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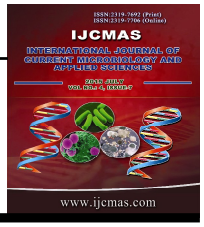

Original Research Article

Screening of Phytochemicals and Testing the Antimicrobial Activity of Different Parts of *Erigeon* sp and its Essential Oil

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A B S T R A C T

Keywords

Erigeon sp, Antibacterial activity, Antifungal activity, Essential oil

Erigeon sp possess different activities such as antibacterial, anti oxidants and anti inflammatory activities. Essential oils are nearly common to all species and the components present in oils like these have been known to possess antimicrobial activity and are generally recognized as safe substances. This study was designed to test the antibacterial activity of essential oil and solvent extracts of *Erigeon sp*. The extracts and essential oil showed a good activity against the bacterial and fungal strains and phyto chemicals present in the extracts also characterized. The obtained results provide a support for the use of this plant in traditional medicine. This plant studied can be a potential source of biologically active compounds as antifungal, antibacterial activity.

Introduction

Many plants synthesize substances that are useful to maintenance of health in humans and other animals (Bruneton and Zaragoza Acribia, 2001). Asia is the largest continent and has 60% of the world's population. It has abundant medicinal and aromatic plant species, well documented traditional knowledge, a long-standing practice of traditional medicine, and the potential for social and economic development of medicinal and aromatic plants (Handa, 2006).

Illness caused by the consumption of contaminated foods has a wide economic and public health impact worldwide (Mead *et al.*, 1999). Many pathogenic microorganisms such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella sp* and *Pseudomonas aeruginosa* have been reported as the causal agents of food borne diseases (McCabe Sellers and Samuel, 2004). Plants provide flavors, fragrances and traditional medicines as part of everyday life. Approximately 3000 plants species contain essential oils, of which only 300 are commercially important. Essential oils and some of their constituents are used not only in pharmaceutical products for their therapeutic activities but also in agriculture as food preservatives and additives (Bakkali *et al.*, 2008).

A large number of *Erigeron species* yield essential oils rich in biologically active

polyacetylenic compounds/terpenoids and are reported to possess diverse biological activity, viz., antibacterial, antifungal, and nontoxic.

The major constituents of *Erigeron species* include monoterpenes, sesuiterpenes, polyacetylenic compounds, diterpenoids and bioactive prone derivatives (Awen *et al.,* 2010). *Erigeron plants* such as *Erigeron annuus, Erigeron philadelphicus* and *Erigeron sumatrensis* have been investigated and shown as it contain monoterpenoids, diterpenoid, sesquiterpenoids, triterpenoids, sterols and phenolic compounds (Miyazawa *et al.,* 1981).

The aim of the present study was to screening of phyto chemicals and testing the antimicrobial activity of different parts of *Erigeon sp* and its essential oil

Materials and Methods

Collection of plant for analysis

Erigeron sp were collected from KR market (flower shop) Bangalore, Karnataka state.

Preparation of extracts

10g of plant powder was taken in 100ml of methanol, aqueous, ethanol, and chloroform and kept in shaker for 24 hours. The extracts of flower, leaf branch, and stem were extracted and stored at 4°C until further use.

Essential oil extraction

250g of air dried flower parts were used for the oil extraction using Clevenger apparatus hydrodistillation was done for 3 hours. The flower extract was dried over anhydrous sodium sulphate and solvent hexane was added. Using separator funnel oil was extracted and preserved in a sealed vial at 4°C for further analysis.

Test for phytochemical analysis

Preliminary screening

Phytochemical analysis for tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids, quinones, coumarin, terpenoids, cardiac glycosides were analysed (Anjali Soni and Sheetal Sosa, 2013) using the extracts of flower, leaf, branch, stem of *Erigeron sp.*

Test for tannins

1ml of plant extracts were taken in test tube and 1ml of 0.008M potassium ferricyanide was added then 1ml of 0.02M ferric chloride containing 0.1N HCL was added and observed for blue black coloration.

Test for phlobatannins

Plant extracts were boiled with 2% aqueous HCL. The deposition of a red precipitate shows presence of phlobatannins.

Test for saponins

Plant extracts was mixed with 5ml of distilled water in a test tube and shaken vigorously and some drops of olive oil was added. Formation of stable foam was taken as an indication for the presence of saponins.

Test for flavonoids

5ml of dilute ammonia solution was added to a portion of the plant extracts followed by addition of concentrated H_2SO_4. Yellow color indicates the presence of flavonoid.

Test for steroids

2ml of acetic anhydride was added to 0.5ml of plant extracts with 2ml of H_2SO_4. Color changes from violet to blue or green in

extracts indicates the presence of steroids.

Test for alkaloids

Plant extracts were mixed with 2ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

Test for quinones

Dilute NaOH was added to the 1ml of plant extracts. Blue green or red coloration indicates presence of quinones.

Test for coumarin

10% NaOH was added to the extracts and chloroform was added forms a yellow colour.

Test for terpenoids (salkowski test)

5ml of extracts was mixed with 2ml of chloroform and 3ml of concentrated H_2SO_4 was added carefully to form a layer. A reddish brown coloration of the interface indicates terpenoids

Test for cardiac glycosides (Keller-kiliani test)

5ml of extracts was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1ml of concentrated H_2SO_4. Brown ring indicates a deoxy sugar characteristic of cardenolides. Violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may form.

Test organisms

Test organisms were collected from MTCC. Bacterial cultures *Escherichia coli* (MTCC581), *Pseudomonas aeruginosa* (MTCC 4673), *Staphylococcus aureus* (MTCC 3160), *Listeria monocytogenes* (MTCC 1143), and fungal cultures *Aspergillus niger, Aspergillus flavus, Mucor sps, Candida albicans, Rhizopus sps, Penicillium sps* were used.

Antibacterial activity test

For the determination of antibacterial activity, Agar well diffusion method is used for that 24 hours fresh bacterial culture were taken for the determination of antimicrobial activity of extracts and oil for all bacteria.

0.1ml of culture was spread on Muller Hinton agar, Luria Bertani agar, and Brain Heart Infusion agar. The wells were cut by sterile well borer. To each well different concentration like 10, 15, 20, 25, 50, 100µl of plant extracts (oil, aqueous, methanol, ethanol, chloroform) were delivered to respective well and the plates were incubated at 37°C for 24 hours. After incubation DIZ (Diameter inhibition zone) were measured by using zone measuring scale.

Antifungal activity

Antifungal activity of the oil, aqueous, methanol, ethanol, chloroform, extracts of the plant sample was evaluated by agar well diffusion method. For that 24 hours fresh fungal culture were taken for the determination of antifungal activity for all fungi.

0.1ml of culture was spread on Sabouraud dextrose agar. The wells were cut by sterile well border. To each well different concentration like 10, 15, 20, 25, 50, 100µl of plant extracts (oil, aqueous, methanol, ethanol, chloroform) were delivered to

respective well and the plates were incubated at room temperature for 48 hours. After incubation, each plate was examined and measured the diameter of the zone of inhibition.

MIC

Minimum inhibitory concentration (MIC) of oil, methanol, aqueous extracts of *Erigeron sp* flower was tested by the two fold serial dilution method. The test samples of oil, methanol, aqueous were incorporated in to 1ml of BHI broth to get a concentration of 1000µg/ml and serially dilute to achieve 500, 250, 25, 62.5 and 31.25 µg/ml. 10-µl standardized suspension of each tested organisms was transferred to each tube. The control tubes contained only bacterial suspension and were incubated at 37°C for 24 hours. The lowest concentration of the test samples, which did not show any growth of tested organisms, was determined as MIC.

Results and Discussion

The extracts of *Erigeron sp* were subjected for phytochemical analysis the results were investigated. Phytochemical screening of the crude extracts revealed the presence of tannins, phlobatannins, saponins, flavonoids, steroids, alkaloids, quinones, coumarin, terpenoids, and cardiac glycosides. The positive and negative results were tabulated in table 1.

Antimicrobial activity of the oil, aqueous, methanol, ethanol, chloroform, extracts of at a different concentration like 10, 15, 20, 25, 50, 100µl showing the results of the zones of inhibition of extracts against the microorganisms were represented in table 2, 3 and figure 1, many plant oils and extracts have been reported to have antimicrobial properties (Hoffman, 1987). Also, the renewal of interest in food industry and increasing consumer demands for effective, safe and natural products means that quantitative data on plant oils and extracts are required.

In recent years, several researchers have also reported that mono- and sesquiterpene hydrocarbons and their oxygenated derivatives are the major components of essential oils from plant origin, which have enormous potential to inhibit microbial pathogens (Filipowicz *et al.,* 2003).

On the basis of the results of this study, *Erigeron sp* may act as an alternative to synthetic bactericides for use in food industries, where bacterial pathogens cause severe destruction. At present, food safety is undoubtedly an important public health problem, and there is a need to develop new methods for eliminating foodborne pathogens and spoiling bacteria. Thus, the essential oil and extracts of *Erigeron sp* might be a valuable food additive. However, if plant oils and extracts are to be used for food preservation or medicinal purposes, issues of safety and toxicity will always need to be addressed.

Dr. Dupuy (1898) who made an examination of this plant, found it to contain essential oil, tannic and gallic acids, bitter extractive, etc. The oil is not astringent to the taste, but has a styptic influence upon the system. It is of a colorless, or pale-yellow color, gradually becoming darker-colored, and may be procured from the plant by distillation with water.

The present result showed that the whole plant and its extracts are effective against microorganisms both bacteria and fungi. The oil which is extracted from *Erigeron sp* flower parts shown high antimicrobial activity. Different solvents have been

reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity. Among the solvents methanol and aqueous extracts might have higher solubility for more phytoconstituents, consequently the highest antibacterial activity of three extracts ethanol, methanol, aqueous and essential oil were more active against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus,* and shows high antifungal activity against these fungal organisms *Aspergillus flavus, Aspergillus niger, Penicillium sp* than the other fungal organisms

Table.1 Phytochemical analysis of *Erigeron sp*

S. No	Test	Methanol extracts				Aqueous extracts			
		Flower	leaf	branch	stem	Flower	leaf	branch	stem
1	Tannin	+	+	+	+	+	+	+	+
2	Phlobatannin	_	_	_	_	+	+	+	+
3	Saponin	+	+	+	+	+	+	+	+
4	Flavonoids	+	+	_	+	+	_	+	+
5	Steroids	_	+	+	_	_	_	+	+
6	Alkaloids	+	+	+	+	+	+	+	+
7	Quinones	_	_	_	_	_	_	_	_
8	Coumarin	+	_	+	+	_	+	+	+
9	Terpenoids	+	_	_	+	_	+	_	_
10	Cardiac glycosides	_	_	_	+	_	+	_	+

+ (positive) - (negative)

Table.2 Antibacterial activity of *Erigeron* sp extracts

S. No	Test Organisms	ZONE OF INHIBITION (mm diameter) LB agar (various concentration)			
		Extracts	10µl	15µl	20µl
1	*Pseudomonas aeruginosa*	BM	10mm	7mm	15mm
		BE	no zone	8mm	10mm
		BC	10mm	11mm	20mm
		BW	no zone	11mm	10mm
2	*Escherichia coli*	FM	No zone	10mm	20mm
		FE	No zone	10mm	15mm
		FC	10mm	10mm	12mm
		FW	No zone	20mm	23mm
3	*Staphylococcus aureus*	SM	no zone	10mm	12mm
		SE	no zone	20mm	23mm
		SC	no zone	10mm	15mm
		SW	no zone	20mm	20mm

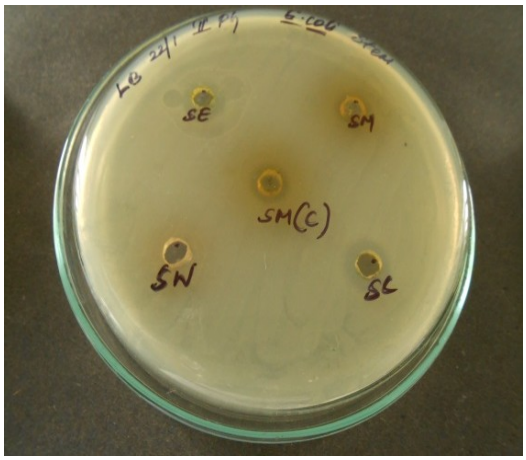
B - Branch, F - Flower, S -Stem, M - Methanol, E - Ethanol, C - Chloroform, W - Water

Table.3 Antifungal activity of *Erigeron* sp extracts

S.No	Test organisms	Extracts	ZONE OF INHIBITION (mm diameter) SDA agar							
			20µl				50µl			
			Leaf	Stem	Branch	Flower	Leaf	Stem	Branch	Flower
1	*Penicillium*	M	20	25	10	10	23	27	15	15
		E	15	15	15	15	19	20	15	20
		C	25	25	25	25	28	28	25	30
		W	25	15	20	10	26	20	20	35
2	*Aspergillus niger*	M	10	10	10	15	15	15	15	19
		E	10	10	10	10	15	15	18	20
		C	20	20	20	20	25	20	18	25
		W	10	10	15	10	15	15	15	20
3	*Aspergillus flavus*	M	10	15	10	15	15	16	16	15
		E	10	15	15	15	20	25	20	10
		C	20	20	20	20	21	20	10	15
		W	10	10	10	10	15	15	20	15
4	*Rhizopus*	M	6	6	7	6	10	10	10	10
		E	6	10	8	10	10	10	10	10
		C	7	8	7	7	9	10	9	10
		W	10	6	7	6	15	10	10	15
5	*Mucor*	M	7	9	10	7	10	20	10	10
		E	7	6	10	7	10	15	10	15
		C	7	6	10	10	10	10	10	10
		W	10	10	9	10	15	10	15	15

M - methanol, E - ethanol, C - chloroform, W - water

Figure.1 Antimicrobial activity of extracts



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