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Original Article

PHYTOCHEMICAL PROFILING OF MEDICALLY SIGNIFICANT CRUDE EXTRACT USING GC-MS ANALYSIS

MARUTHAMUTHU RAJADURAI[1], V. MAITHILI[2], R. ARUNAZHAGI[3], V. YOGESH[4]

PG and Research Department of Biotechnology and Bioinformatics, Bishop Heber College, Tiruchirappalli 620001, India
Email: mr.rajadurai@gmail.com

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ABSTRACT

Objective: The objective of this research is to identify the phytochemical constitutions present in Natural crude extract which obtained from Thumlappati district.

Methods: Kidney stone is one of the most clinical disorder arising nowadays. They are existing due to the depletion of the urine and disproportionate execration of the components such as oxalate, phosphate, uric, cysteine, and struvite. Many alopathy medicine are not effectively curable in the case of kidney stone, consequently people are in need of traditional medicine system. Thus there is a great demand for research on potential inhibitor from natural products for dissolving kidney stone. In present work deals with an unknown crude extract collected from G. Thumlappati, Battalagundu Dindugal district Tamil Nadu. The crude extract of phytochemical are analyzed by using GCMS method.

Results: Thus the sample has some bioactive compound to discharge the stone particles. So we subjected the crude extract sample to GC-MS process which reveals 210 compounds in 21 different peaks.

Conclusion: This studies forms a basis for the biological characterization and importance of bioactive compounds were identified.

Keywords: Kidney stone, Crude extract, GCMS analysis, Bioactive compounds

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INTRODUCTION

History reveals that, every civilization in the world used plants as their derivatives for treatment (or) prevention of diseases. Plants had been used as traditional health care system from the centuries and is a major source of the therapeutic agents for curing the human diseases. In the last few years more than 13000 plants have been studied for the various diseases among these some medicinal properties of plants have been documented by researchers [1-3]. In India the Traditional medicinal system using medicinal plants are Ayurveda, siddha, homeopathy, etc., to treat various diseases [4]. Traditional plant based medicines for primary health care need is followed in underdeveloped countries of about 80% of world's population (WHO) [5]. A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of disease. Traditional medicine have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, and with less side effects. Traditional medicine is the sum of knowledge skills and practices based on the theories, beliefs, and experience indigenous to different culture that are used to maintain health and also to prevent, diagnose, improve or treat physical and mental illness [6]. It is also believed that crude extract from medicinal plants are biologically active than isolated compounds due to their synergistic effects [7]. Therefore Kidney stones are hard deposits made of minerals and salts on the inner lining of the kidney. The world Health Organization reported 35 million peoples are affected by kidney stone [8]. In India states like Andhra Pradesh, Odisha and Tamil Nadu were worst affected by Kidney stone. The scientific drugs are taken by the affected people are not much benefited only during the time of drugs consumption there are reveling the problem and pain. After few month again they were affected by the stone formation due to their food habits and the environment. So we have a small step to completely cure the stone from the urinary tract and not allowing to form the stone again by our unknown crude sample.

In this study the Gas Chromatography Mass Spectroscopy (GC-MS) method was carried out in the methanol of crude extract for the phytochemical analysis followed by qualitative and quantitative determination of the compounds. This crude possess various medicinal properties, the aim of this study was to identify the phytocompounds in the methanol of crude extract and to identify each specific compound with their concentration by GC-MS analysis. Extraction of several active phytocompounds from these extract leadsto some high activity profile drugs.

MATERIALS AND METHODS

Collection of extract

On every Sunday early morning, the 60 y old folker persons (men and women) providing the mixed plant crude extract to the people affected from kidney stones as a liquid medicine at free of cost, They are so delight to do as a service to the public. By hearing the statement we collected sample G. Thumlappati, Battalagundu Dindugal district Tamil Nadu, India, through interview and questionaries' from folker peoples, collected the crude sample for further research.

Preparation of extract

500 ml of crude extract were heated at the temperature not exceeding the boiling point. The fine paste were obtained. Required quantity of the sample was weighted, transferred to the conical flask, and diluted with methanol in the ratio of 1:2 until the paste was fully immersed, the flask was shaken every hour for the first 6 h and incubated overnight, then filtered through what man No.1 filter paper. The methanol sample may be contains polar and non-polar components of the material and 4 µl of methanol sample was employed in GCMS analysis.

GCMS analysis

The GC–MS analysis was carried out using a Clarus 500 Perkin–Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold–Perkin Elmer Turbo mass 5.1 spectrometer with an Elite–1 (100% Dimethyl poly siloxane), 30m x 0.25 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110oC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280°C, at the rate of an increase of 5oC/min, and maintained for 9

min. Injection port temperature was ensured as 250oC and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z).

Identification of phytocompounds

Interpretation on Mass-Spectrum GC-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and retention time of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

At present the crude sample was utilized by tribal people residing at different corners of the district and also by rural and urban persons. We observed that the region of G. Thumalappati has lot of traditional utility of medicinal plants and herbs for diseases. But the folker were not willing to reveal the compounds of the crude sample. GCMS is one of the technique to identify the bioactive constituents of long chain branched chain hydrocarbons, acids, alcohols, esters etc. GCMS analysis was done using the organic solvent methanol it shows the presence of different 210 compounds in the crude sample. The spectrum profile of GCMS confirmed the 21 major peaks with the retention time 10.257,10.781,12.326,13.138,14.657,15.222,16.828, 21.892,23.428,24.654,25.966,28.821,31.006,32.161,33.480,36.490,3 7.941,38.799,40.163,41.134,42.157. The studies on the active principles in the plant crude sample of methanolic extract by GCMS analysis clearly showed the presence of 210 compounds with their retention time (RT), molecular weight (MW) are presented in table 1. The GCMS chromatogram of the 21 peak of the compounds detected was shown in fig. 1. The highest peak area % (15) is 29.742 and the lowest peak area % (1) is 0.010. By comparing the GCMS compound against with traditional plants using Dr. Dukes photochemical and ethanobotanical database, almost maximum number of crude sample compounds are identify in*sarcostemma acidum, Hymenocardia acida, Cicca acida, Rumex aceosella, Phyllanthus acidus, Citrus auratum, Citrus acida, Uncaria acida, Citrus sinesis, Elephantopus scaber, Tribulus cistoides*plants which has a property of Inhibition formation of uric acid.

Table 1: Compound detected in the methanol extract of crude sample

S. No.	Compound name	Retenton time (min)	Molecular weight
1	Benzoic acid	10.257	122
2	Benzoic acid, silver(1+) salt	10.257	228
3	Heptanediamide, N,N'-di-benzoyloxy	10.257	398
4	Benzoic acid	10.257	122
5	Cyclobutane-1,1-dicarboxamide, N,N'-di-benzoyloxy-	10.257	382
6	2,4-Dinitrophennylhydrazone of ribose tetrabenzoate	10.257	746
7	Methanol, oxo-, benzoate	10.257	150
8	4-Piperidinepropanoic acid, 1-benzoyl-3-(2-chloroethyl)-, ethyl	10.257	351
9	Cyclopropanecarboxamide, N-benzoyloxy-	10.257	205
10	1-O-Monoacetyl-2,3-O-dibenzoyl-d-ribofuranose	10.257	400
11	Phenol, 4-ethenyl-, acetate	10.781	162
12	Benzofuran, 2,3-dihydro-	10.781	120
13	4-Ethoxystyrene	10.781	148
14	Benzaldehyde, 4-methyl-	10.781	120
15	Benzene, (ethenyloxy)-	10.781	120
16	Benzaldehyde, 3-methyl-	10.781	120
17	4-tert-Butoxystyrene	10.781	176
18	Benzaldehyde, 2-methyl-	10.781	120
19	6-Methylenebicyclo[3.2.0]hept-3-en-2-one	10.781	120
20	Bicyclo[4.2.0]octa-1,3,5-trien-7-ol	10.781	120
21	dl-Mevalonic acid lactone	12.326	130
22	2-Hexene, 1-methoxy-, (E)-	12.326	114
23	Oxirane, butyl-	12.326	100
24	(2,3-Dimethyloxiranyl)methanol	12.326	102
25	trans-3-Penten-2-ol	12.326	86
26	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (.+/-.)-	12.326	130
27	2-Nonanone	12.326	142
28	Pentane, 1-(2-propenyloxy)-	12.326	128
29	Cyclooctyl S-2-(dimethylamino)ethyl propylphosphonofluoridate	12.326	321
30	2,6-Octadiene-4,5-diol	12.326	142
31	2-Methoxy-4-vinylphenol	13.138	150
32	4-Hydroxy-2-methylacetophenone	13.138	150
33	Ethanone, 1-(2-hydroxy-5-methylphenyl)-	13.138	150
34	4-Hydroxy-3-methylacetophenone	13.138	150
35	3-Methoxyacetophenone	13.138	150
36	Benzene, 1-ethoxy-4-ethyl-	13.138	150
37	Ethanone, 1-[5-(1-hydroxyethylidene)-1,3-cyclopentadien-1-yl]-	13.138	150
38	Phenol, m-tert-butyl-	13.138	150
39	Phenol, 2-(1,1-dimethylethyl)-	13.138	150
40	1-(4-Hydroxymethylphenyl)ethanone	13.138	150
41	1(3H)-Isobenzofuranone	14.657	134
42	Benzoic acid, 2-(hydroxymethyl)-	14.657	152
43	Benzoyl bromide	14.657	184
44	Ethanone, 2,2-dibromo-1-phenyl-	14.657	276
45	Ethanone, 2,2-dihydroxy-1-phenyl-	14.657	152
46	beta.-Benzilmonoxime	14.657	225
47	Benzhydrazide, N2-(2-methoxy-5-nitrobenzylideno)-	14.657	299
48	N,N'-(4,5-Dimethyl-1,3-phenylene) bisbenzamide	14.657	344

49	Benzoic acid, 3,5-difluophenyl ester	14.657	234
50	. alpha.,. alpha.-Dichloroacetophenone	14.657	188
51	Dodecane, 1-chloro-	15.222	204
52	1-Chloroundecane	15.222	190
53	Decane, 1-chloro-	15.222	176
54	Tetradecane, 1-chloro-	15.222	232
55	Nonane, 1-chloro-	15.222	162
56	Hexadecane, 1,16-dichloro-	15.222	294
57	n-Dodecylpyridinium chloride	15.222	283
58	Hexadecane, 1-chloro-	15.222	260
59	Octane, 1-chloro-	15.222	148
60	1-Octadecanesulphonyl chloride	15.222	352
61	1-Undecanol	16.828	172
62	Cyclodecane, methyl-	16.828	154
63	Cyclopropane, nonyl-	16.828	168
64	E-11,13-Tetradecadien-1-ol	16.828	210
65	Cyclodecane	16.828	140
66	1-Decanol	16.828	158
67	3-Tetradecene, (Z)-	16.828	196
68	Cyclooctane, 1,2-dimethyl-	16.828	140
69	3-Dodecene, (E)-	16.828	168
70	Cyclooctane, methyl-	16.828	126
71	3-tert-Butyl-4-hydroxyanisole	21.892	180
72	Ethanone, 1-(3,4-dimethoxyphenyl)-	21.892	180
73	3',5'-Dimethoxyacetophenone	21.892	180
74	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	21.892	180
75	Phenol, 3-(1,1-dimethylethyl)-4-methoxy-	21.892	180
76	(+)-s-2-Phenethanamine, 1-methyl-N-vanillyl-	21.892	271
77	Ethanone, 1-(2,5-dimethoxyphenyl)-	21.892	180
78	(+-)-2-Phenethanamine, 1-methyl-N-vanillyl-	21.892	271
79	2,5-Dimethoxy-4-ethylamphetamine	21.892	223
80	1,2,4-Cyclopentanetrione, 3-(2-pentenyl)-	21.892	180
81	Tetradecane, 1-chloro-	23.428	232
82	Dodecane, 1-chloro-	23.428	204
83	Hexadecane, 1-chloro-	23.428	260
84	1-Chloroundecane	23.428	190
85	Decane, 1-chloro-	23.428	176
86	Hexadecane, 1,16-dichloro	23.428	294
87	1-Octadecanesulphonyl chloride	23.428	352
88	Nonadecane, 1-chloro-	23.428	302
89	Octadecane, 1-chloro-	23.428	288
90	Nonane, 1-chloro-	23.428	162
91	1-Hexadecanol	24.654	242
92	n-Tridecan-1-ol	24.654	200
93	Cyclotetradecane	24.654	196
94	Hexadecen-1-ol, trans-9-	24.654	240
95	3-Hexadecene, (Z)-	24.654	224
96	5-Octadecene, (E)-	24.654	252
97	7-Hexadecene, (Z)-	24.654	224
98	n-Heptadecanol-1	24.654	256
99	1-Undecanol	24.654	172
100	Cetene	24.654	224
101	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	25.966	208
102	2-Propenoic acid, 3-[4-(acetyloxy)-3-methoxyphenyl]-, methyl es	25.966	208
103	1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene	25.966	208
104	2-Propenoic acid, 3-(2,4-dimethoxyphenyl)-, (E)-	25.966	208
105	3,5-Dimethoxycinnamic acid	25.966	208
106	2,3-Dimethoxycinnamic acid	25.966	208
107	1H-1,3-Benzimidazole-6-carboxylic acid, 2-mercapto-, methyl est	25.966	208
108	2,5-Dimethoxycinnamic acid	25.966	208
109	4-Methyl-3,5-dinitrobenzamide	25.966	225
110	3,5-Dimethoxy-4-hydroxycinnamaldehyde	25.966	208
111	1-Octanol, 2-butyl-	28.821	186
112	2-Ethyl-1-dodecanol	28.821	214
113	2-Dodecanol	28.821	186
114	Methoxyacetic acid, pentadecyl ester	28.821	300
115	2-Methyl-1-undecanol	28.821	186
116	1-Dodecanol, 2-methyl-, (S)-	28.821	200
117	Isobutyl tetradecyl carbonate	28.821	314
118	1-Hexadecanol, 2-methyl-	28.821	256
119	2-Hexyl-1-octanol	28.821	214
120	2-Hexadecanol	28.821	242
121	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	31.006	208
122	2-Propenoic acid, 3-[4-(acetyloxy)-3-methoxyphenyl]-, methyl es	31.006	250

123	1,2-Dimethoxy-4-(3-methoxy-1-propenyl)benzene	31.006	208
124	1H-1,3-Benzimidazole-6-carboxylic acid, 2-mercapto-, methyl est	31.006	208
125	2-Propenoic acid, 3-(2,4-dimethoxyphenyl)-, (E)-	31.006	208
126	2,5-Dimethoxycinnamic acid	31.006	208
127	2-Propenoic acid, 3-(2,3-dimethoxyphenyl)-, (E)-	31.006	208
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128	trans-2,5-Dimethoxycinnamic acid	31.006	208
129	3,5-Dimethoxycinnamic acid	31.006	208
130	1,3-Benzenedicarboxylic acid, 4-methyl-, dimethyl ester	31.006	208
131	6,9,12,15-Docosatetraenoic acid, methyl ester	32.161	346
132	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester, trans-	32.161	310
133	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, cis-	32.161	312
134	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	32.161	366
135	Oxiraneundecanoic acid, 3-pentyl-, methyl ester, trans-	32.161	312
136	Methyl 11-hexadecenoate	32.161	268
137	Butyl 6,9,12-hexadecatrienoate	32.161	306
138	Octadecanoic acid, 9,10-dichloro-, methyl ester	32.161	366
139	14-Methylpentadec-9-enoic acid methyl ester	32.161	268
140	Methyl 9-eicosenoate	32.161	324
141	Acetic acid, 2-diacetylamino-1-methyl-1-propenyl ester	33.480	213
142	6,6-Dimethyl-1,4-dioxa-spiro[4.5]dec-7-ene	33.480	168
143	1-Nitro-. beta.-d-arabinofuranose, tetraacetate	33.480	363
144	1-Nitro-2-acetamido-1,2-dideoxy-d-glucitol	33.480	252
145	N,N-Diethyl-N'-(1-naphthyl)ethylenediamine	33.480	242
146	DL-Leucine, N-DL-leucyl-	33.480	244
147	1,16-Cyclocorynan-17-oic acid, 19,20-didehydro-, methyl ester,	33.480	322
148	1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	33.480	352
149	9-Oxabicyclo[3.3.1]nonane-2,6-dione, 2-oxime-6-ethylene ketal	33.480	213
150	Malonodihydrazide, 2-(3-butoxy-2-hydroxypropyl)-	33.480	262
151	trans-13-Octadecenoic acid, methyl ester	36.490	296
152	11-Octadecenoic acid, methyl ester	36.490	296
153	6-Octadecenoic acid, methyl ester, (Z)-	36.490	296
154	10-Octadecenoic acid, methyl ester	36.490	296
155	6-Octadecenoic acid, methyl ester	36.490	296
156	cis-13-Octadecenoic acid, methyl ester	36.490	296
157	13-Octadecenoic acid, methyl ester	36.490	296
158	16-Octadecenoic acid, methyl ester	36.490	296
159	9-Octadecenoic acid (Z)-, methyl ester	36.490	296
160	9-Octadecenoic acid (Z)-, methyl ester	36.490	296
161	trans-13-Octadecenoic acid, methyl ester	37.941	296
162	11-Octadecenoic acid, methyl ester	37.941	296
163	10-Octadecenoic acid, methyl ester	37.941	296
164	cis-13-Octadecenoic acid, methyl ester	37.941	296
165	13-Octadecenoic acid, methyl ester	37.941	296
166	16-Octadecenoic acid, methyl ester	37.941	296
167	6-Octadecenoic acid, methyl ester	37.941	296
168	14-Octadecenoic acid, methyl ester	37.941	296
169	6-Octadecenoic acid, methyl ester, (Z)-	37.941	296
170	9-Octadecenoic acid (Z)-, methyl ester	37.941	296
171	Methyl stearate	38.799	298
172	Heptadecanoic acid, 16-methyl-, methyl ester	38.799	298
173	Tridecanoic acid, 12-methyl-, methyl ester	38.799	242
174	Methyl tetradecanoate	38.799	242
175	Hexadecanoic acid, 15-methyl-, methyl ester	38.799	284
176	Pentadecanoic acid, 15-bromo-, methyl ester	38.799	334
177	Pentadecanoic acid, methyl ester	38.799	256
178	Cyclopentaneundecanoic acid, methyl ester	38.799	268
179	Tetradecanoic acid, 12-methyl-, methyl ester	38.799	256
180	Octadecanoic acid, 17-methyl-, methyl ester	38.799	312
181	Myo-Inositol, 4-C-methyl-	40.163	194
182	Myo-Inositol, 2-C-methyl-	40.163	194
183	. alpha.-d-6,3-Furanose, methyl-. beta.-d-glucohexodialdo-1,4-fur	40.163	192
184	3-O-Methyl-d-glucose	40.163	194
185	D-Epi-Inositol, 4-C-methyl-	40.163	194
186	3-Methylmannoside	40.163	194
187	2-O-Methyl-D-mannopyranosa	40.163	194
188	Scyllo-Inositol, 1-C-methyl-	40.163	194
189	Methyl 4-O-methyl-d-arabinopyranoside	40.163	178
190	Hydroperoxide, 1,4-dioxan-2-yl	40.163	120
191	Heptacosane, 1-chloro-	41.134	414
192	Tritetracontane	41.134	604
193	2-methyloctacosane.	41.134	408
194	Tetracosane, 11-decyl-	41.134	478
195	Tetratetracontane	41.134	618

196	Sulfurous acid, butyl heptadecyl ester	41.134	376
197	Sulfurous acid, butyl tridecyl ester	41.134	320
198	Sulfurous acid, butyl tetradecyl ester	41.134	334
199	Sulfurous acid, pentadecylpentyl ester	41.134	362
200	Sulfurous acid, butyl pentadecyl ester	41.134	348
201	Sulfurous acid, butyl heptadecyl ester	42.157	376
202	Sulfurous acid, butyl octadecyl ester	42.157	390
203	Sulfurous acid, butyl hexadecyl ester	42.157	362
204	Tritetracontane	42.157	604
205	Heptacosane, 1-chloro-	42.157	414
206	Sulfurous acid, butyl pentadecyl ester	42.157	348
207	Sulfurous acid, octadecylpentyl ester	42.157	404
208	Sulfurous acid, butyl tetradecyl ester	42.157	334
209	Sulfurous acid, hexadecylpentyl ester	42.157	376
210	Sulfurous acid, butyl tridecyl ester	42.157	320

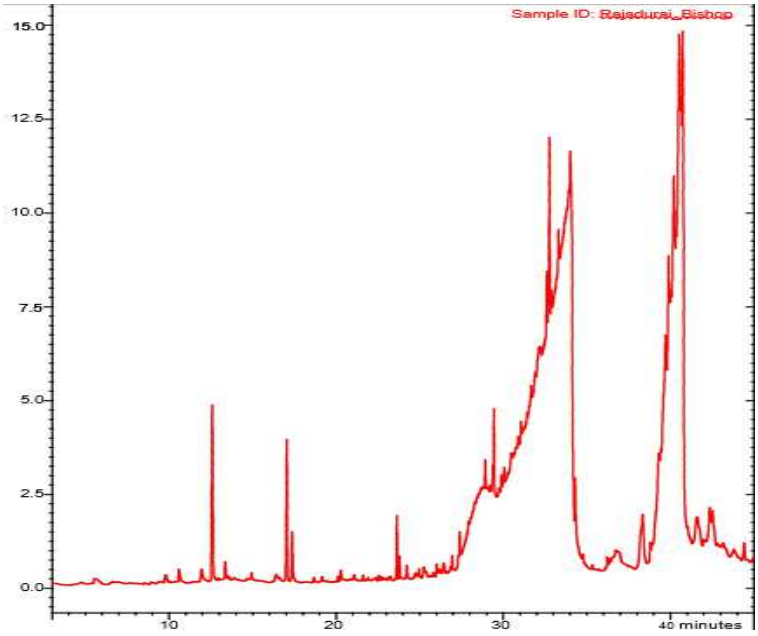

Fig. 1: GCMS analysis of crude extract

CONCLUSION

This shows that the crude sample may be the mixture of these plant extract. Gas Chromatography and mass spectroscopy analysis put on view the available of various compound with variable molecular weight. This experiment showed that the stronger extraction capacity of methanol could have produced number of bioactive constituents which are plays vital role for many biological activities. This various bioactive compounds might be utilized for the expansion for the drug development which used to treat the kidney stone formation without no side effects, purely in traditional way. At this end it can be concluded that the *in vivo* studies on the crude extract open up to new ways for natural drug that can be employed for clinical trials which may generate successful results in future.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICTS OF INTERESTS

All authors have none to declare

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