Test
A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

Test for Terpenoids-Salkowski's Test
1ml of the extract was taken and added 0.5ml of chloroform along with 3-5 drops of conc. H_2SO_4. Formation of the reddish-brown precipitate was observed.

Test for Glycosides-Keller Killiani Test
A small amount of glacial acetic acid and ferric chloride solution were added to the test solution, then combined. After adding concentrated sulfuric acid, formation of two layers were watched. A positive glycoside test would have a lower reddish-brown layer and an upper acetic acid layer that turns bluish green.

Test for Coumarin
To the test sample 10% of sodium hydroxide and chloroform were added. Formation of yellow colour indicates the presence of coumarin.

Tests for Volatile Oils
After hydro-distillation, characteristic odor of the distillates and their non-permanent staining of filter papers indicated the presence of volatile oils.

2.3 Antibacterial Activities of Plant Parts
The antimicrobial profiles of different *E. perfoliatum* plant parts were assessed in comparison to bacterial isolates obtained from MTCC, IMTECH, Chandigarh. Against five Gram positive isolates (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Micrococcus luteus, and Listeria monocytogens*) and five Gram negative isolates (*Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enteric, Escherichia coli, and Aeromonas hydrophila*), the extract from the root, stem, and leaves showed varying levels of activity.

2.3.1 Agar Disc Diffusion Tests
The predetermined battery of antimicrobial discs was dispensed onto the surface of the inoculated agar plate. The discs were prepared by impregnation as four discs- one containing each plant part extract. Then all the discs were dried at 40 ℃ and placed into the bacteria inoculated Petridishes. As reference, antibiotic Azithromycin was used as positive control and distilled water was used as negative control. Each disc was pressed down to ensure complete contact with the agar surface. Disc was placed in a way that they are no closer than 24 mm from centre to centre. Five discs, three containing the extracted metabolite, fourth containing the test antibiotic and fifth containing distilled water were placed on one 90 mm plate and a disc should not be relocated once it has encountered the agar surface. Instead, place a new disc in another location on the agar. All experiments were performed in triplicates.
All Petri dishes after inoculation were allowed to dry for 15-20 min in room temperature. The plates were then incubated in an incubator set to 35±1 °C (Salie *et al*,, 1996) [11]. After 24 to 48h of incubation, each plate was examined. The diameters of the zones of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding using the HiAntibiotic Zone Scale (HiMedia), which was held on the back of the inverted Petri plate (Collins &Lyne, 1987 and Shannon and French, 2002) [13].

2.4 Statistical analysis
The statistical analysis was carried out by using SPSS v 1.6. Mean and standard deviation of all the experiments was intended from triplicates (n=3) and represented in the table (Mean ± S.D) and figures. Variations within the experimental groups was determined by ANOVA.

3. Results and Discussion
3.1 Phytofabrication studies
The phytofabrication analysis of different plant parts of *Eupatorium perfoliatum* has been studied in the present research. The phytofabrication of plant extracts divulged the presence of various bioactive compounds as depicted in the table. The presence of flavonoids, cardiac glycosides and terpenoids is a crucial finding in the terms of pharmacognosy and drug discovery as these compounds are the precursors of known bacterial and viral inhibitor molecules (Derksen *et al*, 2016) [14]. The precursors revealed in our study also give rise to compounds potent enough to block the Main protease (M^{pro}) of the recent pandemic causing virus SARS-CoV-2 (Pandith and Latha, 2020) [15].

Table 1: Table showing presence and absence of phytochemicals "+" indicates presence and "-" indicates absence of the same

Plant Parts	Alkaloid	Flavonoids	Saponin	Tannins	Phenols	Terpenoids	Glycosides	Coumarin	Volatile Oils
Root	+	+	-	+	-	+	+	+	+
Stem	-	+	+	-	+	+	+	+	+
Leaves	+	+	-	+	-	+	+	-	+

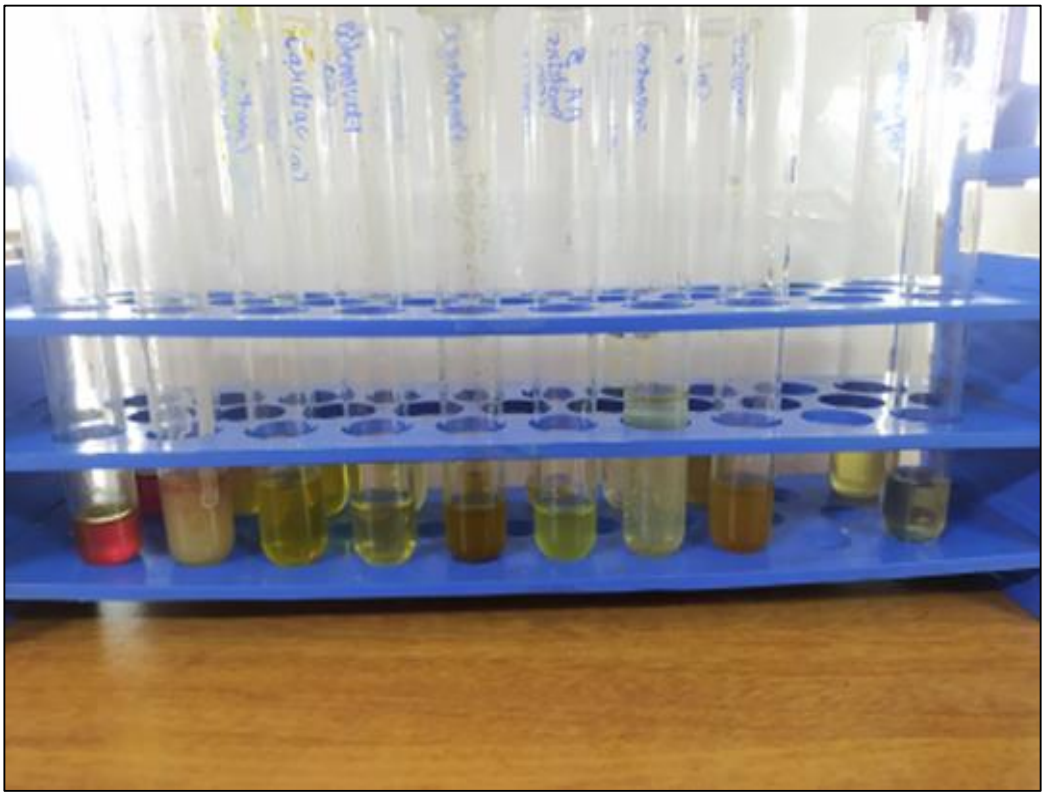

Fig 3: Phytochemical analysis of extracts

3.2 Antibacterial Profiling

The antibacterial profiling of plant part extracts against 5 Gram positive (*Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Micrococcus luteus and Listeria monocytogens*) and 5 Gram negative (*Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enteric, Escherichia coli, Aeromonas hydrophila*) have been analysed in the present study. *Eupatorium perfoliatum* is famously known as a potent homoeopathic medicine against Dengue and Malarial parasites but its activity against bacterial isolates has not been fairly explored yet. The antiplasmodial effect of aerial parts has also been demonstrated previously (*Mass et al.*, 2011) [9]. The antibacterial activity analysed in present study has been demonstrated as below. The experiments were performed in triplicates and result shown in the form of mm ± SD. As it is clear from the table the highest zone of inhibition was demonstrated by leaves extract against *Staphylococcus aureus* (F= 367.58, $p<0.05$) which was nearly equal to the positive control and the lowest activity was demonstrated by stem extract against *Klebsiella pneumoniae* (F=889.89, $p<0.05$).

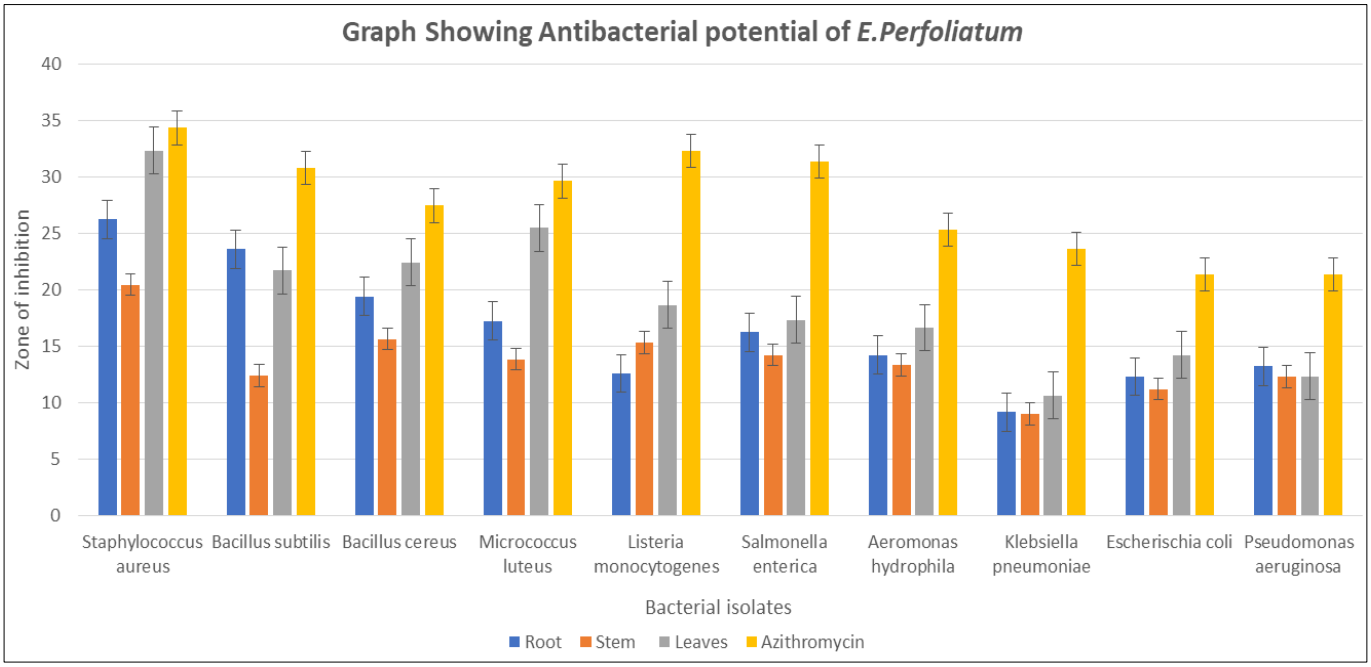

Fig 4: Antibacterial activity of Root, Stem and Leaves Extract of *E. perfoliatum* by Agar Disc Diffusion method. Each bar represents Mean ± SD of three replicate observations

Table 2: Antibacterial Activity of root, stem and leaves extract of *E. perfoliatum* by agar disc diffusion method. Positive control= Azithromycin, Negative control= DW. Values are expressed as Mean ±SD (n=1*3)

S. No		Microorganism	Zone of Inhibition (mm ± SD)				
			Root	Stem	Leaves	Control	
						Positive (Azithromycin)	Negative (DW)
1.	*Gram Positive*	*Staphylococcus aureus*	26.23±0.34	20.45±0.25	32.33±0.56	34.33±0.45	0
2.		*Bacillus subtilis*	23.62±0.69	12.4±0.45	21.75±0.34	30.76±0.65	0
3.		*Bacillus cereus*	19.43±0.32	15.66±0.57	22.42±0.54	27.45±0.48	0
4.		*Micrococcus luteus*	17.25±0.35	13.87±0.23	25.47±0.24	29.62±0.20	0
5.		*Listeria monocytogens*	12.6±0.27	15.33±0.57	18.66±0.46	32.33±0.57	0
6.	*Gram Negative*	*Salmonella enterica*	16.24±0.26	14.25±0.68	17.35±0.34	31.33±0.57	0
7.		*Aeromonas hydrophila*	14.24±0.6	13.34±0.45	16.66±0.52	25.34±0.32	0
8.		*Klebsiella pneumoniae*	9.2±0.20	9±0.32	10.66±0.37	23.66±0.57	0
9.		*Escherichia coli*	12.32±0.32	11.22±0.30	14.22±0.22	21.35±0.35	0
10.		*Pseudomonas aeruginosa*	13.23±0.33	12.34±0.23	13.33±0.57	21.34±0.20	0

4. Conclusion

The present study demonstrates the antibacterial potential of various plant parts of *Eupatorium perfoliatum* and the various phytoconstituents associated with them. With an exponential increase in deadly bacterial and viral pathogens, the urge to find possible antimicrobial molecules has also increased. The present investigation has opened new vistas for discovering novel antibiotic molecules and altering the drug delivery systems. The current investigation is hence a unique effort on its own.

5. Acknowledgement

The authors are grateful to the MATS School of Sciences, MATS University, for providing central laboratory facilities.

6. Conflict of Interest

The authors declare none.

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