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Research Paper

Phyto Constituents in Milk Yam (*Ipomoea digitata* L.) Tuber

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A B S T R A C T

Milk yam (*Ipomoea digitata* L.) is an underutilized medicinal plant having proven pharmacological potential. Its mature tubers harvested at 21 months after planting (MAP) is rich in phytochemical constituents. This study envisages gas chromatography mass spectrum analysis of mature milk yam tubers. The plants were raised in polybags, trailed in a pandal and harvested at 21 MAP for preparing powder. GCMS analysis of the tuber powder revealed the presence of sixteen different chemical constituents of which 2-tetr-Butyl-4-isopropyl-5-methylphenol was abundantly present (8.03 per cent). The study signifies the phytochemical quality of tuber and its scope for using it as a pharmaceutical.

***Key words*:** *Ipomoea digitata*, Antidiabetic, Chemical, Essential, Phytochemical

Milk yam (*Ipomoea digitata* L.) is a medicinal climber having attractive purplish coloured bell shaped flowers, palmately five lobed leaves and tuberous roots having medicinal potential. The plants are naturalized near waterbodies, river banks, lakes, ponds etc. Milk yam tubers are pharmacologically superior with several properties including anti-diabetic, anti-cholesteromic, galactogogic, hypotensive etc. [1]. Antioxidant and anti-diabetic activity of mature milk yam tubers was proved by Sonia and Jessykutty [2]. The tubers are having brownish skin, cream-yellow flesh and when cut horizontally annulations and latex exudation can be observed. Its tubers are having 30 cm or more length, approximate 25 cm girth and more than two kilo gram weight [3]. Moreover, the nutrients rich tubers are a used for curing emaciation in children, diabetic patients and as a rasayana preparation in Ayurveda [4].

Phytochemicals in its tubers include taraxerol, taraxerolacetate, β-sitosterol, scopoletin and scopoline [5] among which the scope of using scopoletin for Alzheimer's disease [6] since, it is proved to possess acetyl choline esterase activity. This compound is isolated and quantified from milk yam tubers [7]. A few more compounds are also reported from the tubers [8-10].

As yet, spectroscopic and chromatographic analysis done for exploring the phytochemicals contained in milk yam tubers are limited. Compound isolation and structure elucidation of β-sitosterol glucoside using H-NMR and C-NMR spectroscopy [11]. FT-IR analysis of ethanolic milk yam extract [12]. Chromatographic analysis of the anti-HIV compound viz. umbelliferone [13] was also accomplished [14-15]. Sulaiman *et al*. [16] isolated and fragmented the compound caffeoyl glucose (C15H18O9) from methanolic tuber extract. GC-MS profiling of milk yam acetone extract [17] and identified palmitic acid (hexadecenoic acid) and other 26 different compounds.

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MATERIALS AND METHODS

Milk yam vines were collected from Instructional Farm, College of Agriculture, Vellayani, its herbarium specimens were made in duplicate and were got identified from internationally recognized Janaki Ammal Herbarium (RRLH) [Accession No.-23207] and authenticated from CSIR-Indian Institute of Integrative Medicine, Jammu. The vines were cut into two nodded cuttings and planted in polybags filled with potting mixture (soil: sand: farm yard manure, 1:1:1) and managed by organic management practices and its tubers were harvested at 21 months after planting. Phytochemical components and amino acid composition of the harvested mature milk yam tubers were done by following methods.

GC-MS analysis of crude methanolic extract of mature milk yam tuber

The phytochemical components in crude methanol extract of mature milk yam tuber were performed in Gas Chromatograph (Thermo-GC TRACE Ultra ver.: 5.0) coupled with a Mass Spectrophotometer (Thermo MS DSQ II). The column used was DB 35 MS capillary standard non polar column, the dimensions were 30 m, ID: 0.25 mm and film thickness: 0.25 µm. Flow rate of the mobile phase gas carrier helium was set at 1 milliliter / minute. The oven temperature was programmed initially at 70°C, then raised to 260°C at

6°C/ minute. Sample injection volume, 1 µL was utilized. Samples were dissolved in methanol and were fully run. The results were compared with the database stored in GC-MS library.

RESULTS AND DISCUSSION

GC-MS profiling of crude methanolic extract of mature milk yam tubers

GC-MS analysis of methanolic milk yam tuber extract (21 MAP) revealed the presence of sixteen different chemical constituents in it. Names of these compounds along with retention time, nature of the compound, molecular formula, molecular weight (g/mol) and peak area (%) are presented in (Table 1). It included five hydrocarbons, three fatty alcohols, three fatty acid esters, two fatty acids, one phenol, one aldehyde and an amide. The hydrocarbon compounds are 1-Hexadecene; 1-Nonadecene; Cyclotetracosane; 10-Heneicosene (c,t) and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- or Squalene. The fatty alcohol compounds were 9-Octadecen-1-ol, (Z)- or Oleyl alcohol; 1-Eicosanol and 1-Octadecanol or Sipol S or Stearyl alcohol. Fatty acid esters were 9-Octadecenoic acid (Z)-, methyl ester; 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester and cis-4,7,10,13,16,19-Docosahexaenoic acid, trimethylsilyl ester. Octanoic acid or Caprylic acid and Decanoic acid or Sebasic acid were the two fatty acids identified. The identified phenol, aldehyde and amide compounds were 2-tert-Butyl-4-isopropyl-5-methylphenol; E-15-Heptadecenal and Octanamide respectively. Among these phytochemicals, 2-tert-Butyl-4-isopropyl-5-methylphenol was abundantly present (8.03%) in optimally mature milk yam tubers. E-15-Heptadecenal and 1-Nonadecene were also found in higher amounts (4.94 and 4.31 per cent respectively), all other compounds followed it and 9-Octadecen-1-ol, (Z)- or Oleyl alcohol identified as scarce (0.3%).

Table 1 Phyto-constituents identified from crude methanolic extract of milk yam (*Ipomoea digitata* L.) tubers through GC-MS analysis

Retention time (Rt) (min)	Compound	Nature of compound	Molecular formula	Molecular weight (g/mol)	Area (%)
13.06	2-tert-Butyl-4-isopropyl-5-methylphenol	Phenol	C14H22O	206	8.03
14.32	Octanoic acid or Caprylic acid	Fatty acid	C8H16O2	144	3.71
14.85	1-Hexadecene	Hydrocarbon	C16H32	224	3.25
18.56	Decanoic acid or Sebasic acid	Fatty acid	C10H20O2	172	2.22
19.13	E-15-Heptadecenal	Aldehyde	C17H32O	252	4.94
23.14	1-Nonadecene	Hydrocarbon	C19H38	266	4.31
24.38	9-Octadecen-1-ol, (Z)- or Oleyl alcohol	Fatty alcohol	C18H36O	268	0.39
25.11	9-Octadecenoic acid (Z)-, methyl ester	Fatty acid ester	C19H36O2	296	1.82
29.87	Cyclotetracosane	Hydrocarbon	C24H48	336	2.77
31.48	1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	Fatty acid ester	C16H22O4	278	1.31
32.37	1-Eicosanol	Fatty alcohol	C20H42O	298	1.45
32.62	1-Octadecanol or Sipol S or Stearyl alcohol	Fatty alcohol	C18H38O	270	1.37
35.61	Octanamide	Amide	C8H17NO	143	1.03
35.92	10-Heneicosene (c,t)	Hydrocarbon	C21H42	294	0.46
36.24	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)- or Squalene	Hydrocarbon (tri-terpenoid)	C30H50	410	1.02
38.73	cis-4,7,10,13,16,19-Docosahexaenoic acid, trimethylsilyl ester	Fatty acid Ester	C25H40O2Si	400	0.43

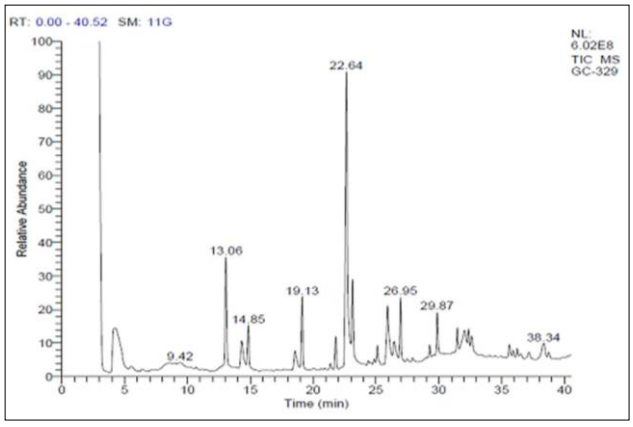

Fig 1 A typical gas chromatogram of crude methanolic extract of optimally mature milk yam (*Ipomoea digitata* L.) tuber

GC-MS profiling crude methanolic extract of mature milk yam tuber

Methanolic extract of mature milk yam tuber revealed the presence of sixteen bio-active compounds through GC-MS analysis (Fig 1). Most abundant bio-active compounds among these sixteen compounds were 2-tetr-Butyl-4-isopropyl-5-methylphenol which is a phenolic compound and a structural analogue of thymol. It is reported as an agonist of $GABA_B$ [Gamma Amino Butyric Acid 'B'] auto-receptor in central nervous system [18]. This compound was already reported from *Antigonon leptopus* leaves by [19].

The five different bio-active hydrocarbon compounds identified from crude methanolic extract of milk yam tuber were 1-Hexadecene; 1-Nonadecene; Cyclotetracosane; 10-Heneicosene (c,t) and 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all- E)- or Squalene. [20] explored out 1- Hexadecene from *Kaempferia galanga* and from *Thesium humile* by [21]. In addition, its antibacterial, antifungal and antioxidant activity were also documented. 1-Nonadecene from bark extracts (methanol, ethyl acetate and acetone) of *Solanum verbacifolium* and from *Ceropegia bulbosa* var. *bulbosa* along with the documentation of its anti-fungal activity [22]. Cyclotetracosane proved for its mosquito repellent activity by [23]. Cyclotetracosane was existed in hexane fraction of *Myristica fatua* leaves [24] and ethyl

acetate fraction of *Gynura segatum* [25]. The presence of 10-Heneicosane (c,t) from acetone extract of *Pseudarthria viscida* leaf extract and ethanolic extract of *Syzygium dhaneshiana* leaves and bark extracts respectively [26-27]. Squalene is an important bio-active compound possessing several pharmacological properties viz., immune-stimulating, hypolipidemic, cholesterol reducing, anti-carcinogenic, anti-tumor, chemo-preventive, anti-microbial and anti-inflammatory properties [28-30]. The presence of squalene from dichloro-methane root extract of *Radermachera xylocarpa* [31], in addition it was investigated from *Clerodendron infortunatum* flower extracts [32] and methanolic extract of *Ardisia elliptica* [33]. The three fatty alcohols obtained in the study were 9-Octadecen-1-ol, (Z)- or Oleyl alcohol, 1-Eicosanol and 1-Octadecanol. Presence of Oleyl alcohol was confirmed in acetone extract of *I. digitata* tubers [34] but its pharmacological activity is not yet reported. *Leea indica* reported to contain 1-Eicosanol and proved for its anti-tumor activity [35]. Anti-microbial and antioxidant activities as well [36]. 1-Eicosanol from ethanolic extract of *Micrococca mercurialis* and ethylacetate extract of *Rostellularia diffusa* [37-38]. Stearyl alcohol or 1-Octadecanol a component in several skin care products and topical applicants were identified from methanolic leaf extract of *Punica granatum* [39] and whole plant extracts (methanol) of *Isodon rugous* [40].

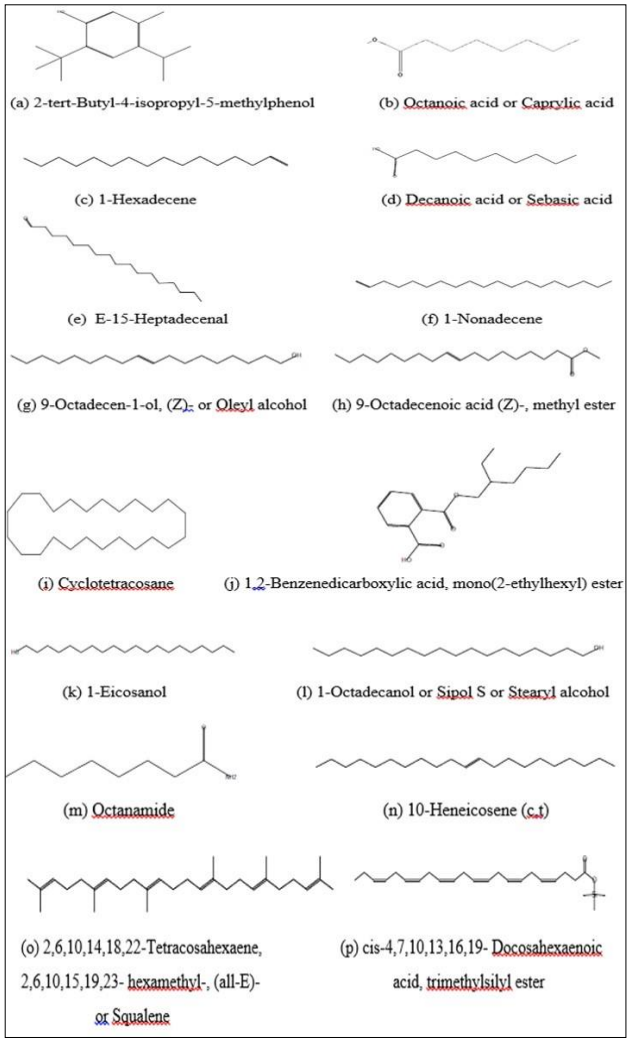

Fig 2 Chemical structure of bio-active compounds in crude methanolic extract of mature milk yam (*Ipomoea digitata* L.) tubers

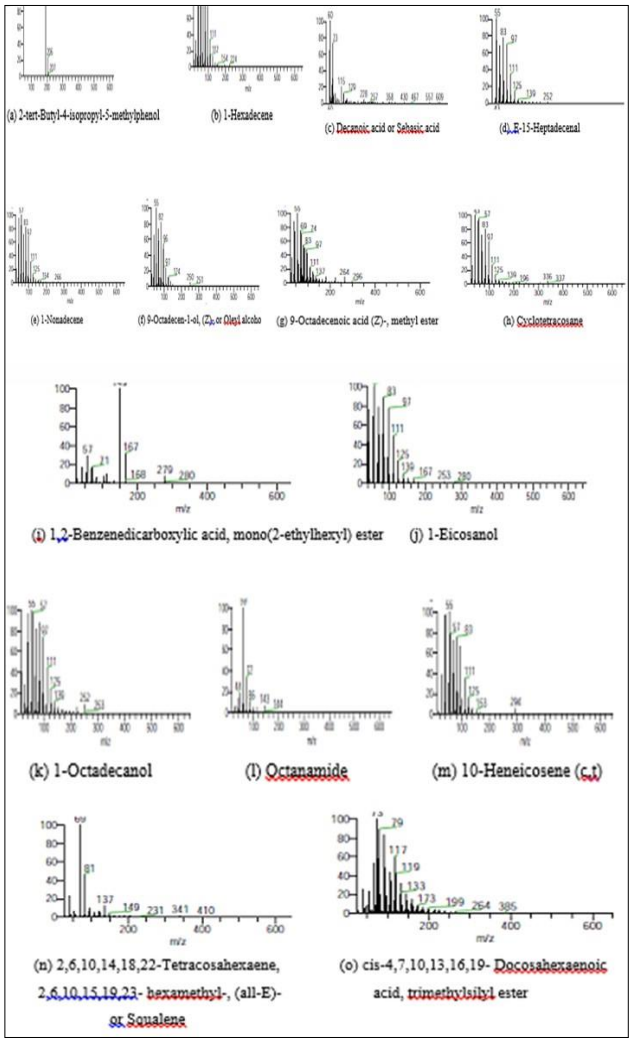

Fig 3 Mass spectrum of bio-active compounds in crude methanolic extract of mature milk yam (*Ipomoea digitata* L.) tubers

The three fatty acid esters identified in this study were 9-Octadecenoic acid (Z)-, methyl ester; 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester and cis-4,7,10,13,16,19-Docosahexaenoic acid, trimethylsilyl ester. Petroleum ether extract of *Cassia glauca* seeds were reported to contain 9-Octadecenoic acid (Z)-, methyl ester [41]. The presence of 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester in ethanolic extract of *Polygonum chinense* and methanolic fruit extract of *Terminalia chebula* respectively [42-43]. Cis-4,7,10,13,16,19-Docosahexaenoic acid, trimethylsilyl ester was identified in the methanolic leaf extract of *Adathoda vasica* [44] by Khan and Bhadauria. 9-Octadecenoic acid (Z)-, methyl ester possessed antimicrobial and nematicidal properties [45]. Cytotoxic activity of 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester against $HepG_2$ and MCF-7 cancer cell lines [46].

Octanoic acid or Caprylic acid, a constituent in mammalian milk, coconut oil, palm oil etc. was identified from optimally mature milk yam tubers too. It was also reported from *Bergenia ciliate* and *Terminalia chebula* [47] as well as [48] identified it in methanolic extract of *Euphorbia lathyrus*, its anti-bacterial activity had been documented as well. Decanoic acid or Sebasic acid is an analgesic and was

reported to be present in chloroform fraction of *Isodon rugosus* [49], *Asparagus racemosus* and *Bergenia ciliate* [50].

E-15-Heptadecenal and Octanamide were the aldehyde and amide compounds identified respectively from crude methanolic extract of mature milk yam tuber. E-15-Heptadecenal were reported to have anti-bacterial activity [51] and had already been identified from acetone extracts of *I. digitata* tubers [52] and methanolic extracts of *Cassia sophera* leaves [53]. Similarly, anti-inflammatory active fractions were reported in *Tribulus terrestris* [54], hexane extract of *Peganum harmala* [55], ethanolic extract of *Syzygium dhaneshiana* leaves [56] and *Tinospora cordifolia* methanolic stem extract (methanol) [57]. Octanamide was reported from *Crataeva nurvala* fruits [58].

To sum up, GC-MS analysis of mature milk proved that it is a potential source of bio-active phytochemicals. The abundant compound in methanolic extract of the tuber was 2-tetr-Butyl-4-isopropyl-5-methylphenol (8.03%) followed by E-15-Heptadecenal (4.94%) and 1-Nonadecene (4.31%), Octanoic acid or caprylic acid (3.71%) and 1-Hexadecene (3.25%) in relatively higher proportions. 1-Eicosanol (1.45%) and Squalene (1.02%) were the two relevant compounds possessing anti-tumor activity among the other pharmacologically beneficial compounds identified. The mass spectrum and chemical structure of the identified bio-active compounds are depicted in (Fig 2-3), respectively. Thus, the potency of milk yam tubers in providing pharmacological benefits to human beings was validated and thus can be an authoritative suggestion for the development of novel drugs.

CONCLUSIONS

GC-MS profiling of the bio-active compounds present in optimally mature (21 MAP) milk yam tubers revealed the presence of sixteen compounds including five hydrocarbons, three fatty alcohols, three fatty acid esters, two fatty acids, one phenol, one aldehyde and an amide. Phenolic compound, 2-tert-Butyl-4-isopropyl-5-methylphenol is abundantly present in it – 8.03 per cent. Milk yam tubers are a very good candidate for using as a dietary supplement since it is rich in phytoconstituents cum nutrients.

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