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Research Article

GC- MS EVALUATION OF CHEMICAL CONSTITUENTS FROM METHANOLIC LEAF EXTRACT OF *Kedrostis foetidissima* (Jacq.) Cogn

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ABSTRACT

Kedrostis foetidissima (Jacq.) Cogn., a medicinal plant belongs to the family of Cucurbitaceae. Traditionally the plant was used in the treatment of small pox and skin diseases. The present study deals with the phytochemical screening and GC-MS evaluation of chemical constituents from the methanolic leaf extract of *Kedrostis foetidissima*. Preliminary phytochemical screening reveals the presence of alkaloids, flavonoids, steroids, phenols, triterpenoids, glycosides and saponins. Fifteen chemical constituents have been identified through GC-MS analysis, among that the major constituents are 7,10- hexadecadienoic acid, methyl ester (CAS) (9.37%), 2-hexadecen-1-ol,3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-(CAS) (5.57%), 1H-1,2,4-triazole-3,5-dicarbaldehyde (5.22%) and docosanoic acid (4.96 %) and other minor constituents were also identified. The compounds identified through the GC-MS analysis were used in various applications as anti-microbial, anti-inflammatory, cancer preventive, flavor and anti-fouling agents.

Keywords: *Kedrostis foetidissima*, Hexadecadienoic acid, GC-MS analysis, phytochemicals

INTRODUCTION

Medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development.[1] In developing countries, it is estimated that about 80% of the population really depends on traditional medicine for their primary healthcare. So there arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies.[2]

Kedrostis foetidissima (Jacq.) Cogn. commonly known as "Appakovai" in Tamil is a medicinal plant found to be growing around the fence and have a very unpleasant smell but cattle feed on it ravenously. It is very effective in the treatment of asthma, chest pain and urinary tract infections,[3] diarrhoea, small pox, skin diseases[4] and snake bite.[5] The leaf extracts were used as anti-fouling agents for the treatment of bloat in cattle's.[6] The leaf juice was used for the treatment of cold in children.[7] With this background information the present study was aimed to identify the chemical constituents in methanolic leaf extract of *K.foetidissima* through phytochemical screening and GC-MS technique.

MATERIALS AND METHODS

Plant Material

The fresh leaves of *Kedrostis foetidissima* (Jacq.) Cogn., were collected from Sivagiri (11°07′N, 77°48′E), Erode (dt), Tamilnadu, India during the month of January-March 2012 and authenticated by Dr.K.Nandakumar, Professor, Department of Botany, Kandaswami Kandars, College, Velur, Namakkal (dt), Tamilnadu, India. The fresh leaves of *K. foetidissima* were collected and washed with distilled water and blotted gently with filter paper and shade dried. The dried leaves were powdered using a mechanical grinder and stored in a sterile container at 4°C until future use.

Preparation of Extract

30gms of dried powdered plant material was extracted successively with methanol in an orbital shaker for 24 hrs at room temperature. The extracts were filtered using Whatman No.1 filter paper to remove extractable substances, at every 3 hrs interval. The combined extracts were then evaporated at 40°C on water bath and the dried extract was stored at 4°C in a sterile container.

Phytochemical Screening [8-11]

Test for alkaloids

To 2 ml of the extract, few drops of Dragendorff's reagent was added, formation of orange brown precipitate indicates the presence of alkaloids.

Test for flavonoids

Few drops of extract were mixed with 1 ml of 5% lead acetate solution. Appearance of white precipitate confirms the presence of flavonoids.

Test for phenols

Few drops of extract were spotted on the filter paper and a drop of 5% phosphomolybdic acid is sprayed on it and then it was exposed to ammonia gas. Appearance of blue colour indicates the presence of phenols.

Test for Tannins

To 2 ml of the extract, 2-3 drops of ferric chloride solution was added, formation of dark blue or greenish black colour indicates the presence of tannins.

Test for Glycosides

Dissolve the extracts in pyridine and added few drops of sodium nitroprusside and 2% alcoholic sodium hydroxide. Appearance of red colour indicates the presence of glycosides.

Test for Triterpenoids

To 5 ml of the extract, 2ml of chloroform and 3ml of conc. H_2SO_4 was added, a monolayer of reddish brown coloration indicates the presence of triterpenoids.

Test for steroids

To 1 ml of the extract, 1 ml of chloroform, 2 ml of acetic anhydride and 2 drops of conc. H_2SO_4 was added. Appearance of dark red colour indicates the presence of steroids.

Test for Saponins

The extract was diluted with 10 ml of distilled water and it was shaken for 15 minutes. Formation of foam indicates the presence of saponins.

GC-MS analysis of methanolic extract of *K.foetidissima*

GC-MS analysis was carried out to identify some of the potent volatile and semi volatile constituents present in the methanolic leaf extract of *K.foetidissima*. GC analysis was carried out using Thermo GC-Trace ultra version: 5.0, Thermo MS DSQ II equipment. The

chromatograph was fitted with DB 5-MS capillary standard non-polar column 30m × 0.25mm internal diameter, with film thickness of 0.25µm. The injector temperature was set at 80-260°C and the oven temperature was initially at 80°C and then slowly the temperature was raised to 260°C at the rate of 5°C/min. Helium was used as a carrier gas with the flow rate of 1.0 ml/min. 1 µl of the sample was injected to the column. The Mass Spectrometer was operated in the Electron Impact (EI) mode at 70eV. Ion source and transfer line temperature was kept at 250°C. The mass spectra were obtained by centroid scan of the mass range from 50 to 650amu. Mass spectra of the separated components from the extracts were compared with the known components in the NSIT database. The name, molecular weight and structure of the components of the test materials were determined.

RESULTS AND DISCUSSION

For the pharmacological as well as pathological discovery of novel drugs, the essential information regarding the chemical constituents are generally provided by the qualitative phytochemical screening of plant extract.[12] The phytochemical screening of methanolic leaf extract of *Kedrostis foetidissima* is depicted in table 1. The result indicates the presence of alkaloids, flavonoids, phenols, tannins, steroids, triterpenoids, saponins and glycosides in the methanolic leaf extract of *K.foetidissima*. Our results supported the previous study conducted by Vasantha *et al.,*[13] who reported the presence of alkaloids, flavonoids, phenols, tannins, steroids, terpenoids, saponins, glycosides and absence of phlobatannins and anthraquinones in leaves, stems and tubers of *K.foetidissima.* These bioactive compounds synthesized by secondary metabolism are chemically and taxonomically diverse compounds with obscure function.[14]

The phytoconstituents like alkaloids, saponins and glycosides were reported to have various biological functions which includes anticancer, anti-inflammatory and antimicrobial activities. Phenolic compounds which are commonly found in both edible and inedible plants are reported to have multiple biological effects, including antioxidant activity[15] and promotion of health benefits.[16]

The active compounds identified in the methanolic leaf extract of *K.foetidissima* by GC-MS analysis was shown in Figure-1.

Totally fifteen compounds have been detected through GC-MS analysis based on retention time, molecular formula, molecular weight and peak area.. The active compounds with their retention time (RT), molecular formula, molecular weight (MW) and concentration (peak area %) are presented in Table-2. The major compounds present in the leaves were 7,10- hexadecadienoic acid methyl ester (CAS) (9.37%), 2-hexadecen-1-ol,3,7,11,15-tetramethyl-[R-[R*,R*-(E)]]-(CAS) (5.57%), 1H-1,2,4-triazole-3,5-dicarbaldehyde (5.22%) and docosanoic acid (4.96 %) etc., other major and minor compounds were also present.

The presence of phytol compounds attributes to the antimicrobial, anti-inflammatory and anticancer property of the plant leaves. The quinolizidine compounds found to have an important role as herbivore repellents and inhibit the growth of various bacteria and fungi providing protection to the plants from herbivores such as insects and grazing mammals[17]. Other minor constituents present in the leaves are well known for their antimicrobial activity which relates the use of *K.foetidissima* leaves as traditional medicine for the treatment of measles

PHYTOCHEMICALS	METHANOLIC LEAF EXTRACT OF *K.foetidissima*
Alkaloids	++
Flavonoids	++
Phenols	++
Tannins	++
Glycosides	++
Triterpenoids	++
Steroids	+
Saponins	++

ILLUSTRATIONS

Table1: Phytochemical screening of the methanolic leaf extract of *K.foetidissima*

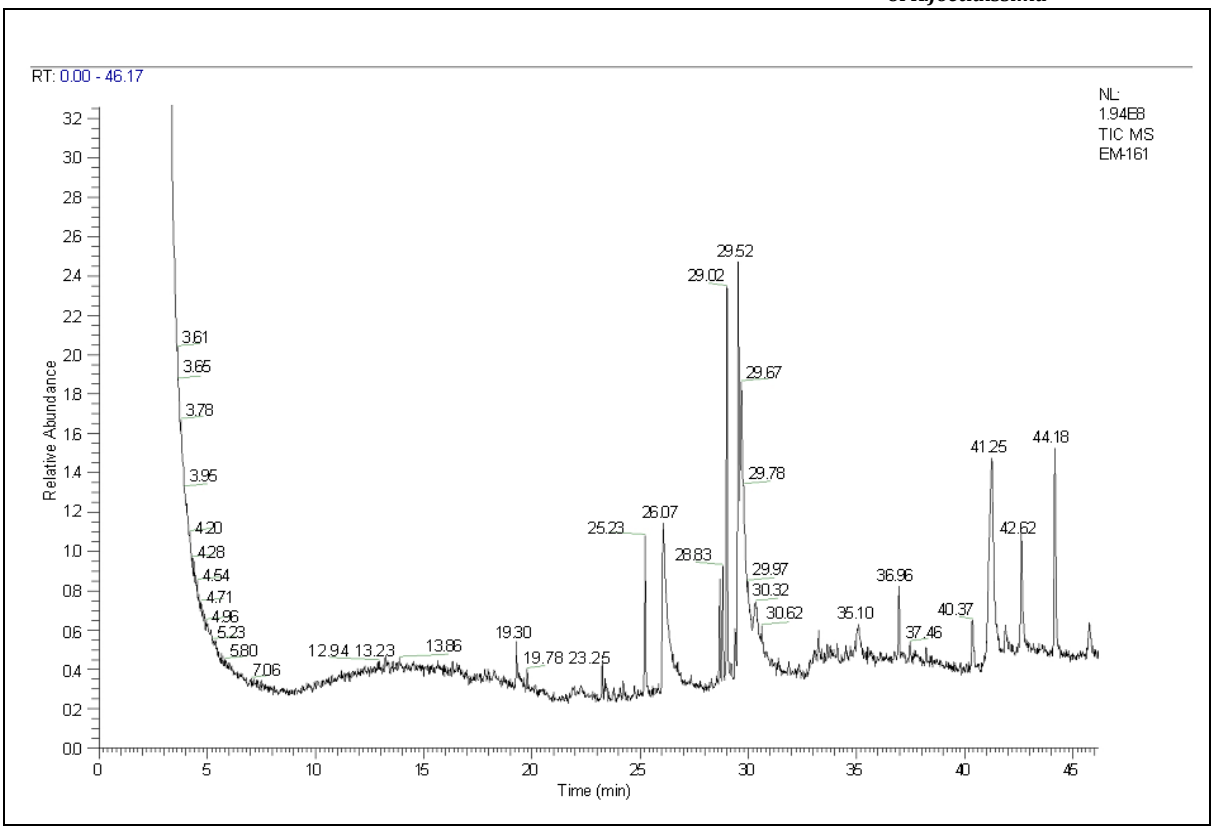

Figure 1: GC-MS Chromatogram of methanolic leaf extract of *K.foetidissima*

Table 2: Bioactive compounds identified from GC-MS analysis of methanolic leaf extract of *K.foetidissima*

No	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1	13.25	1H-Indole, 1-methyl-(CAS)	C_9H_9N	131	0.25
2	19.30	(+-)-4-(1-Cyclohexenyl)-3- (p-toluenesulfonyl)-ë-(3,4)- quinolizidine	$C_{22}H_{29}NO_2S$	371	0.65
3	19.78	3-Methylene-5-methylhepten-5-ol	$C_9H_{16}O$	140	0.18
4	23.25	Phytol Acetate	$C_{22}H_{42}O_2$	338	0.69
5	25.23	Eicosanoic acid, methyl ester(CAS)	$C_{21}H_{42}O_2$	326	2.40
6	26.07	Docosanoic acid	$C_{22}H_{44}O_2$	340	4.96
7	28.83	1,1-Dideutero-,6,10,14-tetra methylpentadeca-9,13-diene-1,3,5-triol	$C_{19}H_{32}D_2O_2$	294	2.07
8	29.02	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,[R-[R*,R*-(E)]]-(CAS)	$C_{20}H_{40}O$	296	5.57
9	29.52	7,10-Hexadecadienoicacid, methylester (CAS)	$C_{17}H_{30}O_2$	266	9.37
10	35.1	2,3-epoxy-propylcyclooctane	$C_{11}H_{20}O$	168	1.03
11	36.96	9-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	1.23
12	37.46	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	0.27
13	40.37	3-Chloro-2-methylidenepropyl isobutyrate	$C_8H_{13}ClO_2$	176	1.16
14	42.62	4,5-Diacetoxy-3-butylpentanal	$C_{13}H_{22}O_5$	258	2.76
15	44.18	1H-1,2,4-Triazole-3,5-dicarbaldehyde	$C_4H_3N_3O_2$	125	5.22

Table 3: Biological activities of the identified bioactive compounds from methanolic leaf extract of *K.foetidissima.*

No	RT	Peak Area	Name of the compound	Compound Nature	Activity**
1	29.52	9.37	7,10-exadecadienoicacid, methylester (CAS)	Unsaturated fatty acid	No activity reported
2	29.02	5.57	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(CAS)	Phytol	Antimicrobial Anticancer Antiinflammatory Diuretic
3	44.18	5.22	1H-1,2,4-Triazole-3,5-dicarbaldehyde	Nitrogen compound	Antimicrobial
4	26.07	4.96	Docosanoic acid	Fatty acid	No activity reported
5	42.62	2.76	4,5-Diacetoxy-3-butylpentanal	Aldehyde compound	Antimicrobial
6	25.23	2.40	Eicosanoic acid, methyl ester(CAS)	Fatty acid	No activity reported
7	28.83	2.07	1,1-Dideutero-,6,10,14-tetra methylpentadeca-9,13-diene-1,3,5-triol	Alcoholic compound	No activity reported
8	36.96	1.23	9-Octadecenoic acid, methyl ester	Oleic acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor
9	40.37	1.16	3-Chloro-2-methylidenepropyl isobutyrate	Chloro compound	Antimicrobia
10	35.1	1.03	2,3-epoxy-propylcyclooctane	Epoxy compound	No activity reported
11	23.25	0.69	Phytol Acetate	Phytol compound	Antimicrobial Antiinflammatory Anticancer Diuretic
12	19.30	0.65	(+-)-4-(1-Cyclohexenyl)-3- (p-toluenesulfonyl)-ë-(3,4)- quinolizidine	Sulfur compound	Antimicrobial
13	37.46	0.27	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Plasticizer compound	Antimicrobial, Anti fouling
14	13.25	0.25	1H-Indole, 1-methyl-(CAS)	Alkaloid	Antimicrobial Antiinflammatory
15	19.78	0.18	3-Methylene-5-methylhepten-5-ol	Alcoholic compound	No activity reported

****Source:Dr.Duke's Phytochemical and Ethnobotanical Databases**

CONCLUSION

Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemo-therapeutants, these are non-phytotoxic, more systemic and easily biodegradable.[18] Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties.[19]

In the present study the phytochemical screening of methanolic leaf extract of *K.foetidissima* reveals the presence of various secondary metabolites like alkaloids, flavonoids, phenols, tannins, triterpenoids, steroids and glycosides which contributes to various biological activities like antioxidant, anti-microbial and anti-tumor activities. The fifteen chemical constituents identified from methanolic leaf extract of *K.foetidissima* by GC-MS analysis were found to have various biological activities like anti-microbial, anti-cancer, anti-fouling, anti-inflammatory, anti-adrenogenic and hypocholesterolemic activities. Thus presence of various bioactive compounds justifies the use of *K. foetidissima* leaf extracts for various ailments by traditional practitioners and further studies on isolation and identification of individual constituents is needed.

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