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PHYTOCHEMICAL PROFILING OF OXALIS CORNICULATA LEAF EXTRACT

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ABSTRACT Elucidation of the phytochemical composition of plant extract is an essential step in developing novel therapeutic agents from medicinal plants. Mass spectrometry, coupled with chromatographic separations (GC/MS), is increasingly applied to analyze plant extracts as this technique has proved to be a valuable method for the analysis of nonpolar volatile components and alkaloids. Fourier transform infrared (FTIR) is being used for the identification of characteristic functional groups present in the plant extract. The absorption spectrum provides information about the structure of the molecules present in the phytoextract. FTIR analysis revealed the presence of phenols, alkane, alkene, carboxylic acid, aromatic compounds, alcohol, and bromo alkane compounds in methanolic leaf extract of *Oxalis corniculata and* GC/MS analysis provide different peaks determining the presence of twenty three phytochemical compounds.

KEYWORDS : Phytochemicals, Oxalis corniculata

INTRODUCTION

The plant *Oxalis corniculata* Linn. belonging to the family Oxalidaceae is commonly known as creeping wood sorrel, is a delicate-appearing, low growing, herbaceous medicinally important plant with abundant distribution in damp shady places, roadsides, plantations and lawns. Various traditional medicinal systems use this plant for the treatment of varied ailments of humans. *Oxalis corniculata* has hypoglycemic, antihypertensive, antipsychotic, nervous system stimulant, chronotropic, and inotropic effects [Achola *et al*., 1995; Raghavendra *et al*., 2006]. Antioxidant and toxicity evaluation of different extracts of *Oxalis corniculat*a was also investigated [Alam *et al*., 2011]. The plant also has antifungal and antibacterial activities [Satish *et al*., 2008]. The plant offers protective effects against nephrotoxicity in CCl_4 induced rats [Khan and Zehra, 2013] and also offers protection against lung injuries induced by CCl_4 [Ahmad *et al*., 2015]. Results of the *in vitro* and *in vivo* studies using aqueous extract of *Oxalis corniculata* indicate that, by virtue of its potent antioxidant property, it can effectively scavenge free radicals and thereby reduce the risk of isoproterenol-induced oxidative stress and myocardial infarction in rats [Abhilash *et al*., 2011]. Research studies carried out on the plant so far have also been reviewed [Badwaik *et al*., 2011; Kathiriya *et al*., 2010], highlighting its diverse medicinal applications.

In view of extensive ethnomedicinal applications of the edible folkloric medicinal plant *Oxalis corniculata*, the study was undertaken to identify the flavonoids and volatile phytochemicals present in the flavonoid rich fraction of *Oxalis corniculata* leaf extract.

Infrared spectroscopy (IR) has the potential to provide biochemical information without disturbing the biological sample. Fourier transform infrared spectroscopy [FTIR], due to its high precision in absorbance and wavenumber measurements, use infrared spectra for the identification of biomolecules [Pillai and Nair, 2014]. It measures the vibrations of bonds predominantly within chemical functional groups and generates a spectrum that can be regarded as a biochemical or metabolic "fingerprint" of the sample. By FTIR, it is possible to identify concrete structures of plant secondary metabolites and offers a rapid and non-destructive investigation to fingerprint herbal extracts or powders [Yang and Yen, 2002 and Ivanova and Singh, 2003].

Gas Chromatography-Mass Spectroscopy [GC-MS] is a very compatible technique used to screen active components from medicinal plants that have efficient protection and treatment role against various diseases and is most commonly used for identification and quantification purpose [Muthuraj *et al*., 2015]. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra [Hites, 1997].

MATERIALS AND METHODS

The shade dried leaves (1 kg) of *Oxalis corniculata* were refluxed in 80% methanol at 65 °C for 24 h. The supernatant was collected and the extraction was repeated twice. The extract was decanted, filtered and concentrated to remove the solvent in a rotary evaporator. The aqueous concentrate was extracted successively with petroleum ether (60–80 °C) and ethyl acetate.

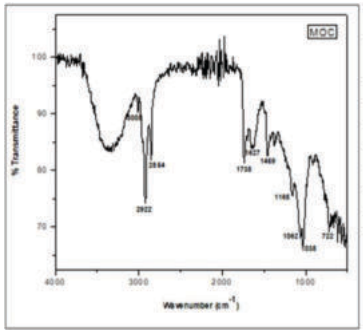
Ethyl acetate fraction gave positive Shinoda test for flavonoids and was used for further phytochemical analysis. The ethyl acetate extract was concentrated and subjected to phytochemical analysis. FTIR analysis was performed using the Perkin Elmer spectrophotometer system which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded.

The GC-MS was performed by using Shimadzu QP2010 Ultra Model gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: The fused silica column was packed with Rxi-5Sil MS (5% Phenyl 95% dimethylpolysiloxane, 30m x 250µm) fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70 eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µl was employed (split ratio of 20:1) injector temperature 260 ºC; ion-source temperature 200 ºC.

The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9 min isothermal at 280ºC. Mass spectra were taken at 70eV, a scan-interval of 0.5 seconds, and fragments from 40 to 450 Da. The total GC running time is 50 min.

The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The identification of components was based on Willey and NIST libraries as well as the comparison of their retention indices.

RESULTS



FTIR Peak Values	Functional group
3008	C-C STRETCH (Phenolics)
2922	-C-H stretch (aldehydic)
2864	-C-H stretch (alkane)
1738	C=O stretch (aldehyde)
1627	C=C stretch
1459	CH_3 bend
1168	C-F
722	C-Br

Figure 1: FTIR spectrum of the *Oxalis corniculata* leaf extract

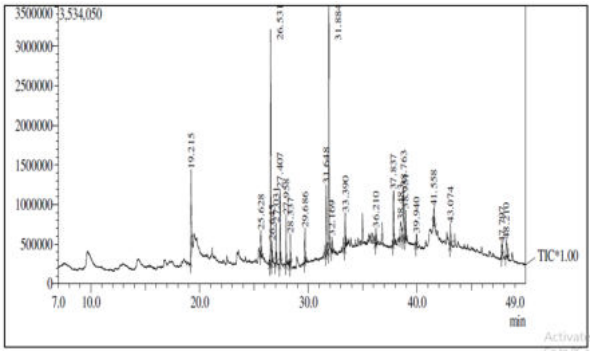


Figure 2 : *GC-MS Chromatogram* of *Oxalis corniculata* leaf extract

Table 2: GC-MS analysis of phytochemical components

Sl. No.	Retention Time	Name of compound	Peak area (%)
1	19.215	2,4-Di-tert-butylphenol	9.77
2	25.628	9-eicosene, (E)-	1.65
3	26.531	Phytol, acetate	15.79
4	26.645	6,10,14-Trimethyl-pentadecan-2-Ol	1.73
5	27.031	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.78
6	27.407	Neophytadiene	5.01
7	27.958	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	3.72
8	28.337	Methyl palmitate	2.18
9	29.686	1-Nonadecene	2.43
10	31.648	Linolenic acid, methyl ester	5.62
11	31.884	Phytol	18.59
12	32.169	Methyl stearate	0.87
13	33.390	1-Tricosene	2.47
14	36.210	Butyl 9,12,15-octadecatrienoate	1.08
15	37.837	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	4.28
16	38.483	Decane, 1,9-bis[(trimethylsilyl)oxy]-	4.04
17	38.763	2-Monopalmitin	7.69
18	38.951	1,2-Benzenedicarboxylic acid	2.36
19	39.940	Octadecyltrifluoroacetate	0.77
20	41.558	2,2-Dimethyl-3-vinyl bicyclo[2.2.1]heptane	1.16
21	43.074	Squalene	1.62
22	47.797	N-hentriacontanol-1	1.33
23	48.210	Tocopherol	3.07

DISCUSSION

The FTIR spectrum helps to identify the functional groups of the active components present in the extract sample based on the peaks values in the region of IR radiation. The broadband at 3008 cm-1 corresponds to O-H stretching H-bonded alcohols and phenolic compounds in the leaf extract. The more intense bands occurring at 2922 cm^{-1} stretch indicate the presence of carbohydrates. The absorption band at 2922 cm^{-1} and 2864 cm^{-1} indicates C-H stretch in methyl ($–CH_3$) and methylene ($–CH_2$) aliphatic saturated (C–H) sharp asymmetric and symmetric stretching. The C=O stretching observed at 1738 cm^{-1} is due to the presence of the carbonyl group. The band at 1627 cm^{-1} shows the presence of aromatic ring (C=C). The peak at 1460 cm^{-1} indicates CH_2 bending vibration of alkane. Peaks at 1168 cm^{-1} confirm the presence of C=O polysaccharide. The peaks 1168, 1038 and 722 cm^{-1} indicate the presence of organic halogen compounds in *Oxalis corniculata* leaf extract. The above infrared functional group characteristics were cited in the literature [Ranjana and Mendhulkar 2015; John C, 2000; Saranya and Sekar, 2016; Liton and Islam, 2006]. The results of the present study confirm the presence of phenol, alkane, alkene, carboxylic acid, aromatic compound, alcohol, and Bromo alkane compounds in methanolic leaf extract of *Oxalis corniculata.*

Figure 2 shows the results of the GC-MS analysis of the flavonoid rich fraction of *Oxalis corniculata* leaf extract. The twenty three major peaks are tabulated in Table-2. The most phytochemical peak area were depicted by phytol (18.59%), 2-Monopalmitin, linoleic acid and neophytadiene. Phytols are diterpenes member of the long-chain unsaturated acyclic alcohols having a wide spectrum of biological effects. 2-Monopalmitin and linoleic acid are the major fatty acid constituents of the leaf extract. The compound neophytadiene detected is a diterpene with anti-inflammatory properties [Bhardwa *et al*., 2020].

CONCLUSION

The FTIR analysis clearly indicates that the leaf extract is high in antioxidant phenolics and flavonoids. The GC-MS study also shows many phytochemicals like tocopherols, 2,4-Di-tert-butyl phenol, ethyl ester, Phytol, Linolenic acid, 1,2-Benzenedicarboxylic acid, 2-Monopalmitin, Squalene, etc., all of which contributes the activities like antimicrobial, antioxidant, anticancer and other pharmacological activities.

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