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Comparative fingerprint and extraction yield of *Prosopis cineraria (Lin.)* Druce. Leaves with phenolic compounds (Gallic acid) & flavonoids (Rutin)

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ABSTRACT

The main objective of this study is to analyse the extraction yield of *Prosopis cineraria* leaf with respect to phenolic compounds (Gallic acid) and flavonoids (Rutin). UV and FTIR spectroscopic methods were employed for qualitative and quantitative analysis of phenolic compounds (gallic acid) and flavonoids (rutin) in the leaf extract of *Prosopis cineraria*. The extraction yield of methanolic extract was found to be superior. The FTIR signals at 675-600, 1225–950, 1540-1870, and 3500–3200 cm^{-1}, are the indicator of presence of phenol (Gallic acid) & flavonoid (Rutin). These signals were used to identify the presence of the functional groups in the extract. This study concludes that data obtained from the UV & FTIR spectroscopy is enough to fingerprint & evaluate extraction yield of *Prosopis cineraria*.

Keywords: UV spectroscopy, FTIR spectroscopy, Methanolic extract, Rutin, Gallic acid, *Prosopis cineraria*.

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INTRODUCTION

Globally the demand is increasing for plant based medicines, pharmaceuticals, tonics, cosmetics and other products. India along with China is the leading country in the world for production, consumption and export of herbal products as well as raw materials. Herbals products are derived from whole plant or plant parts and are used to prevent, relieve and treat illness. Since antiquity they are valued for medicinal, aromatic, nutritional and even rejuvenating qualities, herbs have played crucial role in maintaining the well-being of human body, and for alleviating human suffering across civilizations.[1]

The traditional plant based drugs are useful for health promotion, and have lesser or no side effects as compared to chemically synthesized drugs. The analysis of a these herbal drugs can be accomplished by many techniques including GC, HPTLC, HPLC, ELSD, MS, FT-NIR, NIR, NMR or hyphenation of these techniques. The spectroscopic method like UV-Visible offers an easy technique to identify the main phytochemical, discriminating between the hydrophilic and lipophilic components according to the solvents polarities. FTIR Spectroscopy is highly sophisticated analytical tool to reveal the chemical constituents and for elucidation of the structures of compounds. FTIR is the non-destructive investigating tool which offers a rapid fingerprinting of herbal extracts.[2-7]

The plant *Prosopis cineraria* (Khejri) is a flowering tree of the leguminous family *Fabaceae.* It is a small to medium size thorny, irregularly branched flowering tree, found widely in the Thar desert of Rajasthan, India and plays a vital role in preserving the ecosystem.[8]

It is one of the most important natural resource of arid regions of India because of its economic values (fuel, fodder), ecological role in preventing soil erosion. *Prosopis cineraria* have also been used in indigenous system of medicine as a folk medicine for various ailments. Its bark is dry, acrid, bitter, with sharp cooling taste, it is used as anthelmintic, tonic, cures leprosy, dysentery, asthma, leucoderma, piles, tremors of the muscle and wandering of the mind. The flowers are grounded, mixed with sugar and it is used as safeguard against miscarriage during pregnancy. The ash of bark is rubbed over the skin to remove hair. The smoke of the leaves is good for eye troubles. Its fresh leaves juice mixed with lemon juice is used for dyspepsia; extract of crushed pods is used for earache, toothache, pain relief from fractured bones. Aqueous extracts of bark and leaves are applied externally to treat skin diseases, disinfect wounds and promotes healing.[9-10] *Prosopis cineraria* is mostly used

as a folk medicine due to the presence of numerous phytoconstituents, like alkaloids (Spicigerine, Prosophylline), steroids (Campesterol, Stigmasterol, Sitosterol), tannins, phenolics compound (Gallic acid), flavone derivatives (Prosogerin A, B, C, D, and E), etc in it . 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. Synthetic antioxidants have toxicity, so interest in natural antioxidant especially from plant originated has greatly increased in recent years.[10]

MATERIALS AND METHODS

Fresh leaves of *Prosopis cineraria* were collected in month of November from Artiya Khurd village of Jodhpur, Rajasthan. A voucher specimen (JJ No. 847889) was submitted in the Herbarium of Botanical Survey of India, Jodhpur for authentication. BSI issued a certificate of authentication with reference number. BSI/AZRC/1.12014/Tech/2016-17-(Pl. Id)/1062. Leaves were collected in bulk, washed, shade dried, coarsely powdered and extracted with petroleum ether, chloroform, ethyl acetate, methanol, and water for 48 hrs sequentially in a soxhlet assembly. The filtrates were then concentrated using rotary evaporator (*Heidolph instrument GmbH & Co.kg, Germany*) and stored at 4^0C prior to use.

UV-Spectra and Calculation of Extraction Factor

The UV spectrum were recorded (400—200 nm) for extracts i.e. Petroleum ether, chloroform, ethyl-acetate, methanol, and water using UV-1800 Shimadzu Spectrophotometer. The maxima wavelengths and absorbance values were recorded for each extract. The absorption spectrums for the standards (Gallic acid & Rutin) were also recorded. The extraction yield in different solvents was calculated by extraction factor (EF). It was calculated by considering the absorption values (A λmax) recorded, multiplied with the dilution factor (d).

The formula applied was $EF = A(\lambda max)d$.

The results are expressed as mean values of samples in duplicate.

Considering the calibration curve with pure gallic acid (5 to 35 µg/ml) & rutin (10 to 50 µg/ml) in methanol

FT-IR measurements

The FTIR spectra was recorded with for each extracts, in-between 4000 cm^{-1} to 500 cm^{-1}, and maximum of 24 scans were accumulated for each spectrum using the Eco-ATR single reflection ATR module equipped ZnSe , a ALPHA FTIR Spectrometer (Bruker Optik GmbH, Germany). The spectral data were processed using the OPUS 7.5 build 7, 5, 18/ DB 7, 5 18, 424 Software. Total phenols & flavonoids were determined by FTIR method, using the intensity of peak at 1714 cm^{-1} & from the area of the region 1225–950cm^{-1}, 1540-1870 cm^{-1}, 3400–3200 cm^{-1}.

RESULT

UV Spectra

The UV spectrums of the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts were recorded and compared, methanol was taken as standard solvent to extract phenols from the plants. Based on their spectra, the mean values of extraction factors [EF] were calculated for each solvent absorption maxima (λmax). To have an integrated image of the differences between extract, solvent type and concentrations of bioactive molecules extracted, the EF mean values at 240-270 nm for phenol acid derivatives, 320-370 nm for flavonoid derivatives for each extract were calculated. The absorption spectrum for standard Gallic acid and rutin were also recorded. The maximum absorption was recorded in methanolic extract as compared to other extract. The extraction factor of methanol has been noticed superior to petroleum ether, chloroform, ethyl acetate & aqueous extracts, for phenols compounds & flavonoids.

Based on the differences in polarity between the five solvents used, EF values for methanolic extract was found rich in polar molecules (Gallic acid, Rutin), while petroleum ether and chloroform extracts had less amount of phenols & flavonoids. High EF values in methanol were indication of polar active molecules whereas petroleum ether, chloroform, ethyl-acetate and aqueous extract had less amount of galllic acid & rutin which showed the less concentrations of phenols & flavonoids. However, the methanolic extract showed high absorptions at 266.2, 344.4, 346.4nm wavelength, which is attributed due to high concentrations of phenol, flavonoids and poly-phenolic compounds. Methanolic extract was found rich in gallic acid & rutin. This indicates the presence of higher concentration of antimicrobial bioactive molecules in Methanolic extract.(Table 1.)

Table 1. The absorption maxima λmax (nm) of leaves extracts in different solvent from UV spectra and the mean values calculated for extraction factor EF

Solvent	λmax	Abs	Extraction factor
Petroleum ether	269.4	0.207	20.7
	264.2	0.208	20.8
	244.6	1.4	140
	240.2	1.479	147.9
Chloroform	342.4	0.11	2.2
	338.2	0.109	2.18
	331.8	0.112	2.24
	324.2	0.112	2.24
	274.8	0.104	2.08
	241.4	0.129	2.58
Ethyl acetate	321	0.825	82.5
	267.8	0.944	94.4
Methanol	266.2	2.015	403
	346.4	1.51	302
	344.4	1.516	303.2
Water	264.2	0.804	80.4
	254	0.782	78.2

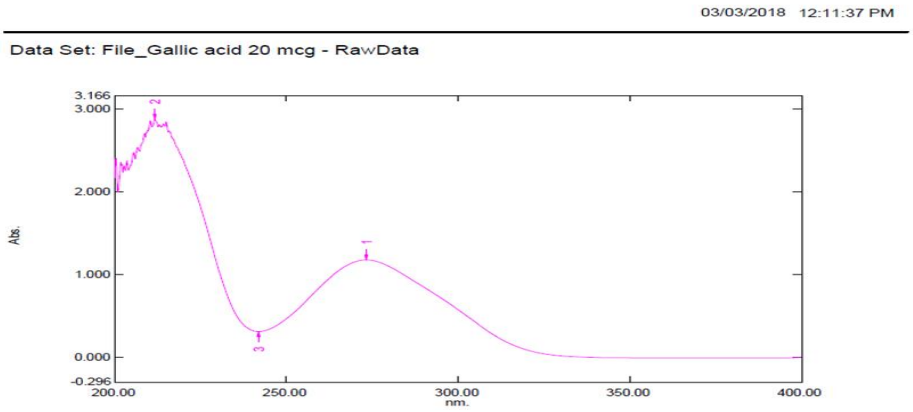

Figure 1.1 UV Spectrum of Gallic acid

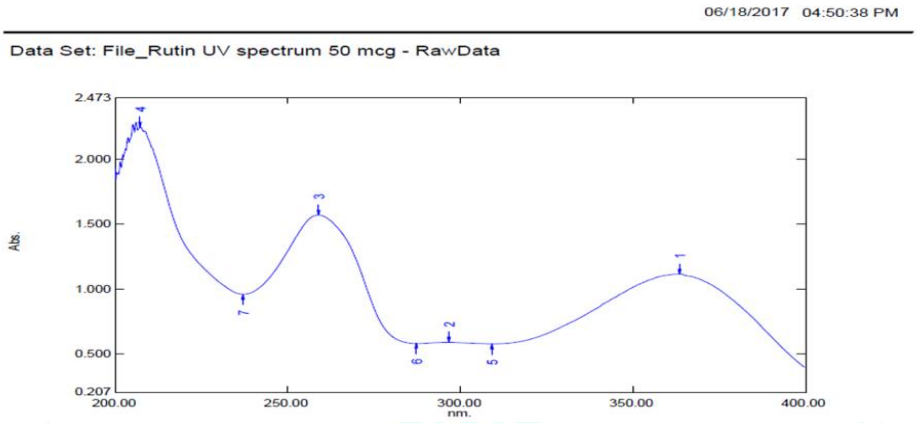

Fig. 1.2 UV spectrum standard rutin

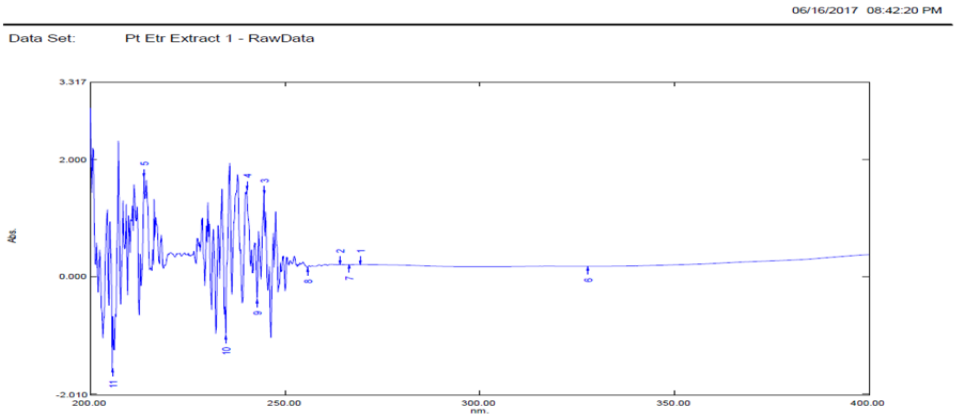

Fig. 1.3 UV spectrum petroleum ether extract

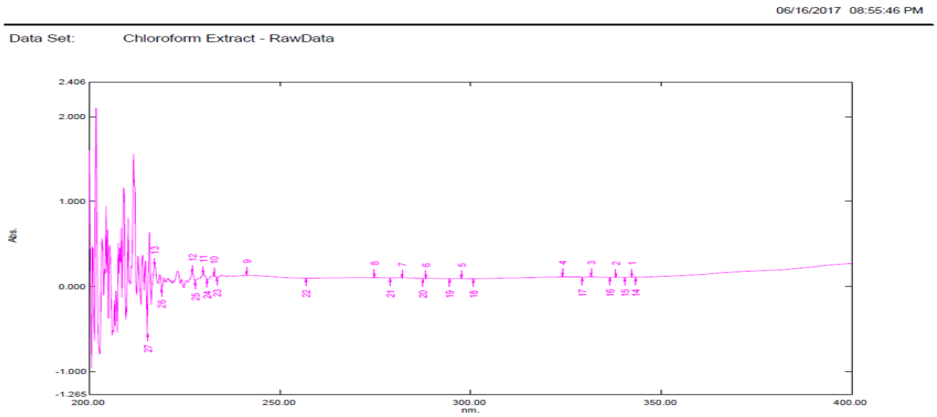

Fig. 1.4 UV spectrum chloroform extract

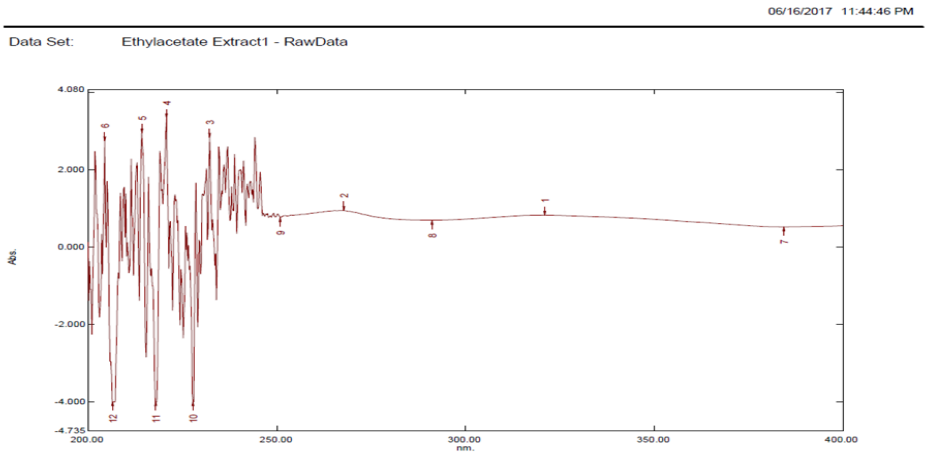

Fig. 1.5 UV spectrum ethyl acetate extract

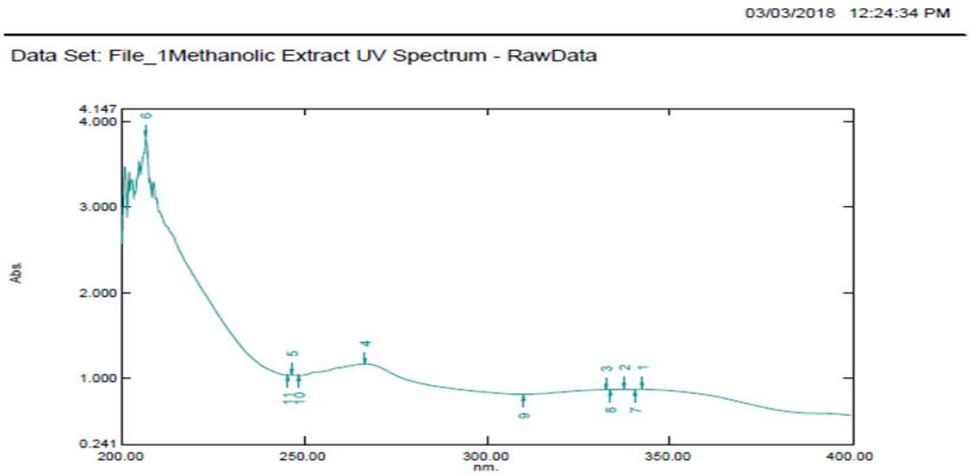

Fig. 1.6 UV spectrum methanolic extract

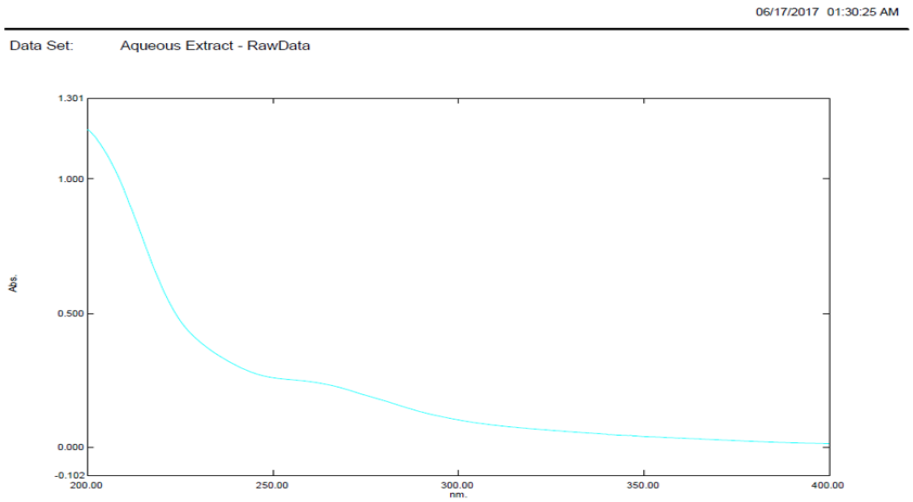

Fig. 1.7 UV spectrum aqueous extract

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 FTIR spectrums in between 4000- 500cm^{-1} for all the extracts were obtained and the effective peaks were compared with standard gallic acid and rutin spectrums. The FTIR spectrum of standard gallic acid contained 12 major peaks at 698.17, 1020.57, 1252.79, 1379.55, 1426.74, 1608.98, 1697.22, 2987.59, 3276.52 3490.84 cm-l and rutin contained 8 major peaks at 728.04, 1008.35, 1062.8, 1502.5, 1599.24, 1654.57, 2878.39, 3344.08 cm^{-1}. In the FTIR spectrum of methanolic extract we observed peaks at 876.33, 1043.87, 1086.10, 1268, 1382.08, 1650.49, 2888.91, 3346.69 cm^{-1}. (Table 2.) This finding clearly indicates the presence of more amount of phenolic compounds and flavonoids in methanolic extract, as

compared to other extracts. The resuts of FTIR spectrums of leaf extracts of *Prosopis cineraria* indicate that the petroleum ether leaf extract confirms the presence of aromatic compounds, aliphatic amine, nitriles, ethers, esters, carboxylic acid, alkanes and phenolic compounds [Figure. 2.3]. The chloroform leaf extract confirms the presence of aromatic compounds, phenol, ether, and saturated alkanes [Figure 2.4]. The ethyl acetate leaf extract confirms the presence of aromatic compounds, carboxylic acids, ethers and phenols [2.5]. The methanolic extract confirms the presence of aromatic compounds, ether, carboxylic acid and phenol [Figure 2.6]. The aqueous extract confirms the presence of nitriles, alkanes, ether, aliphatic amines, carboxylic acid and phenolic compounds. [Figure 2.7].

Table 2 Absorption peak areas of different regions of FTIR spectra recorded for the entire leaf extracts of *Prosopis cineraria*

Extracts	Peak intensity	Functional groups
Petroleum ether	668	Aromatic ring C-H stretching poly nuclear ring
	844.57	Aromatic ring C-H bend out of plane
	953.3	Aromatic ring C-H bend in plane
	1132.94	Aromatic ring C-H bend in plane/ether/Aliphatic amine
	1359.27	Phenol C-O stretching /Methyl C-H Asym./sym. Bend ((−CH3))
	1512.71	Methyl C-H Asym./sym. Bend (−CH3)
	1602.29	Aromatic ring stretch (C=C-C)
	1798.09	Aromatic compound with different substitution (carboxylic acid)
	1987.66	Aromatic compound with different substitution
	2142.96	Alkynes (C≡C Stretch)
	2309.88	Nitril/Alkynes (C≡C Stretch or C≡N Stretch)
	2382.45	Nitril/Alkynes (C≡C Stretch or C≡N Stretch)
	2877.69	Saturated aliphatic (Alkane/alkyl) group -Methylene (CH2) C-H asym./sym. Stretch
	2942.70	Saturated aliphatic (Alkane/alkyl) group -Methylene (CH2) C-H asym./sym. Stretch
	3565.3	amine (Aliphatic primary amine N-H stretch)
Chloroform	669.04	Phenol (GA)/Aromatics ring (C-H stretching in Polynuclear ring)
	744.79	Aromatics ring -Methylene -(CH2) n- rocking (n≥3)
	1214.23	Aromatics ring Phenol /ether , C-O stretch
	2921.69	Saturated aliphatic (Alkane/alkyl) group
	3084.18	Aromatic C-H stretch
Ethyl Acetate	756.73	Aromatics ring -Methylene -(CH2) n- rocking (n≥3)
	1043.45	Aromatic C-H in-plane bend (several)
	1232.68	Phenol (C-O stretch)
	1371.36	Phenol (O-H Bending)
	1736.30	Carboxylic acid/ Phenol(GA)
	2984.83	Carboxylic acid /Methyl (−CH3) C-H
Methanol	876.33	Phenol (GA)/Aromatic C-H out-of-plane bend(several)
	1043.87	Aromatic C-H in-plane bend (several)
	1086.10	Ether/ Aromatic C-H in-plane bend (several)
	1268	Phenol (C-O stretch)
	1382.08	Phenol (O-H Bending)
	1650.49	Carboxylic acid/ Phenol - Carbonyl C=O Strech
	2888.91	Alkyl group/ Carboxylic acid (Methyl (−CH3) C-H)
	3346.69	Phenol (Normal "polymeric" OH stretch)
Aqueous	768.17	Aromatics ring -Methylene -(CH2) n- rocking (n≥3)
	1038.64	Aromatic C-H in-plane bend (several)
	1109.95	Aromatic ring C-H bend in plane/ether/Aliphatic amine

	1214.60	Aromatics ring Phenol /ether , C-O stretch
	1331.94	Phenol (O-H Bending)
	1551.42	Aromatic ring stretch (C=C-C)
	1612.80	Carboxylic acid/ Phenol - Carbonyl C=O Strech
	2311.71	Nitril/Alkynes (C≡C Stretch or C≡N Stretch)
	2888.45	Alkyl group/ Carboxylic acid (Methyl (−CH3) C-H)
	3118.82	Aromatic C-H stretch
	3497.38	Phenol (Dimeric OH stretch)
	3746.73	Phenol/Alcohol (Internally O-H stretch)
Gallic acid	698.17	Phenol (GA) Aromatic C-H out-of-plane bend(several)
	1020.57	Phenol(GA)Aromatic C-H in-plane bend (several)
	1200.75	Phenol(GA) C-O stretch
	1252.79	Phenol(GA)
	1309.74	Phenol(GA)
	1379.55	Phenol(GA)Phenol or tertiary alcohol, O-H Bending
	1426.74	Phenol(GA)Methyl C-H Asym./sym. Bend ((−CH3)),
	1608.98	Aromatic ring stretch (C=C-C)
	1697.22	Phenol(GA)/Carbonyl C=O Strech
	2987.59	Carboxylic acid (Methyl (−CH3) C-H asym./sym. Stretch)
	3276.52	Phenol(GA) Normal "polymeric" OH stretch
	3490.84	Phenol(GA) Normal "polymeric" OH stretch
Rutin	728.04	Aromatics ring
	1008.35	Phenol(GA)
	1062.8	Ether/ Aromatics ring
	1502.5	Aromatic ring C=C-C stretching
	1599.24	Aromatics ring /Carbonyl
	1654.57	Aromatics ring /Carbonyl
	2878.39	Alkyl group/ Carboxylic acid (Methyl (−CH3) C-H)
	3344.08	Phenol (Normal "polymeric" OH stretch)

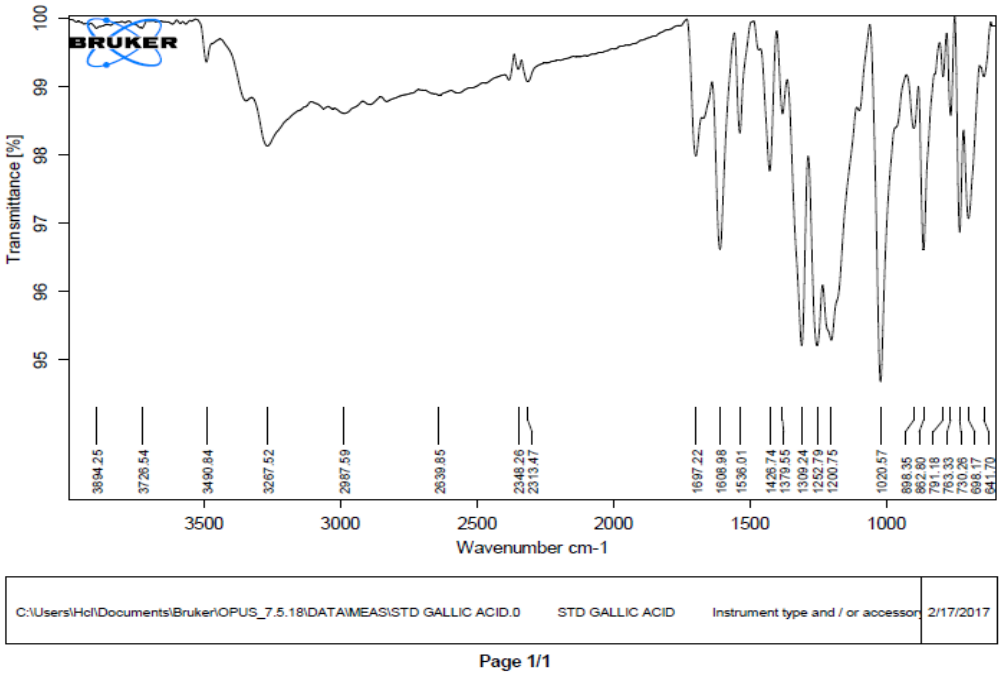

Fig. 2.1 FTIR spectrum of standard gallic acid

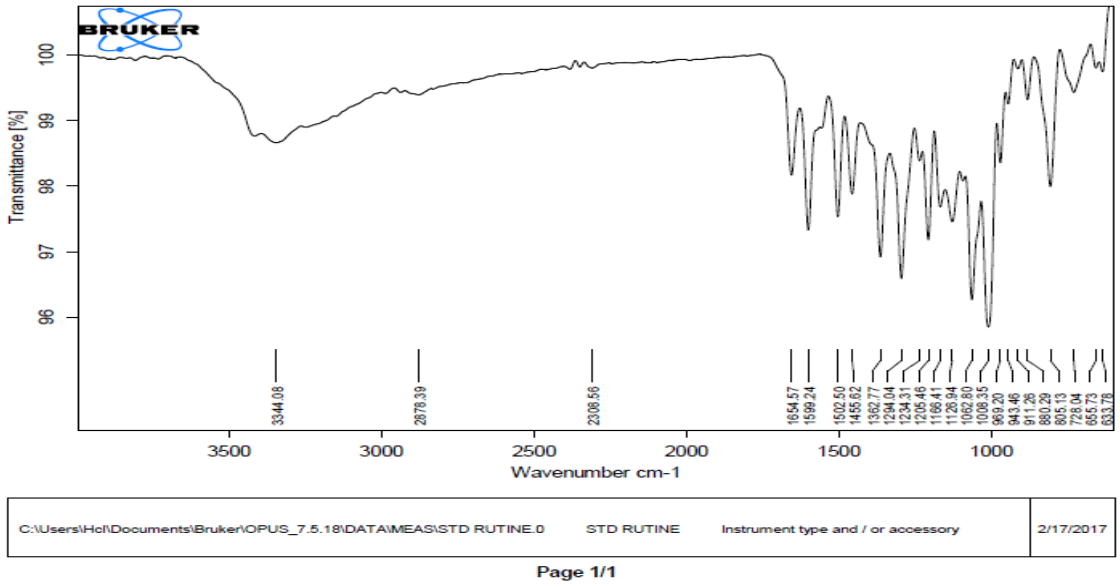

Fig. 2.2 FTIR spectrum of standard rutin

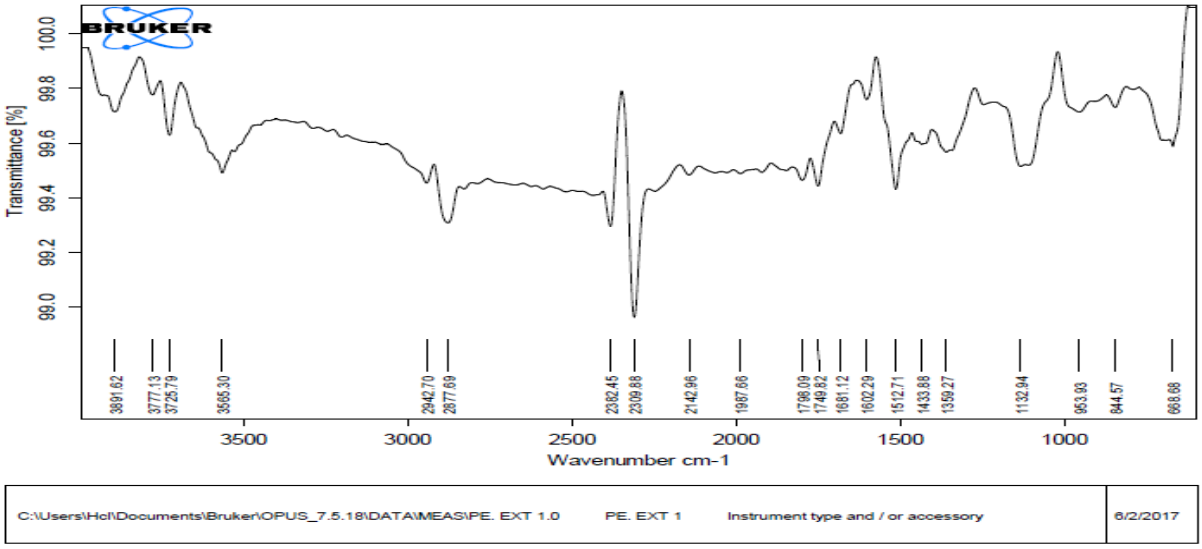

Fig. 2.3 FTIR spectrum of petroleum ether extract

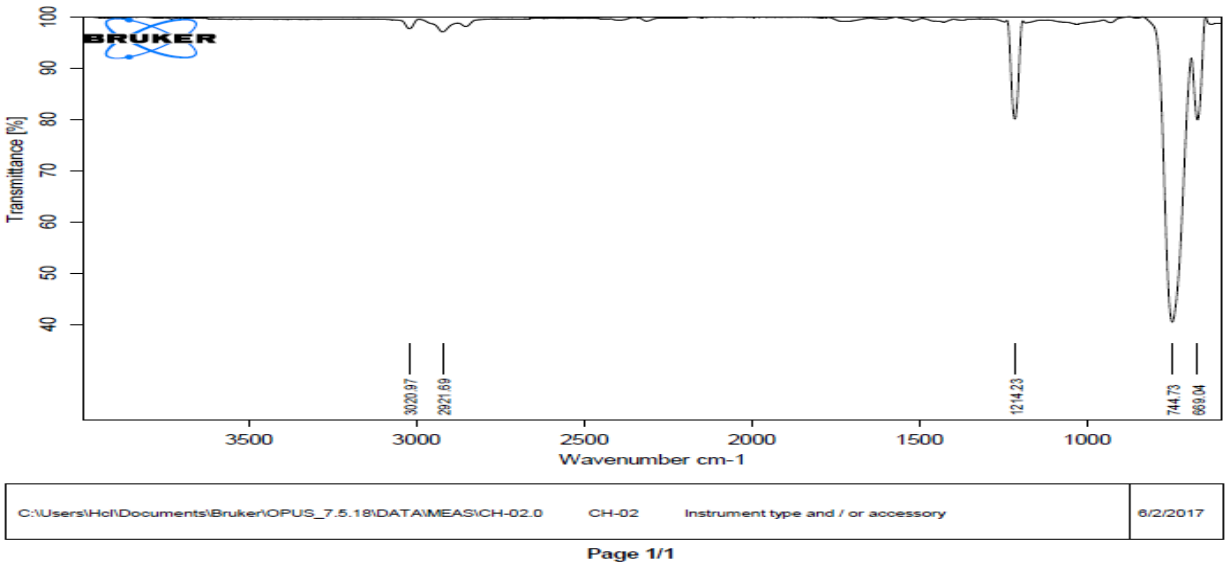

Fig. 2.4 FTIR spectrum of chloroform extract

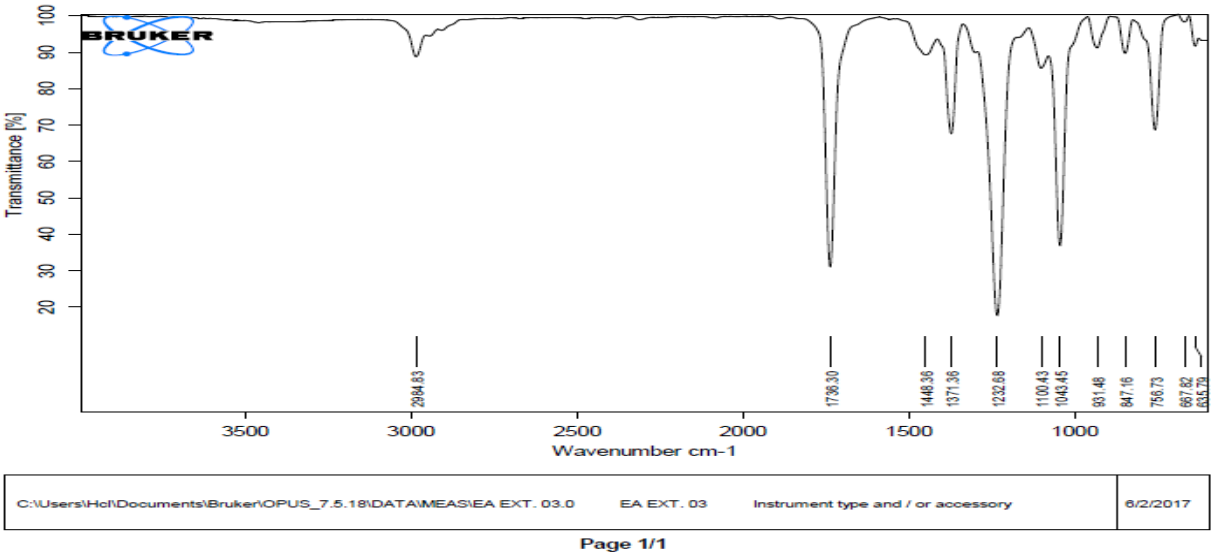

Fig. 2.5 FTIR spectrum of ethyl acetate extract

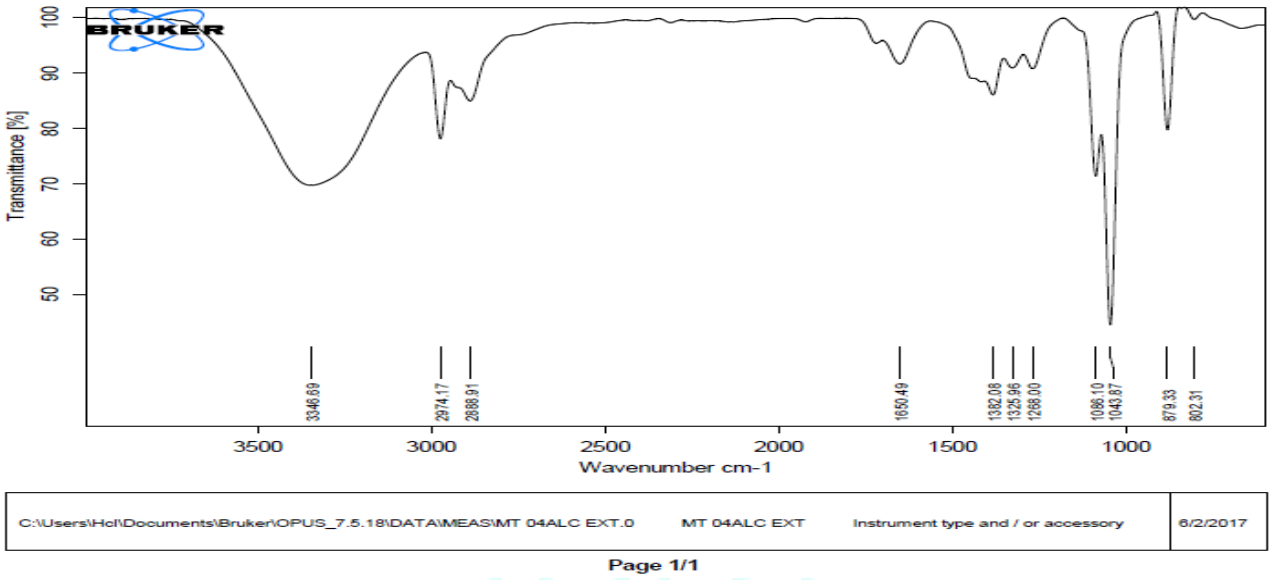

Fig. 2.6 FTIR spectrum of methanolic extract

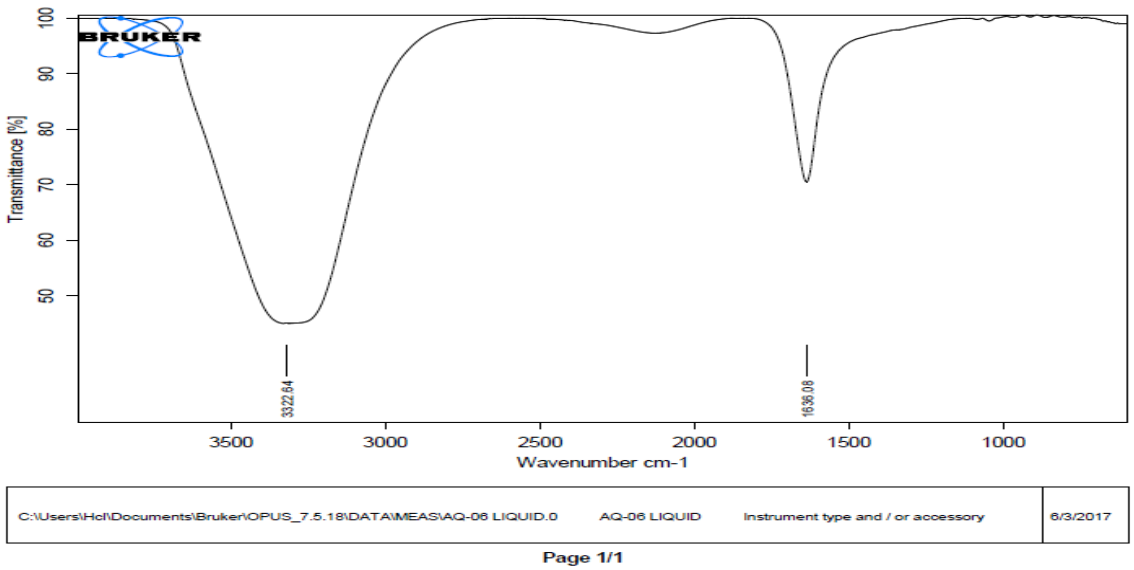

Fig. 2.7 FTIR spectrum of aqueous extract

DISCUSSIONS

The FTIR spectrum is a tool for differentiating, classifying and discriminating closely related plants and other organisms. Noticeably the presence of wavelength numbers, in FTIR spectral peaks of gallic acid (698, 1020, 1252, 1379, 1608, 1697, 2987 and 3490 cm^{-1}), and spectral peaks of rutin (728, 1062, 1599, 1654, 2878 and 3344 cm^{-1}) were analysed in *Prosopis cineraria*. This proves that FTIR technique is an efficient tool for measuring polyphenols in natural products. Spectral differences are the indication of compositional differences of extracts. By FTIR spectrum, we can determine the composition of different extracts accurately and effectively, trace the constituents in the extracts, Can identify medicinal materials correctly and even can evaluate the qualities of medicinal materials. So, FTIR spectrum reflecting objectively the panorama of chemical constituents in complex system. It is a most credible method to validate, identify the mixed substance systems such as traditional medicine and herbal medicine. Therefore, the present work on *Prosopis cineraria* 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

This study concludes that data obtained from the UV spectrometry & FTIR spectroscopies are enough to fingerprint & evaluate extraction yield of *Prosopis cineraria*. UV spectrometry revealed that, the extraction yields were superior in methanol when compared to other solvents. Based on the differences in polarity between the five solvents used, higher extraction yields were obtained in methanol, it found more rich in phenolic compounds (Gallic acid) & flavonoids (Rutin) than other derivatives. Fingerprint region between 900-4000 cm^{-1} were located using FTIR and the specific functional groups were identified. Thus FTIR and UV methods were validated as good techniques to investigate and to determine yield of the phenols and flavonoids composition in *Prosopis cineraria*.

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