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Research Article

LC-MS ANALYSIS OF *KIGELIA PINNATA* (JACQ) DC. ROOT BARK- A MULTI-POTENT MEDICINAL PLANT

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ABSTRACT

Phytochemicals of the plants have significant role in drug development for treatment of human diseases. Majority population in Africa, Asia and Latin America use plant based medicines. *Kigelia pinnata* DC. is a reputed African folklore drug and now abundantly found throughout India. It belongs to the family Bignoniaceae and used in different disease conditions traditionally. Out of various parts used, root bark owns medicinal properties like gynaecological complaints for fibroid, cancer of uterus and also reported biological activities such as antimalarial activity, antiprotozoal activity, antioxidant activity etc. The study was carried out for detailed exploration of biological active components in root bark extract of *K. pinnata* through Liquid chromatography/Mass Spectroscopy (LC/MS). It was performed on ESI ionization mode using both polarities. Total 63 known compounds are found from positive and negative mode. The molecules are derived from class group as terpenoid, flavonoids, phenols, glycoside and others which possess antioxidant, anti-inflammatory, antimicrobial, antihypertensive, anti-diabetic type of biological activities. This work therefore uncovers the phytochemical profile of *K.pinnata* root bark emphasizing their medicinal properties against important human diseases.

INTRODUCTION

Phytochemistry is the study of phytochemicals which are chemicals derived from plants, particularly the secondary metabolites. Plants synthesize phytochemicals for many reasons including protecting themselves against insect attacks, planting diseases etc. Bioactive molecules of the plants have played a major role in discovery of lead compounds for the development of drugs for treatment of human diseases.[1] More than half of the world population in Africa, Asia and Latin America use plant based medicines.[2]

Kigelia pinnata (Jacq) DC. syn *Kigelia africana* (Lam.) Benth. (Family Bignoniaceae), is a reputed african folklore plant which found abundantly throughout the India now. Various parts of *K. pinnata* DC. are used in approximately 76 different disease conditions (singular or in combination) traditionally.[3] The root bark is known to possess a broad spectrum of medicinal properties like gynecological complaints such as fibroid and uterus cancer, also in tapeworm infections, gastrointestinal problems, venereal diseases, rheumatism etc. The reported pharmacological activities of root are antimalarial activity, antiprotozoal activity, antioxidant activity, antibacterial and antifungal, cytotoxic activity, anticancer activities etc. These activities and medicinal properties are owing to the presence of secondary metabolites such as alkaloids, glycosides, flavonoids, tannins, saponins, quinines, carbohydrates, iridoids, terpenes, steroids, coumarins, etc.[4,5-8]

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Liquid chromatography-Mass Spectroscopy (LC/MS) Analysis

Liquid chromatography Mass Spectroscopy is an analytical technique for identification, quantisation and mass analysis of a wide variety of non-volatile or semi-volatile organic or inorganic compounds in a mixture. Liquid chromatography coupled with mass spectrometry (LC/MS) is also a powerful technique for

the analysis of complex botanical extracts.[9] Among hyphenated techniques, it is highly sophisticated and considerably powerful tool for detection of low and high molecular weight analyses.[10] A great advantage of ESI is its ability to provide soft ionization in LC-MS.[11]

To the best of our knowledge, very little has been reported on the phytochemistry and medicinal use of the root of *K. pinnata*, despite its many traditional applications. Hence the main objective of this study was to use Liquid chromatography/Mass Spectroscopy, a powerful analytical technique for detailed exploration of biological active components in root bark extract of *K. pinnata*.

MATERIALS AND METHODS

Drug collection and authentication

The root bark of *K. pinnata* was collected by the scholar herself from Naranpur village area near Ranjit Sagar dam of Jamnagar district, Gujarat state, by following guidelines of Good Field collection practices by NMPB. Identification of the standard sample was confirmed by comparing its characters with various floras, classical references and standard herbarium sample deposited at the Pharmacognosy Laboratory, I.T.R.A., Jamnagar. Plant sample was authenticated by taxonomist Dr. Jadeja from Maharshi Dayanand Science College, Porbandar (Fig. 1).

Drug Processing

The root bark was washed with water. Then it was shade dried for 10 days. The dried plant materials have been pulverized to prepare coarse powder and it was stored in polythene air tight containers at room temperature for further use (Fig. 2).

Preparation of Extracts

The shade dried coarse powders of root bark (5 gm.) of *Kigelia pinnata* DC. extracted with methanol (50ml) keeping it for overnight. After 24 hours the crude extract was filtered and evaporated to dryness on a water bath set at 100°C. The dried residue of crude extract was collected for analysis. Efficiency and solubility of compounds is higher in Methanol. Delineation of compounds in methanolic extract is quintessential. (Fig.3).

LC –MS Technique

The LC-MS method was performed using TOF/ Q-TOF Mass Spectrometer system in Sophisticated Analytical Instrument Facility (SAIF) IIT, Mumbai equipped with a Dual AJS ESI (electro spray ionization) source. The gradient elution at a flow rate of 0.300ml/min was operated for 35.00 min stop time. The full-scan mass spectra were obtained within a range of m/z, amu 120- 1,200 at 1.00 scan rate. Valve switch time 1 was enabled with 5.00µl injection volume.

Solvent Composition

Channel	Ch. 1 Solv.	Name 1	Ch2 Solv.	Name 2	Selected	Used	Percent
A	100.0% Water V.02	0.1% FA in water	100.0% Water V.02		Ch. 2	Yes	100.0%
B	100.0% Methanol V.03	90% ACN + 10% H2O+ 0.1% FA	100.0% Acetonitrile V.02		Ch. 2	Yes	0.00%

The principle of this technique is to reduce the background, such as fault peaks and noise from the methanolic extracts.

Data interpretation, Identification of Components and their activities

Software used for processing of mass spectra and chromatograms was MassHunter by Agilent technologies. The spectrum of the unknown component was compared with the spectrum of the known components stored in the IIT, Mumbai library. The active molecules name, molecular mass, Retention time and m/z ratio were ascertained. Databases used for characterisation various molecules were HMDB, KEGG, LMP, METLIN. The derived class of molecules are obtained from PubChem online database and biological activities from authentic search engines articles.

RESULTS AND DISCUSSION

The LC-MS analysis of the methanolic root extract of *K.pinnata* revealed the presence of 63 known (one is unknown) compounds. Out of them, 27compounds found in positive mode while 36 are in negative mode. The active principles with their molecular formula, molecular mass (Mass), Retention time (RT) and m/z ratio (Mass-to-charge ratio) are presented in Table 1-2. The positive and negative ESI were obtained shown in Fig. 4 and Fig. 5

Table 1: Compounds identified in methanolic extract of *K. pinnata* DC root bark by LC MS (+ve mode)

S.No	Name	Formula	Mass	RT	m/z
1.	Helminthosporoside A	C39 H64 O22	884.3756	19.572	885.3825
2.	Premithramycin A3	C41 H52 O18	832.3079	17.76	833.3146
3.	Harderoporphyrinogen	C35 H42 N4 O6	614.3208	18.232	615.3292
4.	24-Acetyl- 25-cinnamoylvulgaroside	C36 H48 O8	608.3352	19.356	631.3244
5.	Tiapamil	C26 H37 N O8 S2	555.1928	7.592	556.1999
6.	Glimepiride	C24 H34 N4 O5 S	490.2337	12.829	491.2406
7.	24,25-Epoxywithanolide D	C28 H38 O7	486.2617	12.488	509.2511
8.	6-beta-D-Glucopyranosyl-4',5-dihydroxy-3',7-dimethoxyflavone	C23 H24 O11	476.1297	8.038	477.1366
9.	Senampeline A	C25 H31 N O8	473.2105	5.298	496.1996
10.	3-alpha-hydroxy-5-alpha-androstane-17-one 3-D-glucuronide	C25 H38 O8	466.2559	17.127	489.2452
11.	Sulprostone	C23 H31 N O7 S	465.1816	4.23	466.1887
12.	(14alpha,17beta,20S,22R)-14,20-Epoxy-17-hydroxy-1-oxowitha-3,5,24-trienolide	C28 H36 O5	452.257	16.5	475.2464
13.	Ginkgolide C	C20 H24 O11	440.1318	6.83	463.1209
14.	4-O-Methylmelleolide	C24 H30 O6	414.2043	14.871	437.1934
15.	Armillarivin	C23 H28 O5	384.194	14.411	407.1832
16.	3',4',5,7,8-Pentamethoxyflavanone	C20 H22 O7	374.1361	14.928	397.1254
17.	Licoagrodione	C20 H20 O6	356.1239	6.041	357.1309
18.	Gibberellin A95	C19 H22 O5	330.1476	14.052	353.1368
19.	Libanorin	C19 H20 O5	328.1313	14.678	351.1206
20.	Cimifugin	C16 H18 O6	306.1111	7.205	329.0999
21.	Emedastine	C17 H26 N4 O	302.2103	13.111	325.1995
22.	Formononetin	C16 H12 O4	268.0721	9.963	269.0794
23.	Ticlopidine	C14 H14 Cl N S	263.0563	6.808	286.0454
24.	1,2-Dihydroxy-7-hydroxymethylnaphthalene	C11 H10 O3	190.0617	8.339	191.0689
25.	1,3,8-Naphthalenertriol	C10 H8 O3	176.0461	7.548	177.0533
26.	2-Benzofurancarboxaldehyde	C9 H6 O2	146.0356	7.332	147.0428
27.	2-Methyl-2-pentenoic acid	C6 H10 O2	114.069	4.482	137.0582

Table 2: Compounds identified in methanolic extract of *K. pinnata* root bark by LC MS (-ve mode)

S.No	Name	Formula	Mass	RT	m/z
1.	Hellicoside	C29 H36 O17	656.1949	1.153	701.1952
2.	Isoacteoside	C29 H36 O15	624.2118	7.101	623.2048
3.	10-Acetoxyoleuropein	C27 H34 O15	598.1964	8.589	597.1892
4.	Prunus inhibitor b	C30 H24 O11	560.1336	6.757	559.1281
5.	7-Dehydrologanin tetraacetate	C25 H32 O14	556.1846	6.645	555.1773
6.	(2S,2''S,3S,3''R,4S)-3,4',5,7 Tetrahydroxyflavan (2->7,4->8)-3,4',5,7-tetrahydroxyflavan	C30 H24 O10	544.1382	7.448	543.1332
7.	Flocoumafen	C33 H25 F3 O4	542.1713	5.961	541.1653
8.	Pilosanol A	C29 H32 O10	538.1801	6.522	537.1665
9.	6'-O-E-Caffeoyl-mussaenosidic acid	C25 H30 O13	538.1751	7.575	537.1678

10.	Aprepitant	C23 H21 F7 N4 O3	534.1488	5.997	593.1623
11.	Mahuannin D	C30 H24 O9	528.1441	7.468	573.1432
12.	Barbatoflavan	C24 H28 O13	524.1596	6.745	523.1524
13.	2'',6''-Di-O-acetylononin	C26 H26 O11	514.1488	5.252	513.143
14.	Scutellarioside II	C24 H28 O12	508.165	7.371	507.1579
15.	Cappariloside B	C22 H28 N2 O11	496.1644	5.637	555.1771
16.	TyrMe-TyrMe-OH	C26 H26 N2 O8	494.1681	4.873	493.1609
17.	Silidianin	C25 H24 O10	484.1374	4.827	483.1321
18.	Pranlukast	C27 H22 N5 O4	480.1682	6.292	525.1665
19.	TyrMe-HoPhe-OH	C26 H26 N2 O7	478.1719	5.29	523.1713
20.	Linalool oxide D 3-[apiosyl-(1->6)-glucoside]	C21 H36 O11	464.2246	11.264	523.2384
21.	His His Arg	C18 H28 N10 O4	448.23	12.797	507.2436
22.	Desmethylastemizole	C27 H29 F N4 O	444.2339	14.094	489.2321
23.	Met Arg Met	C16 H32 N6 O4 S2	436.1938	15.119	435.1852
24.	(4R,5S,7R,11S)-11,12-Dihydroxy-1(10)-spirovetiven-2-one 11-glucoside	C21 H34 O8	414.224	16.855	473.238
25.	6alpha-Fluoroprednisolone	C21 H27 F O5	378.1863	14.041	423.1846
26.	Arg Asp Ser	C13 H24 N6 O7	376.1716	14.168	421.1697
27.	1-O-(8R-hydroxy-8-methyl-3Z,9-decadienoyl)-beta-D-glucopyranose	C17 H28 O8	360.1771	15.086	405.1748
28.	Arg Ala Asp	C13 H24 N6 O6	360.1764	14.729	405.174
29.	Scopolin	C16 H18 O9	354.0948	2.85	399.093
30.	Sudan III	C22 H16 N4 O	352.134	14.34	351.1266
31.	Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside	C13 H24 O10	340.1353	10.041	339.1279
32.	Sulfaquinoxaline	C14 H12 N4 O2 S	300.0671	9.292	299.0596
33.	Triethyl citrate	C12 H20 O7	276.1188	11.582	321.1169
34.	Panaquinquecol 4	C17 H22 O3	274.1599	11.507	273.1527
35.	(S)-Flurbiprofen	C15 H13 F O2	244.0912	13.5	289.0895
36.	3-hydroxy-4-methoxymandelate	C9 H10 O5	198.0537	4.423	197.0465
37.	Unknown compound	C10 H12 O4	196.073	4.541	241.0729

Out of 63 compounds both in positive and negative mode, 1 compound each from aldehydes, Prostaglandins, alkaloids, Phenylpropanoids, Polyketides, Porphyrins, Sulfones, Thienopyridine, Ketone, 2 from amines, Benzimidazoles, Benzofurans, Steroids, 3 from lipid, Naphthalenes, Carbohydrate, Carboxyl group, Peptide, 4 in phenol and Glycoside, 6 from Flavonoids and majority i.e., 10 compounds from Terpenoid were found. Here identity of one molecule is unknown. (Table 3)

Table 3: Nature of phyto-components identified in the methanolic extract of *K.pinnata* root bark

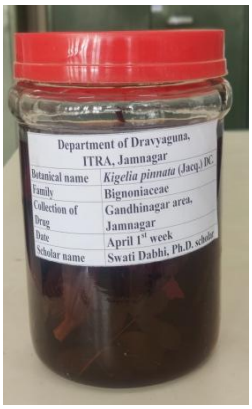
Nature of compound	Name of the compounds
Terpenoid	(2S,2''S,3S,3''R,4S)-3,4',5,7-Tetrahydroxyflavan(2->7,4->8)-3,4',5,7-tetrahydroxyflavan, 7-Dehydrologanin tetraacetate, 10-Acetoxyoleuropein, (4R,5S,7R,11S)-11,12-Dihydroxy-1(10)-spirovetiven-2-one 11-glucoside, Ginkgolide C, Armillarivin, Helminthosporoside A, 24-Acetyl- 25-cinnamoylvulgaroside, 4-O-Methylmelleolide, Gibberellin A95
Flavonoids	Formononetin, 3',4',5,7,8-Pentamethoxyflavanone, 6-beta-D-Glucopyranosyl-4',5-dihydroxy-3',7-dimethoxyflavone, Prunus inhibitor b, Pilosanol A, Silidianin
Phenols	Premithramycin A3, Mahuannin D, 2'',6''-Di-O-acetylononin, 3-hydroxy-4-methoxymandelate
Glycoside	Isoacteoside, Barbatoflavan, Cappariloside B, Linalool oxide D 3-[apiosyl-(1->6)-

	glucoside]
Lipid	(14alpha,17beta,20S,22R)-14,20-Epoxy-17-hydroxy-1-oxowitha-3,5,24-trienolide, 2-Methyl-2-pentenoic acid, 3-alpha-hydroxy-5-alpha-androstane-17-one 3-D-glucuronide
Naphthalenes	1,2-Dihydroxy-7-hydroxymethylnaphthalene,1,3,8 Naphthalenertriol, Flocoumafen
Carbohydrate	Methyl-2-alpha-L-fucopyranosyl-beta-D-galactoside, 1-O-(8R-hydroxy-8-methyl-3Z,9-decadienoyl)-beta-D-glucopyranose, Scopolin
Carboxyl group	(S)-Flurbiprofen, Triethyl citrate, 6'-O-E-Caffeoyl-mussaenosidic acid
Peptide	Met Arg Met, Arg Asp Ser, Arg Ala Asp
Amines	Tiapamil, Sulfaquinoxaline
Benzimidazoles	Emedastine, Desmethylastemizole
Benzofurans	2-Benzofurancarboxaldehyde, Pranlukast
Steroids	24,25-Epoxywithanolide D, 6alpha-Fluoroprednisolone
Aldehydes	Licoagrodione
Alkaloids	Senampeline A
Prostaglandins	Sulprostone
Phenylpropanoids	Libanorin
Polyketides	Cimifugin
Porphyrins	Harderoporphyrinogen
Sulfone	Glimepiride
Thienopyridine	Ticlopidine
Ketone	Panaquinquecol 4

FIGURES

Fresh Root

Fresh Rootbark



Museum sample

Fig. 1 Collection of *K.pinnata* rootbark

Rootbark after shade dried

Coarse powder

Fig. 2 Drug processing

Fig. 3 Preparation of Methanol extract

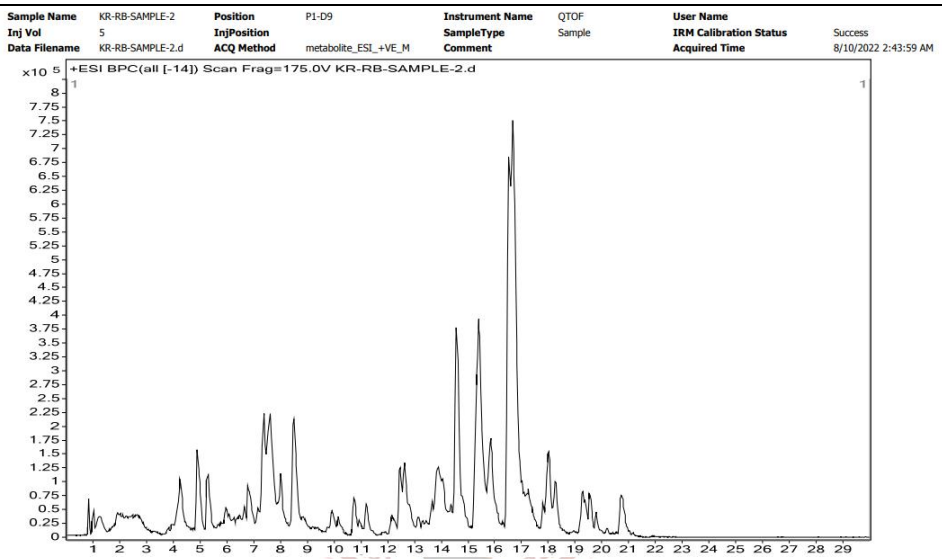


Fig. 4: LC-MS Positive mode analysis chromatogram of *K.pinnata* root bark

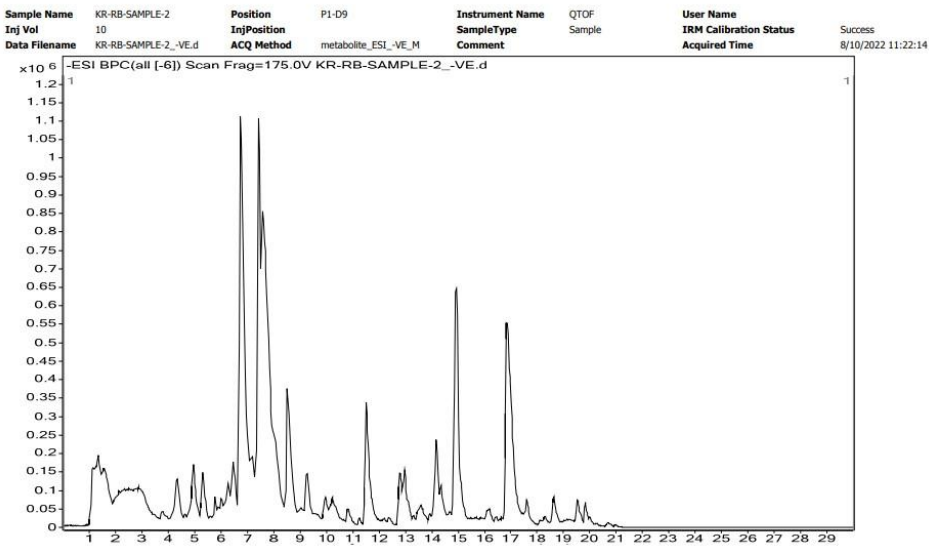


Fig. 5: LC-MS Negative mode analysis chromatogram of *K.pinnata* root bark

The maximum molecular compounds were found from Terpenoid class which having- anticancer, antimicrobial, anti-inflammatory, antioxidant, and antiallergic;[12] then Flavonoid- antioxidative activity, cardioprotective, antidiabetic, anti-inflammatory, anti-allergic, antiviral activities, anti-cancer agent;[13] Phenol- antioxidant, anti-inflammatory, anti-proliferative, antimicrobial, anti-mutagenic, anti-

oangiogenic, and neuroprotective;[14] Glycoside- antiviral, anticonvulsant, antidepressant, sedative, vasorelaxant, laxative, cytotoxic;[15] Peptide- antimicrobial, antithrombotic, antihypertensive, opioid, immunomodulatory, mineral binding, antioxidative;[16] Carboxyl group- nonsteroidal anti-inflammatory drugs (NSAIDs), antibiotics, anticoagulants, and cholesterol-lowering statins;[17] Carbohydrate- antitumor, antioxidant, antidiabetic, antiviral, hypolipidemic and immunomodulatory activities; [18] Naphthalene- anticancer, antimicrobial, anti-inflammatory, antiviral, antitubercular, antihypertensive, antidiabetic, anti-neurodegenerative, antipsychotic, anticonvulsant, antidepressant[19] are reported biological activities respectively. Tiapamil is a phenylethylamine derivative that acts as a calcium antagonist showing hemodynamic effects in patients with acute myocardial infarction.[20] Emedastine which is reported as Immunotherapeutic Agent.[21] Glimepiride is a long-acting third-generation sulfonylurea with hypoglycemic activity, anti-diabetic Agent.[22] in addition, obtained Sudan III molecule coumpound from Azo group which is used as a colouring agent.[23]

CONCLUSION

In the present study, LC-MS based diverse bioactive compound profiling of methanolic extract of *K.pinnata* root appear to be useful for distinguishing between known to unknown compounds. Approximately 63 compounds have been isolated belong to terpenoids, flavonoids, phenols, glycosides, naphthoquinones, steroids category which have a wide range of biological activities. The present study reveals the presence of various biological active components in *K.pinnata* which support its use in folk medicine for a variety of diseases. As a result, this plant is indicated as a valuable therapeutic herb with pharmacological value.

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Abbreviations

K.pinnata- *Kigelia pinnata* DC., LC-MS- Liquid chromatography-Mass Spectroscopy, Mass- molecular mass, RT- Retention time, m/z- mass per charge ions, FA- Formic acid, ACN-Acetonitrile

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