ABSTRACT

The objective of this research is to identify the phytochemical constituents 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 excretion of the components such as oxalate, phosphate, uric, cysteine, and struvite. Many dolapathy 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

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 130000 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 under-developed 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 reliving 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 phyto-compounds 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 Garus 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), 30 m x 0.25 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110°C, 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 5°C/min, and maintained for 9
Identification of phytocompounds

The ionization voltage was 70 eV. The flow rate as one ml/min. Injection port temperature was ensured as 250°C and Helium 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 lots of traditional utility of medicinal plants and herbs for diseases. But the folk 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. GOMS 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, 24.116, 32.161, 33.480, 36.490, 37.941, 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 0.01. 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 insarcostemma acidum, Hymenocardia acida, Cica acida, Rumex accessela, Phyllanthus acidus, Citrus auratum, Citrus acida, Uncaria acida, Citrus sinesis, Elephantopus scaber, Tribulus cistoidesplants has which a property of Inhibition formation of uric acid.

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

| S. No. | Compound name                                      | Retention time (min) | Molecular weight |
|-------|---------------------------------------------------|----------------------|-----------------|
| 1     | Benzoic acid                                      | 10.257               | 122             |
| 2     | Benzoic acid, silver (+) salt                    | 10.257               | 208             |
| 3     | Heptanediame, N,N'-di-benzoxyloxy                 | 10.257               | 398             |
| 4     | Benzoic acid                                      | 10.257               | 122             |
| 5     | Cyclobutane-1,1-dicarboxamide, N,N'-di-benzoyloxy | 10.257               | 382             |
| 6     | 2,4-Dinitrophenylhydrazone of ribose tetabenzoate | 10.257               | 746             |
| 7     | Methanol oxide, benzoate                          | 10.257               | 150             |
| 8     | 4-Piperidinepropanoic acid, 1-benzoyl-3-(2-chloroethyl), ethyl | 10.257               | 351             |
| 9     | Cyclopropenebenzoximoxy, N-benzoyloxy             | 10.257               | 205             |
| 10    | 1-O-Monoacetyl-2,3-0-dibenzoyl-d-ribofuranos       | 10.257               | 400             |
| 11    | Phenol, 4-ethenyl, acetate                        | 10.781               | 162             |
| 12    | Benzoifuran, 2,3-diathyro                        | 10.781               | 120             |
| 13    | 4-Ethoxy styrene                                  | 10.781               | 148             |
| 14    | Benzaldehyde, 4-methyl                            | 10.781               | 120             |
| 15    | Benzene, 4-ethoxy                                | 10.781               | 120             |
| 16    | Benzaldehyde, 3-methyl                           | 10.781               | 120             |
| 17    | 4-tert-Butoxy styrene                             | 10.781               | 176             |
| 18    | Benzaldehyde, 2-methyl                            | 10.781               | 120             |
| 19    | 6-Methylneobicyclo[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-Dimethoxy)trimethylene                      | 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-propenyl oxide                       | 12.326               | 128             |
| 29    | Cycochocyl 5-2-(dimethylamino)ethyl propylphosphonofluoridate | 12.326               | 321             |
| 30    | 2,6-Diethene-4,5-diol                            | 12.326               | 142             |
| 31    | 2-Methoxy-4-vinylphenol                          | 13.138               | 150             |
| 32    | 4-Hydroxy-2-methylacetophene                      | 13.138               | 150             |
| 33    | Ethanone, 1-(2-hydroxy-5-methylphenyl)            | 13.138               | 150             |
| 34    | 4-Hydroxy-3-methylacetophene                      | 13.138               | 150             |
| 35    | 3-Methoxyacetophenone                            | 13.138               | 150             |
| 36    | Benzene, 1-ethoxy-4-ethyl                         | 13.138               | 150             |
| 37    | Ethanone, 1-[5-(1-hydroxymethylidene)-1,3-cyclopentadien-1-yl]- | 13.138               | 150             |
| 38    | Phenol, m-tert-buty1                              | 13.138               | 150             |
| 39    | Phenol, 2-(1,1-dimethylethyl)                     | 13.138               | 150             |
| 40    | 1-(4-Hydroxymethyl)phenylethenone                 | 13.138               | 150             |
| 41    | 1-(3H)-1-benzofuranone                           | 14.657               | 152             |
| 42    | Benzoic acid, 2-(hydroxymethyl)-                 | 14.657               | 152             |
| 43    | Benzyl 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-Benzilimonoxime                             | 14.657               | 225             |
| 47    | Benzyalcoholinate, N,N-(2-methoxy-5-nitrobenzidene)- | 14.657               | 299             |
| 48    | N,N'-[4,5-Dimethyl-1,3-phenylene] bisbenzamide   | 14.657               | 344             |
| No. | Chemical Name                                                                 | Retention Time (min) |
|-----|-------------------------------------------------------------------------------|---------------------|
| 49  | Benzoic acid, 3,5-dihydroxyphenyl ester                                        | 14.657              |
| 50  | 1,2-Dichlorobenzene, 1,4-dichlorobenzene                                      | 14.657              |
| 51  | Dodecane, 1-chloro                                                            | 15.222              |
| 52  | 1-Chloroundecane                                                              | 15.222              |
| 53  | Decane, 1-chloro                                                              | 15.222              |
| 54  | Tetradecane, 1-chloro                                                         | 15.222              |
| 55  | Nonane, 1-chloro                                                              | 15.222              |
| 56  | Hexadecane, 1,16-dichloro                                                     | 15.222              |
| 57  | n-Dodecylpyridinium chloride                                                   | 15.222              |
| 58  | Hexadecane, 1-chloro                                                          | 15.222              |
| 59  | Octane, 1-chloro                                                              | 15.222              |
| 60  | 1-Undecanole, 1-undecane                                                       | 15.222              |
| 61  | Cyclohexane, methyl                                                           | 16.828              |
| 62  | Cyclohexane, methyl                                                           | 16.828              |
| 63  | Cyclohexane, 1-methyl                                                         | 16.828              |
| 64  | E-11, 13-Tetradecadien-1-ol                                                    | 16.828              |
| 65  | Cyclooctane, methyl                                                           | 16.828              |
| 66  | 1-Decanol                                                                    | 16.828              |
| 67  | Hexadecane, 1-chloro                                                          | 16.828              |
| 68  | Hexadecane, 1-chloro                                                          | 16.828              |
| 69  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 70  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 71  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 72  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 73  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 74  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 75  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 76  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 77  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 78  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 79  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 80  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 81  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 82  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 83  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 84  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 85  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 86  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 87  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 88  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 89  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 90  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 91  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 92  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 93  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 94  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 95  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 96  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 97  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 98  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 99  | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 100 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 101 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 102 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 103 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 104 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 105 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 106 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 107 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 108 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 109 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 110 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 111 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 112 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 113 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 114 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 115 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 116 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 117 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 118 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 119 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 120 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 121 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
| 122 | 1-Octadecanole, 1-octadecane                                                   | 16.828              |
123 1,2-Dimethoxy-4-[3-methoxy-1-propenyl]benzene 31.006 208
124 1H-1,3-Benzimidazole-6-carboxylic acid, 2-mercapto-, methyl ester 31.006 208
125 2-Propanoic acid, 3-(2,4-dimethoxyphenyl), (E)- 31.006 208
126 2,5-Dimethylcinnamic acid 31.006 208
127 2-Propanoic acid, 3-(2,3-dimethoxyphenyl), (E)- 31.006 208
128 trans-2,5-Dimethylcinnamic acid 31.006 208
129 3,5-Dimethylcinnamic 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 Cyclopropane-1-pentanoic acid, 2-undecyl-, methyl ester, trans- 32.161 310
133 Oxiranoundecanoic acid, 3-pentyl-, methyl ester, cis- 32.161 312
134 Cyclopropane-dodecanoic acid, 2-octyl-, methyl ester 32.161 366
135 Oxiranoundecanoic 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 32.161 268
139 14-Methylpentadec-9-enoic acid methyl ester 32.161 268
140 Methyl 9-icosenoate 32.161 324
141 Acetic acid, 2,2-diacetylamino-1-methyl-1-propenyl ester 33.480 213
142 6,6-Dimethyl-1,4-dioxo-2-pentene 33.480 168
143 1-Nitro-$\beta$-d-arabinofuranose, tetraacetate 33.480 363
144 1-Nitro-2-acetamido-1,2-dideoxy-$\beta$-glucitol 33.480 252
145 NN-Diethyl-N-[1-naphthyl]ethylenediamine 33.480 242
146 DL-Leucine, N-DL-leucyl- 33.480 244
147 1,16-Cyclohexanone-17-oxo acid, 19,20-didehydro-, methyl ester, 33.480 322
148 1-Nitro-2-acetamido-1,2-dideoxy-$\beta$-mannitol 33.480 352
149 9-Oxobicyclo[3.3.1]nonane-2,6-dione, 2-oxime-6-ethylene ketal 33.480 213
150 Makonoldehydrazide, 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 cis-13-Octadecenoic acid, methyl ester 37.941 296
168 13-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 38.799 290
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 Octadecanoic acid, 17-methyl-, methyl ester 38.799 312
180 Myo-Inositol, 4-C-methyl- 40.163 194
181 Myo-Inositol, 2-C-methyl- 40.163 194
182 . alpha.-d-6,3-Furanose, methyl- beta.-d-glucosodialdo-1,4-fur 40.163 192
183 3-O-Methyl-d-glucose 40.163 194
184 D-Epi-Inositol, L-4-C-methyl- 40.163 194
185 3-Methylmannoside 40.163 194
186 2-O-Methyl-D-mannopyranosa 40.163 194
187 Sclavo-Inositol, L-1-C-methyl- 40.163 194
188 Methyl 4-O-methyl-d-arabinopyranoside 40.163 178
189 Hydroperoxide, 1,4-dioxan-2-yl 40.163 120
190 Heptacosane, 1-chloro- 41.134 414
191 Tritetracontane 41.134 604
192 2-methyltetracosane 41.134 408
193 Tetracosane, 11-decyl- 41.134 478
194 Tetratetracontane 41.134 618
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

REFERENCES
1. Ayitey Smith E, Addae Mensah IW. Afr J Pharmacol Drug Res 1997;4:7-8.
2. Gill LS. Ethnobotanical uses of plants in Nigeria: university of Benin press: Benin city; 1992. p. 350.
3. Banso A, Adeyemo So. Evaluation of antibacterial properties of tannins isolated from dichrostachys cinerea. Afr J Biotechnol 2007;6:1785-7.
4. Pushpangadan P, Atal CK. Ethano-medico-botanical Investigations in. Kerala I. Some primitive tribal of Western Ghats and their herbal medicine. J Ethnopharmacol 1984;11:59-77.
5. WHO, IUCN, WWF. Guidelines on the conservation of medicinal plants. Switzerland: IUCN Gland; 1993.
6. Home page on the Internet, World Health Organization. Available from: http://www.who.int/medicines/areas/traditional/definitions/en/. [Last accessed on 05 Jun 2018]
7. Jana S, shekhawat GS. Phytochemical analysis and antibacterial screening of in vivo and in vitro extracts of Indian medicinal herb: Anethum graveolens. Res J Med Plant 2010;4:206-12.
8. Levey AS, Atkins R, Coresh J. Chronic kidney disease as a global public health problem: approaches and initiatives-a position statement from kidney disease improving global outcomes. Kidney Int Aug 2007;72:247-59.