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FTIR spectroscopic studies and antimicrobial activity in populations of *Eryngium foetidum* L.

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ABSTRACT

The Fourier Transform Infra Red spectroscopic studies have been used to investigate the variations in the phytochemical constituents of two diverse populations of *Eryngium foetidum* L. from India. Based on the FTIR analysis and preliminary phytochemical investigations it was possible to understand the various functional groups and bioactive components present in the methanolic extracts of both populations. The FTIR pattern highlighted sharp peaks for OH, aldehydes and aminoacids in the extracts. Characteristic strong peaks were observed in the fingerprint region confirming the presence of aromatic compounds, alkanes, alkenes, alkynes, aliphatic primary amines, phenols, carbohydrates and halogenated compounds. The extracts were also tested for their antimicrobial efficacy against *E. coli, S. aureus, K. pneumoniae, P. aeruginosa and A. faecalis* using Disc diffusion assay at various concentrations .It was observed that the extracts showed significant activity against *S. aureus* and moderate activity for *E. coli* and *P. aeruginosa.*

Key words: *Eryngium foetidum* L., Apiaceae, FTIR, *Staphylococcus aureus*, ecotypes, hydroxyl group.

INTRODUCTION

Eryngium foetidum L. (Apiaceae) is an endemic medicinal and aromatic herb found in diverse, specific geographic locations in India [1]. It has taken its origin in the Caribbean and is widely distributed in Continental tropical America, South West Africa and South East Asian countries [2]. Commonly referred to as 'culantro' or 'wild coriander' the plant is exclusively used as a leafy vegetable and as condiment in continental dishes owing to its aroma similar to that of the normal coriander [3, 4]. It has been accredited with numerous uses in traditional medicine like in treatment of colds, coughs, fever, arithritis, hypertension, seizures, female reproductive disorders, scorpion stings and ear pain [5, 6]. The scientific validations for the medicinal attributes of the plant have been obtained for its analgesic and anti-inflammatory properties [7, 8], antioxidant activity [8, 9] and anticlastogenic effect [10]. The anti-helminthic property of the plant (Eryngial) against parasytic trypanosome *Strongyloides stercoralis* has been patented [11] .The plant contains essential oils rich in aromatic and long chain aliphatic aldehydes making them indispensable in perfumery industry at domestic and international markets [12,13].

With the advent of usage of aromatic and medicinal plants in various industries, it becomes imperative to have detailed information of their properties and the effects of eco-climatic conditions, if any, on the ecotypes [14]. In the present investigation, populations of *E. foetidum* have been collected from two distinct geographical locations (Karnataka and Tamil Nadu) and their ecotypic variations with respect to their chemical content and bioactivity have been dealt with. The Fourier Transform Infrared (FTIR) spectroscopic studies have been used to analyze the chemotypic diversity among the populations. The fingerprinting characters and extensive applicability to the samples has made this

technique indispensable to pharmaceutical research in recent years [15, 16]. In our study this tool has been effectively used to elucidate the bioactive groups of compounds present in both populations. The ecotypes have also been screened for their antimicrobial activity against five human pathogens.

MATERIAL AND METHODS

Collection and preparation of plant material: The plant populations of *E. foetidum* were collected from Hassan (Karnataka) and Nadugani (Tamil Nadu), identified and voucher specimens deposited at Regional Research Institute, Bangalore. The leaves of both the populations were excised, dried under shade and powdered coarsely into a powder. About 10g of dried plant material was extracted with 100 ml of methanol with occasional agitation at room temperature for 24 h. Thereafter it was filtered, centrifuged at 6000g for 12 min. The supernatant was collected and the solvent evaporated to obtain one fifth of the original volume. This methanolic extract was stored in air tight bottles at 4^0 C till further use.

Preliminary phytochemical Investigation: The qualitative phytochemical investigations of the methanolic extracts of both populations were carried out using standard tests like Mayer's test for alkaloids, Liberman –Burchard test for steroids, Salkowski test for terpenoids, Keller-Kilani test for glycosides, Shinoda's test for flavonoids and Ferric chloride test for tannins and phenol [17, 18,19].

Sample preparation and test chemicals for FTIR: The methanolic extracts were mixed with naphthalene (at a ratio of 1/100), and the mixture was subjected to a pressure of $5x10^6$ Pa in evacuated die to produce a naphthalene pellet for use in FTIR studies. The AR grade alcohol and naphthalene were procured from Sigma Aldrich Company, Bangalore, India and were used for the experiments.

Spectroscopic analysis: The FTIR spectra were recorded on a FTIR 460 plus Jasco. The naphthalene pellets prepared as above were scanned at room temperature (26 $\pm 2^0$C) at 4000-400cm^{-1} spectral range. About 100 interferograms were averaged with a spectral resolution of ±4 cm^{-1} for each spectrum to improve the signal to noise ratio. The background spectra which were collected under identical conditions were subtracted from sample spectra. Therefore, in the present study it is possible to directly relate the intensities of the absorption bands to the concentration of the corresponding functional groups [20]. Each sample was scanned with six different pellets all under identical conditions. Each pellet was of 13mm diameter and 1mm thickness and was applied with the same pressure.

Bacterial cultures: The test organisms selected for screening included *Escherichia coli* MTCC 40, *Klebsiella pneumoniae* MTCC 661, *Pseudomans aeruginosa* MTCC 424, *Staphylococcus aureus* MTCC 96, *Alcaligenes faecalis* MTCC 126. These were procured form IMTECH, India and used for the present study.

Disc diffusion method: A loopful of the bacteria to be tested was inoculated into sterilized nutrient broth and incubated at 37^0C for 24h to obtain a pre culture (stock culture). The antimicrobial sensitivity test was done using Kirby-Bauer's disc diffusion method [21] and recommendations of NCCLS [22] on Mueller Hinton media. The stock culture was adjusted to 0.5 McFarland standard turbidity and used for assay. 0.1ml aliquot of this pre culture bacteria was spread onto agar medium using sterile swab sticks for development of a lawn of bacteria. The sterile discs, 6mm in diameter, dipped in various concentrations (1mg/ml, 2mg/ml, 3mg/ml, 4mg/ml and 5 mg/ml) of the extracts were placed onto the cultured Mueller Hinton agar plates equidistant from each other. Gentamycin (1mg/ml) and methanol were used as positive and negative control respectively. The plates were incubated at 37^0C for 24h and then measured for zones of inhibition. All the assays were performed in triplicates.

RESULTS AND DISCUSSION

The preliminary phytochemical investigations carried out in the extracts of *E. foetidum* populations revealed the presence of secondary metabolites like alkaloids, sterols, terpenoids, glycosides, saponins, flavonoids, tannins and phenols (Table 1). This is in accordance with the previous reports on the phytochemical constituents of *E. foetidum* [23]. Variations observed in the phytochemical contents of both the populations were minimal indicating their fidelity irrespective of their geographic location. The FTIR analysis of the extracts of the populations indicated the presence of various components in the populations of *E. foetidum* (Fig. 1 and Fig. 2), the results of which have been tabulated in Tables 1 and 2. The position of the band based on their wavenumber (cm^{-1}) and the corresponding compounds based on their functional groups have been detailed in the tables. The peaks at 3186, 3313, 3390, 3480 cm^{-1} (Karnataka sample) and 3558, 3396, 3232, 3145 cm^{-1} (Tamil Nadu sample) indicate the presence of hydroxyl (OH) group. Those at 3558 could be derived from phenols and alcohols [15]. The

peak at 3855 cm^{-1} indicates vibration due to O-H stretching [24]. The N-H stretching of amines and amides is found at 3390 and 3396 cm^{-1} in both extracts indicating the presence of amino acids. [16, 25] The C-O bond can be detected at 2362 and 2345 cm^{-1} [16]. The C=C asymmetric stretching in alkynes and the presence of silicon compounds were attributed to peaks at 2039 and 2031 cm^{-1} [16]. Presence of diketones, aromatic compounds and α, β-unsaturated amides could be observed due to peaks at 1637 and 1667 cm^{-1} in both extracts [15]. The strong peaks at 1400 and 1408 cm^{-1} can be related to nitrosamines [16]. The isopropyl groups and C-N stretching of alkyl amines could be detected from bands at 1059 and 1013 [26]. The characteristic peaks between 800-1100 at 878, 835, 900, 1013 and at 2947 cm^{-1} could be strongly corresponded to carbohydrates and their phosphodiester bonds. The presence of C-C, C-O deoxyribose and C-O-C bonds were very strong in this region. Presence of C=O, C–H, C=C and C–O, C–C and C–O were identified. These bonding structures are responsible for the presence of alkyl groups, methyl groups, alcohols, ethers, esters, carboxylic acid, anhydrides and deoxyribose [27]. The presence of α- glucose was observed from the absorption peak at 835 cm^{-1} [26]. The peaks at 670, 500 and 430 cm^{-1} directed towards the presence of alkyl halides (C-Cl, C-Br, C-I) [28]. The C-S linkages were clearly observed at 670 cm^{-1} [16]. The C-H out of plane bending vibrations could be observed at 600, 612 and 666 cm^{-1} [16]. Torsion of C=C and ring structure of phenols were detected at 543 and 456 cm^{-1} [29].

The major difference in the FTIR pattern among the populations was in the region 2700-2950 cm^{-1}. This region constitutes stretching vibrations of C-H, CH_2, CH_3 groups and alkanes [27] and was predominant in the extract obtained from Tamil Nadu population. The peaks at 2892 and 2947 cm^{-1} correlates to lipophilic components [30].

The components that could be deduced to be present from the above study included amino acids, alkanes, alkenes, aromatic compounds, nitrates, phenols, organic halogen compounds and carbohydrates.

The antibacterial activity of the methanolic extracts of the *E. foetidum* populations was determined by measuring the zone of inhibition. It was observed that the extract obtained from Karnataka populations (A) showed moderate activity against *E.coli* and *S. aureus* at all concentrations. There was a weak activity of the extract against *P.aeruginosa* at 4mg and 5 mg (Table 4). The other extract from Tamil Nadu population (B) a debilitated activity against *E.coli* but evoked a significant response towards *S. aureus* producing a maximum zone of inhibition for all concentrations (Table 4). However *K. pneumoniae* and *A. faecalis* remained resistant to both the extracts.

The present investigation has brought about a thorough profile of the phytochemical content of two populations of *E.foetidum* collected from Hassan, Karnataka and Nadugani, Tamil Nadu. The qualitative phytochemical tests and FTIR analysis of the methanolic extracts of these populations showed similar functional groups and bioactive compounds. The only variation in the IR spectroscopic studies was the presence of a strong peak for alkanes at 2700-2900 cm^{-1} in the extract from Tamil Nadu populations (B). The strong antibacterial activity of the extract B against *S.aureus* could also be explained based on the presence of higher amount of alkanes in the extract B. (Table 3). It has been demonstrated by Sharma, 2012 [31] that homologous series of alkanes are highly effective against *S.aureus*. The essential oils of the aerial parts of *E. foetidum* are predominated by long chain aliphatic aldehydes [12, 13] which could be attributed to the anti bacterial efficacy against *S. aureus.* Similar studies on antibacterial potential of *E. maritimum* and *E.caucaseum* against *S. aureus* and *E.coli* have been carried out. [32, 33]

A sudden resurgence in the popularity of natural food and medicine has created immense demand for medicinal plants in India and abroad. However the unavailability of scientific validation regarding their qualitative and quantitative content has been a great setback in the journey towards herbalism. The effects of climate and other ecological conditions on the medicinal plants may affect secondary metabolite production which needs to be incessantly monitored to maintain the potency of the plant drugs [34]. Therefore a concerted effort is required to assess the content of the medicinal plants obtained from various locations. In this context FTIR has proven to be an effective tool in reflecting the broad spectrum of components present in two different populations of *E. foetidum.*

CONCLUSION

This **s**tudy highlights the usefulness of FTIR technique in explicating the phytochemical constituents of natural products. Accordingly the FTIR spectrum indicated the presence of carbohydrates, alcohols, phenols, aldehydes, polysaccharides, amides, and lipids representing the plethora of components in both of the populations. It was also possible to understand the effects of

geographical conditions on the biosynthesis of various components in the ecotypes. The antimicrobial screening assay performed has provided a fundamental scientific validation for further use of the plant extracts in pharmaceutical preparations. However a thorough research has to be taken up to isolate, purify and identify the bioactive components in the plant to expand the vistas to higher levels.

Table 1- Preliminary phytochemical investigation of the methanolic extracts of the populations of *E. foetidum*

Secondary metabolites	Extract (Karnataka)	Extract (Tamil Nadu)
Alkaloids	+	+
Sterols	-	-
Terpenoids	+	+
Glycosides	+	+
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Phenols	+	+

The secondary metabolites present are represented as '+' and those not present as '-'.

Table 2- FTIR analysis of the methanolic extract of *E. foetidum* collected from Hassan, Karnataka

Sl No.	Frequency range(cm^{-1})	Assignment of functional groups
1.	3700-3800	OH stretching
2.	3100-3550	OH bonds
3.	3390	N-H, stretching of amines and amides
4.	2362	C-O bond
5.	2039	C=C symmetric stretching of
6.	1637	Diketones, aromatic ring, C=O stretching (lipids)
7.	1408	Nitrosamines, alkanes
8.	1341	Isopropyl group, amide III band region
9.	1059	Alkyl amine
10.	1017	Aliphatic amine, vibration of C-O in alcohol hydroxyl group
11.	800-1300	Phosphodiester region
12.	765	Aromatic compounds, alkyl halides
13.	600-900	C-H out of plane bending vibrations
14.	600-700	C-S linkage
15.	513	Torsion and ring torsion in phenol
16.	432	Alkyl halides

Table 3- FTIR analysis of the methanolic extract of *E. foetidum* collected from Nadugani, Tamil Nadu

Sl. No.	Frequency range(cm^{-1})	Assignment of functional groups
1.	3100-3550	OH group
2.	2947	C-H, CH_2, CH_3
3.	2892	CH_3, C-H stretching, cycloalkanes
4.	2769	C-H, aldehydes
5.	2345	C-O bond, C-N
6.	2115	C=C stretching bond of alkynes
7.	2031	Silicon compounds
8.	1938	C=C asymmetric stretch

9.	1667	Diketones, α, β- unsaturated amide
10.	1400	Alkanes, α- CH_2 bending
11.	1333	Isopropyl group
12.	900-1100	Phosphodiester region
13.	1013	Alkyl amines
14.	723	Halogen compounds(C- Cl)
15.	600-900	C-H out of plane bending vibrations
16.	543	Torsion and ring torsion in phenol
17.	456	Alkyl halides

Table 4- Disc diffusion assay of the extracts of the populations of *E. foetidum* against five test organisms.

Target organism	Extract	Diameter of the zones of inhibition against various concentrations and standard (in mm)					
		1mg	2mg	3mg	4mg	5mg	Standard
E. coli	A	10	12	12	13	13	22
	B	08	08	08	09	09	22
S. aureus	A	13	13	14	15	15	30
	B	26	28	28	28	28	32
K. pneumoniae	A	00	00	00	00	00	24
	B	00	00	00	00	00	24
P.aeruginosa	A	00	00	00	08	08	26
	B	00	00	00	00	08	26
E. faecalis	A	00	00	00	00	00	22
	B	00	00	00	00	00	22

Extract A-Karnataka; Extract B-Tamil Nadu
The zones of inhibition (in mm) includes the diameter of the disc (6mm).
The assay was performed in triplicates and the results are the mean of the three values.

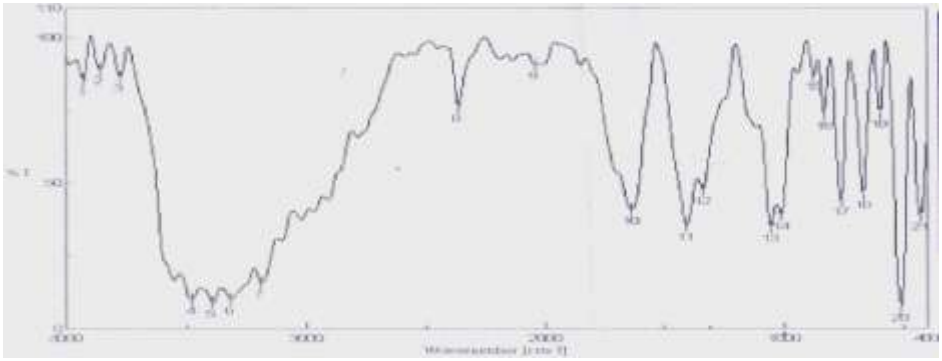

Fig. 1 FTIR spectrum of *E.foetidum* (Karnataka)

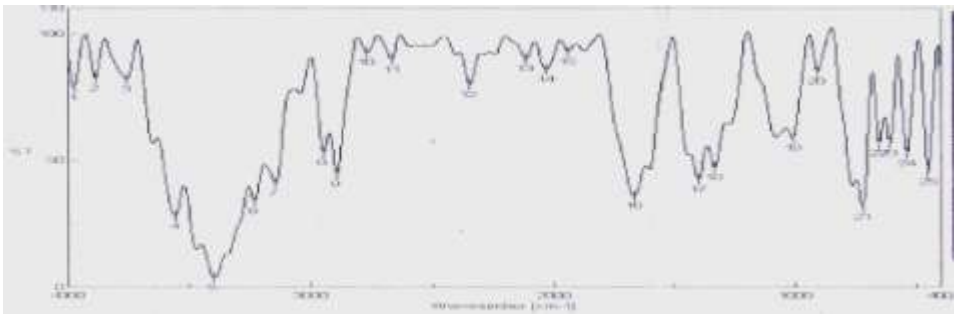

Fig. 2. FTIR spectrum of *E.foetidum* (Tamil Nadu)

REFERENCE

1. Chandrika R, Thara Saraswathi KJ, Shivakameshwari MN. Int J Plant Repro Biol, 2013; 5(1): 89-91.
2. Adams CD. University of West Indies Press, Mona, Jamaica WI, 1971; 256-264.
3. Wörz A. Diekmann H. Plant Divers. Evol, 2010; *128* (3-4): 387-408.
4. Ignacimuthu S, Arockiasamy S, Antonysamy M and Ravichanran P. Pl. Cell Tissue Org. Cul., 1991;56: 131-137.
5. Rajith NP and Ramachandran V. Ind J. Nat. Prod. Res. 2010; 1(2):249-253.
6. Paul JH, Seaforth CE, Tikasingh T. Fitoterapia. 2011; 2(3):302-8.
7. Saenz MT, Fernandez MA, Garcia MD. Phytotherapy Research, 1997; 11(5): 380-383.
8. Mekhora C, Muangnoi C, Chingsuwanrote P, Dawilai S, Svasti S, Chasri K, Tuntipopipat S. Asian. Pac. J Cancer Pre, 2012; 13: 653-664.
9. Singh S, Singh DR, Banu S, Salim KM., Proc. Natl. Aca. Sci, India - Sec B: Biol Sci. 2012;
10. Promkum C, Butryee C, Tuntipopipat S, Kupradinun P. As Pac J Cancer Prev, 2012; 13: 3343-334.
11. Forbes WM & Steglich C 2009 Methods of treating infectious diseases, Patent Application Number-20090047342.
12. Pino JA, Rosado A and Fuentes B. J. Ess.. Oil Res, 1997; 9: 467-468.
13. Wong KC, Feng MC, Sam TW and Tan GL. J Ess. Oil Res, 1994; 6: 369-374.
14. Faravani M. Bakar B. and Gholami B. J Appl Environ Bio Sci , 2011; (7) :115-125.
15. Meenambal M, Phugalendy K, Vasantharaja C, Prapakaran S, Vijayan P. Int J Chem Anal Sci, 2012,;3(6): 1446–1448
16. Muruganantham, M, G. Anbalagan N, Ramamurthy, Romanian J. Biophys, 2009; 19(4): 285–294.
17. Sofowara A. Medicinal plants and Traditional medicine in Africa. Spectrum Books Ltd, Ibadan, Nigeria. 1993; 289.
18. Trease GE, Evans WC. Pharmacognosy.13th edn. Bailliere Tindal Ltd, London. 1989; 176- 180.
19. Harborne, J. B. Phytochemical method. 3rd edn. Chapman and Hall, London. 1993; 135-203.
20. Cakmak G, Togan F, Severcan, Aqua Toxi, 2006; 77:53-63.
21. Bauer AW, Kirby WMM, Sherris JC, App Spect Rev, 2008; 43:134–179.
22. NCCLS (1997). Performance Standards for Antimicrobial Disk Susceptibility Tests: Approved Standard M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA, USA.
23. Okon J, Edet E, Esenowo G, Umoh N. Int J Mod Biol Med, 2013: 3(2): 69-77.
24. Praveena P, Sudarshanam D, Amudha. Int J Recent Scientific Res, 2012; 3(5): 382–383.
25. Komal kumar J, Deviprasad A G. .Romanian J. Biophys, 2011; 21(1): 63–71.
26. Chen RJ, Xu GY, Wang HY, Xu HZ, Liu YM, Chem Bul , 2001; 64 (6): 372–374.
27. Baseri MK, *B*aker S. Romanian J. Biophys, 2011; 21(4): 277–284.
28. Anand T, Gokulakrishnan K. J Pharm, 2012; 2(3): 520–524.
29. Deepashree CL, Komal Kumar J, Devi Prasad AG, Zarei M, Shuba G. Romanian J. Biophys, 2012; 22(3).
30. Xie, JX, Chang JB, Wang XM, Infrared Spectrum, Science Press, Beijing, 2001; 77: 212–309.
31. Sharma RK. Bioorg. Chem, 1993; 21: 49.
32. Kholkhal W, Ilias F, Bekhechi C, Bekkar AF. Cur. Res. J. Bio Sci. ; 2012 ;4(4): 437-443,
33. Alipour M, Khanmohammadi O. Af. J. Microbio. Res, 2011; 5(19): 2909-2911.
34. Figueiredo AC, Barroso JG, Pedro LG and Scheffer JJC. Flav. Frag. J., 2008; 23:213–226.