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Comparative fingerprint and extraction yield of *Diospyrus ferrea (willd.) Bakh*. root with phenol compounds (gallic acid), as determined by uv-vis and ft-ir spectroscopy

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ABSTRACT

Objective: To analyze the comparative finger print and extraction yield of *D.ferrea* root with phenol compound (Gallic acid), as determined by UV-Vis spectroscopy and FTIR spectroscopy. **Method:** The UV Vis spectroscopy and FTIR spectroscopy are adequate techniques to fingerprint comparatively and to evaluate the extraction yield of *D.ferrea* root extract. The higher extraction yield was recorded in ethanol comparatively superior and richer in phenol (gallic acid). Gallic acid has therapeutic application for inflammatory allergic diseases due to its ability to inhibit histamine. Finger print region was recorded between 500-3500 cm-1 for each extract and functional groups were identified and compared with the standard. **Result:** The extraction factor was superior in ethanol (270 nm) rich in polar molecules. The FTIR signal at 900, 1500, 1714, 3000, 3100cm-1 considered as a good indicator of phenol (gallic acid).The functional groups of each extract were identified.**Conclusion:** The UV and FTIR method was validated as a good tool to investigate the finger print and to predict the composition of different root extract of *D.ferrea*.

1. Introduction

The traditional herbal medicine since centuries has beneficial effects on health promotion, which are out of side effects when compared with synthetic drugs. The identification of phytochemical fingerprint by chromatography and spectroscopy provide effective information about qualitative and quantitative composition of herbal medicines and their pattern recognition by chemometry [1].The evaluation of a herbal product by metabolomic fingerprinting can be accomplished by appropriate methods, including HPLC with UV (DAD), ELSD, MS detection or GC-MS, HPTLC-densitometry, FT-MIR, NIR, NMR or a combination of these techniques[2-7] .The UV-Vis spectroscopy offers a simple, technique to identify the main phytochemicals, discriminating between the lipophilic and hydrophilic components in relation to the polarity of the solvent. Fourier Transform Infrared Spectroscopy is a high-resolution analytical tool to identify the chemical constituents and elucidate the structural compounds. FTIR offers a rapid and nondestructive investigation to fingerprint herbal extracts or powders. Therefore in our present study UV and FTIR techniques are employed to evaluate the amount of phenolic compounds (gallic acid) present the various root extracts of *Diospyrus ferreae*.

Diospyrus ferrea (Willd.) Bakh. is a small bonsai tree, belongs to the family Ebenaceae, grows in most areas of western and southern regions of Tamil Nadu as ornamental. They occur near the coastal region also in the Deccan plateau, dry evergreen forests of India. Leaf is alternate, entire, obovate and rounded at the apex. Flowers minute, unisexual, cup shaped with male and female flowers occurring in dense axillary cymes. Fruit is fleshy red in color, edible. The bark is thin, grey to black in color; wood is grey with dark streak[8]. The roots extract which is generally used as a folk medicine due to the presence of quinones, terpenoids and steroids. Of various kinds of natural antioxidants, flavonoids and phenolic compounds

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have received much attention. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated. In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant and anti inflammatory activity. Currently, the possible toxicity of synthetic antioxidants has been criticized. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.

Gallic acid is a bitter plant polyphenol found in a variety of foods and herbs (blueberries, walnuts, apples, flax seed, and tea). Gallic acid is effective against neuronal death, prevent cellular mutations and prove to be an effective in treatment of prostate, colon and lung cancer. Gallic acid when consumed helps those suffering from cancer to fight against the disease and prevent the damage of healthy cells. Gallic acid also has anti-viral, anti inflammatory, anti-fungal and anti oxidative properties[9]. Gallic acid has therapeutic applications for inflammatory allergic diseases, such as asthma, allergic rhinitis, sinusitis due to its ability to inhibit histamine release and the expression of proinflammatory cytokine[10]. Gallic acid as a remote astringent works to constrict tissue and help in the treatment of prolonged menstrual periods. When administered internally, is beneficial in the treatment of uterine, pulmonary, and nephritic hemorrhages, as well as all hemorrhages of a passive character.

Hence the present study aimed to compare the fingerprint of different root extracts of Diospyrus ferrea with gallic acid. The dependence of the extract composition on the solvent polarity (hexane chloroform, methanol, ethanol and water) was studied by UV-Vis spectrometry and Infrared (FT-IR) finger prints.

MATERIALS AND METHODS

Fresh roots of Diospyrus ferrea were collected from Southern Western Ghats, South India. A voucher specimen (FLOR 24. 144) was deposited in the Herbarium of Botanical Survey of India Coimbatore for authentication. Roots were collected in bulk, washed, shade dried, macerated and extracted with hexane, chloroform, methanol, ethanol and aqueous for 48 hrs sequentially in a Soxhlet assembly. The filtrates were then concentrated using rotary evaporator and stored at 4 ^{0}C prior to use.

UV-Vis spectra and calculation of extraction factors

The UV-Vis spectra was recorded (700-200 nm) for each extract (hexane chloroform, methanol, ethanol and aqueous) using a Jasco V 530 Spectropho-tometer. The maxima wavelengths and OD values were recorded for each extract. The absorption spectrum for the standard (gallic acid) was also recorded. The extraction yield of different solvent was calculated by extraction factor (EF). It was calculated by considering the ab-sorption values (Aλmax) recorded for each λmax, multiplied with the dilution factor (d). The formula applied was EF = A (λmax) x d. The results were expressed as mean values of five sam-ples in duplicates.

FT-IR measurements

The Fourier Transform Infrared spectrum (FTIR) of each extract was recorded in the IR region, from 4000 to 500 cm-1, and maximum of 27 scans were accumulated for each spectrum using the Horizontal Attenuated Total Reflection (HATR) device, using a Shimadzu Prestige 2 FTIR spectrometer (with apodization Happ-Genzel). The spectral data were processed using the IR solution Software Overview (Shimadzu) and Origin R 7SR1 Software. Total phenolics were deter-mined by FTIR method, either using the intensity of the peak at 1714 cm-1 or from the area of the region 950-1900 cm-1, 3000-3100 cm-1 considering the calibration curve with pure gallic acid (5 to 10 mg/ml ethanol).

RESULT

UV-Vis spectra

TABLE[1]

THE ABSORPTION MAXIMA λmax (NM) OF ROOT EXTRACT OF DIFFERENT SOLVENT FROM UV-VIS SPECTRA AND THE MEAN VALUES CALCULATED FOR EXTRACTION FACTOR-EF

Solvents	λ max(nm)	Extraction factor
Hexane	410	24±05
	370	60±12
	268	42±12
	215	0
Chloroform	274	32±05
	227	40±22
	219	0
Methanol	260	50±78
	227	49±67
Ethanol	271	99±45
	227	20±34
	370	45±21
Aqueous	272	0
	224	22±77
	244	31±34
Gallic acid	272	150±22
	220	23±11

The comparative UV-Vis spectra of the hexane, chloroform methanol, ethanol and aqueous were recorded, as well pure ethanol being considered a "reference" solvent known to extract phenolics from the plant. Based on their specific spectra, the absorption maxima [(λmax.] and the mean values of extraction factors [EF] were calculated for each solvent [Table 1]. To have an integrated image of the differences between extract, solvent type and concentrations of bioactive molecules extracted, the EF mean values at 270-290 nm for phenol acid derivatives, 317-340 nm for flavonoid derivatives for each extract were calculated [Figure 1].The absorption spectrum for standard gallic acid was also recorded. The maximum absorption spectrum was reported in ethanol extract[Figure1.5] compare to other extra ct.

It has been noticed that extraction factors in ethanol were superior to methanol, chloroform, hexane, especially for phenolic acids comparing to flavonoid and other derivatives. Based on the differences of polarity between the five solvents used EF values for ethanol extract was rich in polar molecules (gallic acid), while hexane and

chloroform extract had less amount of phenol content. More amounts of EF values in ethanol was an indication of polar active molecules where as hexane, chloroform, methanol, and aqueous extract had less amount of gallic acid which showed the reduced concentrations of phenol. [Figure1.1, 1.2, 1.3, 1.4, 1.6]. However, the ethanolic extract showed high absorptions at 270 and 275 nm, which attributed to higher con-centrations of phenol and poly phenolic compounds. For therapeutic reasons it has been considered that ethanol extracts rich in gallic acid can provide higher concentrations of bioactive molecules.

TABLE[2]

ABSORPTION PEAK AREAS OF DIFFERENT REGIONS OF FTIR SPECTRA RECORDED FOR THE ENTIRE FIVE ROOT EXTRACT OF DIOSPYRUS FERREAE.

Solvent	Peak intensity	Functional groups
Hexane	721.38	Benzene
	1029.99	Aliphatic amine
	1093.64	Aliphatic amine
	1180.44	Fluoroalkane
	1249.8 7	Aromatic ether
	1450.47	Phenol (GA)
	1637.56	Carbonyl
	1718.58	Ester
	2866.22	Carboxylic acid
	2922.16	Alkanes
	2937.59	C=CH2
	3221.12	Secondary amine
	3200	Phenol (GA)
Chloroform	596	Bromoalkane
	1033.85	Aliphatic
	1238.3	Ether
	1712.79	Ester
	2864.29	Alkanes
	3084.18	Phenol
	3398.57	Amine
	3410.15	Amine
Methanol	611.43	Unknown
	754	Aromatic Benzene
	1047.35	Aliphatic amine
	1217.08	Ether
	1620.21	Phenol(GA)
	1714.72	Carboxylic acid
	2945.32	Alkanes
	3369.64	Amines
	3402.45	Amines

FT-IR fingerprint

The FTIR spectrum was used to identify the functional groups of the active components based on the peak value in the region of infrared radiation. The FT-IR spectra (4000-500cm-1) of different root extract were registered and the specific wave numbers and intensities were considered. The FTIR spectrums of all the five extracts were obtained and the effective peaks were compared with that of standard [Figure2, Table2]. The FTIR spectrum of standard gallic acid contained 8 major peaks at the range of 1022.27, 1234.44, 1448.54, 1622.13, 1714.22, 3043.67, 3280.92, 3365.78cm-1 whereas the FTIR spectrum of ethanol was also recorded with the same number of peaks lying between 1714.72, 1022.56, 1622.35, 1714.72, 3043.11, 3280.18, 3365.86 cm-1 respectively. This finding clearly implies that more amount of gallic acid reported in ethanol extract when compared to other extract.

Solvent	Peak intensity	Functional groups
Ethanol	1041.56	Aliphatic amine
	1334.53	Unknown
	1448.75	Aromatics
	1535.55	Unknown
	1622.35	Phenol(GA)
	1714.72	Carboxylic acid
	2926.01	Alkyl
	2981.95	Alkanes
	3086.11	Phenol
	3138.18	Phenol
	3153.61	Phenol
	3211.48	Phenol
	3230.77	Phenol
	3251.98	Phenol
	3261.63	Phenol
	3277.06	Phenol
	3280.49	Phenol
	3344.57	Alkynes
	3365.93	Amine
	3417.86	Amine
	3439.08	Amine
	3475.73	Amine
Aqueous	503.42	Alkyl halides
	1060.85	Aliphatic amine
	1643.35	Alkanes
	2353.16	Amine
	2738.92	Amine
	2914.44	Alkanes
	3427.51	Amine
	3468.01	Amine
	3550.95	Amine
	3572.17	Amine
Gallic acid	694.37	Phenol (GA)
	1022.27	Phenol(GA)
	1234.44	Phenol(GA)
	1334.74	Unknown
	1448.54	Phenol(GA)
	1517.98	Unknown
	1535.34	Unknown
	1622.13	Phenol(GA)
	1714.22	Phenol(GA)
	2665.62	Unknown
	3043.67	Phenol(GA)
	3280.92	Phenol(GA)
	3365.78	Amine

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The five different root extract of Diospyrus ferrea was passed into FTIR and the functional components were separated based on its peak ratio. The hexane root extract confirms the presence of aliphatic amine fluoro alkanes,

ethers, esters, carboxylic acid, alkanes, secondary amine and phenolic compounds. The chloroform root extract confirms the presence of bromo alkane, aliphatic amine, fluoroalkane, ether, ester, carboxylic acid, alkanes, and phenol [Figure 2.3]. The methanol extract confirms the presence of aromatic benzene, aliphatic amine, ether, carboxylic acid, alkanes and phenol [Figure 2.5]. The ethanol root extract contain most of the phenolic phytoconstituents such as aliphatic amine, ether, carboxylic acid, alkyl, alkanes and most of the phenolic compounds [Figure 2.4]. The aqueous extract confirms the presence of alkyl halides, aliphatic amines, alkanes, amines and absence of phenol compounds [Figure 2.5].

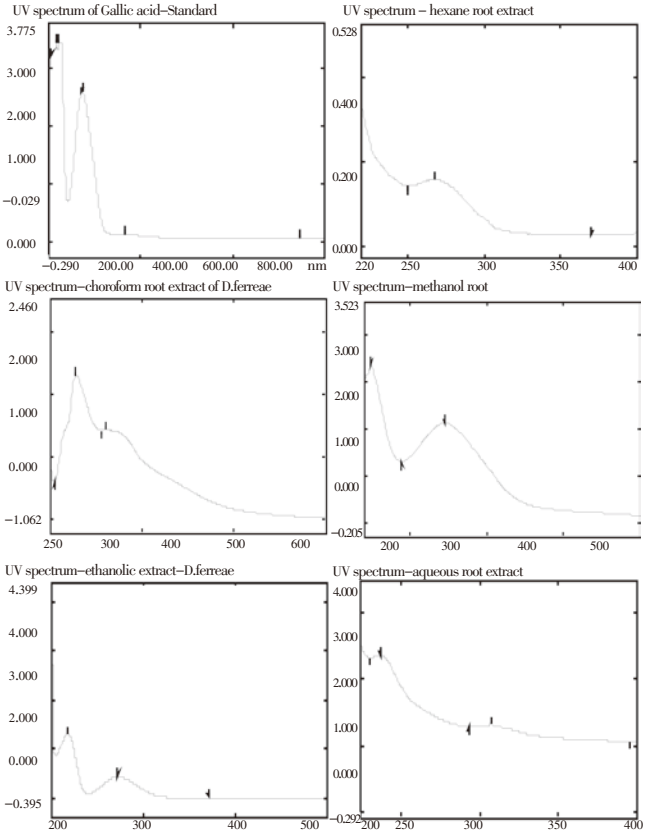

Figure 1. Comparative UV spectrum of different type of root extracts of D. ferreae

The functional groups identification was based on the FTIR peaks attributed to stretching and bending vibrations. The peak areas [Figure 2] were identified in the IR domain and the fingerprint region was localized between 900 and 1500 cm−1. Area 1 [< 1000 cm−1] corresponds to C–H bending vibrations from isoprenoids, area 2 [997–1130 cm−1] to stretching vibrations C–O of mono–, oligo– and carbohydrates, with signals at 1030, 1054, 1104, and 1130 cm−1, while area 3 [1150–1270 cm−1] corresponds to stretching vibrations of carbonyl C–O or O–H bendings. Area 4 [1300–1450 cm−1] correspond to stretching vibrations C–O (amide) and C–C stretchings from phenyl groups, while area 5 [1500–1600 cm−1] to aromatic domain and N–H bending vibrations. Area 6 is a complex one [1600–1760 cm−1], corresponding to bending vibrations N–H [amino acids], C=O stretchings [aldehydes and acetones, esters] as well to free fatty acids [1710 cm−1] and glycerides [1740 cm−1]. Area 7 [2800–2900 cm−1], corresponds to C–H stretching vibrations specific to CH3 and CH2 from lipids, methoxy derivatives, C–H [aldehydes], including cis double bonds. Area 8 [3350–3600 cm−1] corresponds to stretching vibrations of OH groups (from water, alcohols, phenols, carbohydrates, peroxides) as well from amides [3650 cm−1][10–12].

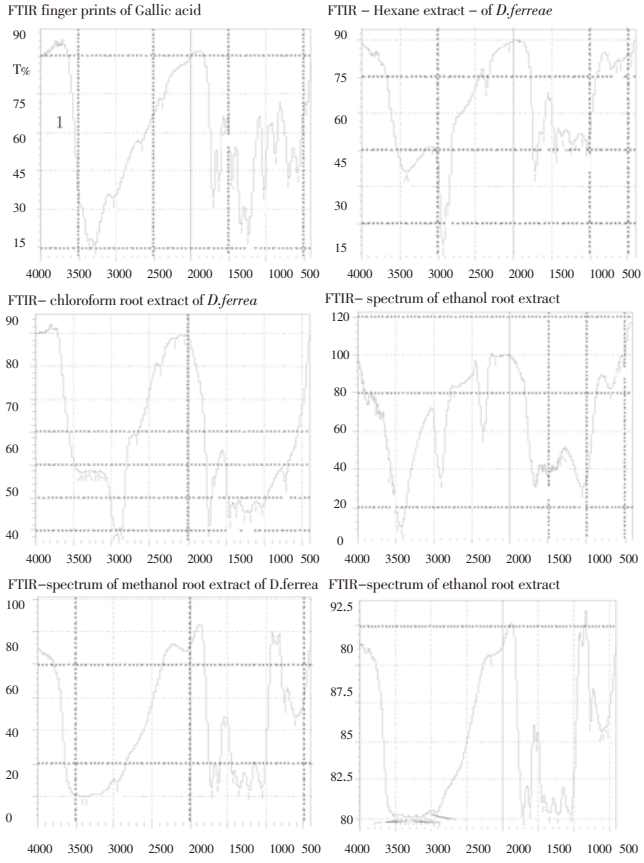

Figure 2. Comparative FTIR spectrum of different type of root extracts of D .ferrea

The bands in the 3400–3500 cm−1 region are assigned to different OH functional groups (from carboxyl or phenols) and those between 2800 and 2980 cm−1 belong to stretching vibrations of the aliphatic and aromatic –CH [Table 2]. The peaks at 1707 cm−1 and 1246 cm−1 indicate the presence of carboxylic groups. The aromatic character of the compound is confirmed by the absorption bands at 1600–1620 cm−1, along with the intense absorption at 864–868 cm−1. The bands between 1200 and 1270 cm−1 represent the C–O deformation vibrations of phenols and carboxyl group[13]. In alcoholic extracts there are absorption peaks in the domain 1300–1800 cm−1, more than in hexane, e.g. at 1558, 1517 and1467 cm−1 , as well in the region 1380–1450 cm−1. Carbonyl groups have specific signals at 1743 cm−1. The region 1714 cm−1 specific to phenols has been noticed higher peak areas in ethanol, corresponding to the result of UV–spectra. In the other IR regions no significant differences between the five solvents extracts were noticed. The ethanol extract was significantly more charged in molecules than that of hexane chloroform methanol and aqueous extracts. The results of the present study confirmed that Diospyrus ferrae is a wealthy resource of phyto–constituents (Gallic acid) which can be isolated and examined for bio–efficacies and pharmacological activities.

DISCUSSIONS

Many workers applied the FTIR spectrum as a tool for differentiating, classifying and discriminating closely related plants and other organisms. Similar work was also reported by Ragupathi Raja Kannan [2011] in IR spectra from the mid-infrared region (4000-400 cm-1) for aqueous methanol extract of sea grasses. Samples of six sea grasses in the region of polyphenols showed slight variation in bands than the standards. Noticeably the presence of wavelength numbers of FTIR spectra of Gallic acid at 669, 763, 1025, 1100 and 1654 cm-1, Tannic acid at 669, 860, 1172, 1511 and 1627 and p-Coumaric acid at 669, 1124, 1171, 1508 and 1638 cm-1 were also observed in all sea grasses analyzed. Wavelength FTIR spectra corresponding for Vanillin was 668, 1498, 1534, 1617, 1654 and 3392 cm-1, among them 668 cm-1 is present in all the sea grass and 1498, 1534, 1617 and 1654 cm-1 were present in *H. pinifolia* only[14]. This is the first study that proves FTIR technique is an efficient tool for measuring polyphenols in sea grasses. Spectral differences are the objective reflection of componential differences. By using the macroscopic fingerprint characters of FT-IR spectrum, we can judge the origin of different extracts accurately and effectively, trace the constituents in the extracts, identify the medicinal materials true or false and even evaluate the qualities of medicinal materials[15]. So, FT-IR spectrum reflecting objectively the panorama of chemical constituents in complex system. It is a most credible method to validate, identify the mix-substance systems such as traditional medicine and herbal medicine[16,17].Therefore, the present work on Diospyrus ferrae displayed novel phytochemical markers as useful analytical tool to check not only the quality of the extract but also to identify the medicinally important plant. Further advanced spectroscopic studies are required for the structural elucidation and identification of specific phenol compounds.

CONCLUSION

The data presented in this study showed that UV-Vis spectrometry and FT-IR spectroscopy are adequate techniques to fingerprint comparatively and to evaluate the extraction yield of medicinal herbs with anti oxidant and anti inflammatory potential. UV spectrometry revealed that, the extraction yields were superior in ethanol when compared to other extract. Increased in phenol acids (gallic acid) were reported in ethanol extract compare to other derivatives. Based on the differences of polarity between the five solvents used, higher extraction yields were obtained for ethanol extract, more rich in phenol acids (gallic acid) than other derivatives. Fingerprint region between 900, 1500 and 3000 cm-1 was located using FTIR and the specific functional groups were identified. The ethanol FTIR data was correlated and further validated with the detailed HPTLC analysis and the amount of gallic acid content in ethanolic root extract was determined. Thus FTIR method was validated as a good tool to in-vestigate and to predict the phenolic composition of *Diospyrus ferreae* a potent Indian medicinal plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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