Original Research article

Identification of Phytoconstituents Present in *Himalayan Garcinia (Garcinia xanthochymus)–Through* GC-MS Studies

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Abstract: *Garcinia xanthochymus* (Clusiaceae) is one of important medicinal plant of this large genus that is native to some Asian countries. The plant has been traditionally used in medicines for treating a wide variety of ailments viz. diarrhoea, dysentery, nausea and vomiting and its fruits are edible and is used in a variety of ways. The present study was intended to check the phytoconstituents of the leaves of this species using GC-MS studies and to find out the biological/pharmacological activity of each of the phytoconstituent through literature corroboration. GCMS-2010 with Restek-5MS column (30m X 0.25mm film thickness 0.25µm) was used for the study. The injector temperature was 260°C. Helium was used as carrier gas with flow rate of 1.21 ml/min. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and technology (NIST), Dr. Duke's phytochemical ethnobotanical databases and previous literature studies. GC-MS studies showed a total of 35 compounds and a total of 10 major peaks were obtained. The major compounds found were Lanosterol (19.23%), Squalene (14.20%), 4,4a,6b, 8a,11,11,12b,14a-octamethyl-eicosahyd (6.14%), lupeol (3.13%), Olean-12-en-28-oic acid, 2-β-3-β-23-trih (2.33%), Stigmasterol (1.97%), Vitamin E (1.54%), Tricyclo [5.4.3.0(1,8)]tetradecan-3-ol-9-one,4-E (1.38%), Solanesol (1.34%) and N-hexadecanoic acid (1.17%). Some minor phytoconstituents such as Neophytadiene (0.89%), γ-tocopherol (0.82%), Humulane-1,6-dien-3-ol (0.80%), 2-Hexadecan-1-ol 3,7,11,15-tetramethyl R-[R](0.79%), Isolongifolol heptafluorobutyrate (0.71%) were also reported. The present study throws a light on the potential of such phytocompounds present in this species that could play a pivotal role in future herbal medicines and newer drug discoveries with minimal side effects.

Key words: *Garcinia xanthochymus,* GC-MS, lanosterol, phytoconstituent

Introduction

The genus *Garcinia* L. is the largest genus in the family Clusiaceae having more than 652 species and subspecies as recorded in the International Plant Name Index (IPNI; http://www.ipni.org). The latest estimation was of 200 species (Stevens, 2007) which is smaller than the earlier estimates of 400 species by (Whitmore, 1973; Jones, 1980). The number of accepted species for *Garcinia* is 390 with a total of 611 species along with subspecies were documented in the www.theplantlist.org. 400 species were distributed across the tropical region of Asia, Africa and Polynesia. About 200 species have been reported to be native to South Asia ranging from southern parts of the Thailand and Peninsular Malaysia to Indonesia. Out of the recorded *Garcinia* species, 35 species have been reported from India, viz. in the 'Konkan' region of Maharashtra, Goa, coastal areas of Karnataka and Kerala, evergreen forests of Arunachal Pradesh, Assam, Khasi,

Jantia Hills, West Bengal and Gujarat (Parthasarathy *et al.*, 2013). About 15 species have been reported in the North Eastern parts of India such as *Garcinia xanthochymus, Garcinia pedunculata*, *Garcinia kydia*, *Garcinia cowa* and *Garcinia lancifolia* (Parthasarathy *et al.*, 2013). A total of 09 species have been recorded in "Materials for the flora of Arunachal Pradesh. Vol-1" from Arunachal Pradesh. Some of the species of this genus such as *Garcinia cambogia*, *Garcinia indica* and *Garcinia cowa* grows mostly in a semi-wild condition (Hassan *et al.*, 2013). Traditionally they are used in garnishing curries and also as a replacement for tamarind especially in the parts of southern India (Parthasarathy *et al.*, 2013). In North Eastern India, the sundried slices of the fruits are used for culinary purposes and as folk medicine. Many species of *Garcinia* have fruit with edible arils and are eaten locally. The seeds of *G. indica* fruits yield valuable edible fat known as '*kokum butter*'. The fruits of *Garcinia* are a food source for several animals (CSIR, 1956). Most species in *Garcinia* are known for their gum resin which is used as purgative or cathartic. Fruits of some *Garcinia* species are also one of the richest sources of red pigments in the plant kingdom. They also contain a high amount of Vitamin C and is used as a heart tonic.

Among these large genus, *Garcinia xanthochymus* Hook.f. ex T. Anderson is one of the important tree species abundant in North Eastern region of India. The species is native to Polynesia, South East Asia, Africa, Australia, North Thailand, Myanmar and Yunnan of China (Hassan *et al.*, 2018). It is commonly known as '*yellow mangosteen*' and '*gamboge*'. Sometimes, it is also called '*false mangosteen*' as it fruits are similar to mangosteen but differ in colour (Murmu *et al.*, 2016). Some of the literature have also named the species as '*Himalayan Garcinia*,''*Mysore gamboge*', '*Sour mangosteen*', '*Asam kandis*' and also '*Egg tree*' (Lim, 2012). It is locally called as '*Tarak*' among the *Adi* communities of Arunachal Pradesh. The plant yields edible fruits that serves in a wide variety of uses viz. eaten fresh, as a preservative in jams, used to make yellow dye for watercolours and fabric,used to make vinegar, beverages, and other products (Acuna *et al.*, 2012, Liu *et al.*, 2016, Chen *et al.*, 2017).

Traditionally, *G. xanthochymus* has been widely used in medicines for treating diarrhoea, dysentery, nausea and vomiting (Fu *et al.*, 2012). Besides, it is also used to dispel worms and in removing food toxins (Muharni *et al.*, 2011). Owing to the rich medicinal utility of this species, at present it is being cultivated in countries such as Africa, South America & Australia while it is reported to be the most cultivated species in Brazil (Silva *et al.*, 2015). Majority of the compounds of this species have been reportedly belong to xanthones. However other classes of compounds such as flavonoids, benzophenones, depsidones are also reportedly present (Hassan *et al.*, 2013).

In spite of its high medicinal utility no such studies have been done to assess the phytoconstituents present in the leaves of this species till date. The present study is therefore mainly intended to find out the phytoconstituents of the unexplored East Himalayan varieties of *G. xanthochymus* through gas chromatography - mass spectroscopy (GC-MS) analysis and to find out the biological/pharmacological activity of each of the phytoconstituent through literature corroboration.

Materials and methods

Collection & preparation of plant material

G. xanthochymus plants were collected from Lower Dibang Valley district of Arunachal Pradesh. Herbarium was prepared following the method of Jain & Rao (1978). The specimen were authenticated at Botanical Survey of India (BSI), Arunachal Pradesh Regional Circle, Itanagar (ARUN), and the voucher specimen No. GX/042/2019 was prepared, labelled and deposited at Herbarium of Department of Botany, Rajiv Gandhi University (HAU), Rono Hills, Doimukh, Arunachal Pradesh, India for future reference. The taxonomic characters and distribution pattern was verified through standard flora such as Flora of British India (Hooker, 1872) and Materials for the Flora of Arunachal Pradesh (Chowdhury, 1996). The accepted name and synonyms were verified through website of www.theplantlist.org (The plant list, version 1.1., 2013) hosted by Royal Botanical Garden, Kew UK and Missouri Botanical Garden USA.

Sample preparation and processing for GC-MS studies

Leaves of *G. xanthochymus* were shade dried with frequent checking of any fungal, yeast or mould growth. After complete shade drying the samples were finely powdered in a grinding mill. The dried powder samples of *G. xanthochymus* were initially extracted with methanol by cold maceration process, wherein the powdered samples were soaked in methanol for 24 hours. This process was repeated twice and the filtrate was collected after filtering through Whatmann No 1 filter paper. The samples were then concentrated on a rotary evaporator (IKA Model No. GS90A24), set at temperature 70°C, rpm - 40. The concentrated samples were then further subjected to dry in water bath (I THERM Model No. BTI 57) and then in hot air oven (I THERM Model No. BTI 29) at 70°C, until the constant weight of extracts were observed. The concentrated extract was then subjected for GC-MS analysis.

Gas Chromatography- Mass Spectroscopy (GC-MS) Analysis

GC-MS analysis of the leaf extracts of the *G. xanthochymus* were carried out in Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), New Delhi. GC-MS was performed in a GCMS-2010 Shimadzu instrument operating in EI mode at 70eV with Restek-5MS column (30m X 0.25mm film thickness 0.25µm). The oven temperature was programmed as follows: kept at 60°C for 2 min, then increased to 210°C, at 3°C min^{-1} and from 210°C to 280°C at the rate of 8°C/min and held for 8min at 280°C. The injector temperature was 260°C with normal injection mode. The flow rate of carrier helium gas was 1.21ml/min. For the plant samples, the temperature program was set according to Jiang H et al., 2005. Mass spectra were acquired using full scan monitoring mode with a mass scan range of 40-650 m/z. The chromatogram and mass spectra were evaluated using the Xcalibur™ software embedded in the GC-MS/MS system. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns and Dr. Duke's phytochemical ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained. Determination of the name, molecular weight and structure of the components of the test materials were done. Comparison of average peak of each component with respect to the total areas were done to find out the relative percentage amount of each component present in the sample (Table 1).

Table 1: The list of 35 compounds identified from leaf extracts of *Garcinia xanthochymus* using GC-MS are listed below along with their molecular formula, molecular weight, retention time (min), peak area (%)

Sl No	Compound Identified	Molecular formula	Molecular Weight	Retention time(min)	Area%
1	Copaene	$C_{15}H_{24}$	204	13.449	0.11
2	Dodecanoic acid	$C_{12}H_{24}O_2$	200	16.253	0.16
3	Neophytadiene	C_20H_{38}	278	20.128	0.89
4	Hexadecanoic acid methyl ester	$C_{20}H_{38}$	278	21.238	0.15
5	N-Hexadecanoic acid	$C_{17}H_{34}O_2$	270	21.723	1.17
6	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-µl) cyclotetra	$C_{16}H_{32}O_2$	256	21.778	0.22
7	Palmitic Acid, TMS Derivative	$C_{20}H_{32}$	272	22.683	0.10
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{40}O_2$	328	23.264	0.11
9	6-Octadecenoic Acid, Methyl Ester, (Z)-	$C_{19}H_{34}O_2$	294	23.342	0.16
10	2-Hexadecan-1-ol 3,7,11,15-tetramethyl R-[R]	$C_{19}H_{36}O_2$	296	23.471	0.79
11	Cis-9-hexadecenal	$C_{20}H_{40}O$	296	23.803	0.39

12	Octadecanoic acid	$C_{16}H_{30}O$	238	24.058	0.11
13	Squalene	$C_{18}H_{36}O_2$	284	31.045	14.20
14	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-Hexa	$C_{30}H_{50}$	410	31.161	0.13
15	Solanesol	$C_{30}H_{50}O$	426	31.758	1.34
16	Geranyl linalool isomer	$C_{45}H_{74}O$	630	31.833	0.52
17	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,1	$C_{20}H_{34}O$	290	31.911	0.50
18	Isolongifolol heptafluorobutyrate	$C_{30}H_{50}O$	426	31.952	0.71
19	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-Hexa	$C_{19}H_{25}F_7O_2$	418	32.285	0.31
20	Ethyl Trans-4a,Cis-4b,trans-8a,cis-10a-Perhydro-Trans-2,4a,8a	$C_{20}H_{34}O$	290	32.545	0.22
21	β-tocopherol	$C_{30}H_{50}O$	426	32.688	0.35
22	γ-tocopherol	$C_{20}H_{32}O_2S$	336	32.837	0.82
23	1-Naphthalenol, 1,2,3,4,4a,7,8,8a-Octahydro-1,	$C_{28}H_{48}O_2$	416	33.142	0.34
24	Tetracosyl trifluoroacetate	$C_{28}H_{48}O_2$	416	33.307	0.39
25	Vitamin E	$C_{15}H_{26}O$	222	33.570	1.54
26	Phytyltetradecanoate	$C_{26}H_{49}F_3O_2$	450	33.753	0.31
27	Stigmasterol	$C_{29}H_{50}O_2$	430	35.048	1.97
28	Lupeol	$C_{30}H_{50}O$	426	36.193	3.13
29	Tricyclo [5.4.3.0(1,8)] tetradecan-3-ol-9-one,4-E (1.38%)	$C_{32}H_{52}O_2$	468	37.030	1.38
30	Lanosterol	$C_{30}H_{50}O$	426	37.343	19.23
31	17-(1,5-Dimethyl-3-Phenylsulfanyl-Hex-4-Eny	$C_{29}H_{50}O_2$	430	38.774	0.39
32	Flavone 4'-Oh,5-Oh,7-Di-O-Glucoside	$C_{27}H_{30}O_{15}$	594	39.106	0.25
33	4,4a,6b, 8a,11,11,12b,14a-octamethyl-eicosahydo- picen-3-one	$C_{36}H_{54}OS$	534	39.501	6.14
34	Olean-12-en-28-oic acid, 2β-3-3β-2,3-trihydroxy-,methyl ester	$C_{27}H_{30}O_{15}$	594	39.791	2.33
35	Humulane-1,6-dien-3-ol	$C_{30}H_{50}O$	426	39.974	0.80

Table 2. Major Compounds identified by GC–MS in the *G. xanthochymus* leaf extract and their biological activities.

Sl No	Major Compounds Identified	Biological Activities
1.	Lanosterol	Retards the exit of trapped glucose from phospholipid vesicles, protein aggregation in cataracts (Yeagle *et al*., 1977; Zhao *et al*., 2015)
2.	Squalene	emollient and antioxidant modulation of carcinogen activation (Huang *et al*., 2009; Desai *et al*, 1996)
3.	4,4a,6b, 8a,11,11,12b,14a-octamethyl-eicosahydo- picen-3-one	No activity reported
4.	Lupeol	Anticancer, antiprotozoal, anti-inflammatory, chemopreventive activities (Gallo *et al*., 2009)
5.	Olean-12-en-28-oic acid, 2β-3-3β-2,3-trihydroxy-,methyl ester	Antibacterial activity (Marwani *et al*., 1997)
6.	Stigmasterol	Used for synthesis of numerous synthetic and semi-synthetic compounds for pharmaceutical industry; acts as a precursor in the synthesis of progesterone and acts as an intermediate in the biosynthesis of androgens, estrogens, corticoids and in the synthesis of vitamin D3; Anti-osteoarthritic activity, anti-hypercholestrolemic activity, cytotoxicity, anti-tumor, hypoglycemic activity, antimutagenic activity, CNS activity antioxidant, anti-inflammatory activities (Kaur *et al*., 2011)
7.	Vitamin E	Antioxidant, have potent role in biosignaling of some specific proteins (Burton and Ingold, 1981; Fluohe and Traber, 1999)
8.	Tricyclo [5.4.3.0(1,8)] tetradecan-3-ol-9-one	No activity reported
9.	Solanesol	widely used in the pharmaceutical industry as an intermediate for the synthesis of ubiquinone drugs, such as coenzyme Q10 and vitamin K2; antibacterial, antifungal, antiviral, anticancer, antioxidant, anti-inflammatory, and anti-ulcer activities; Solanesol derivatives can also be used for the treatment of cardiovascular disease, osteoporosis, acquired immune deficiency syndrome, and wound healing (Yan *et al*., 2015).
10.	N-Hexadecanoic Acid	Antioxidant, hypo-cholesterolemic, pesticide, anti-androgenic factor (Duke, 2014)

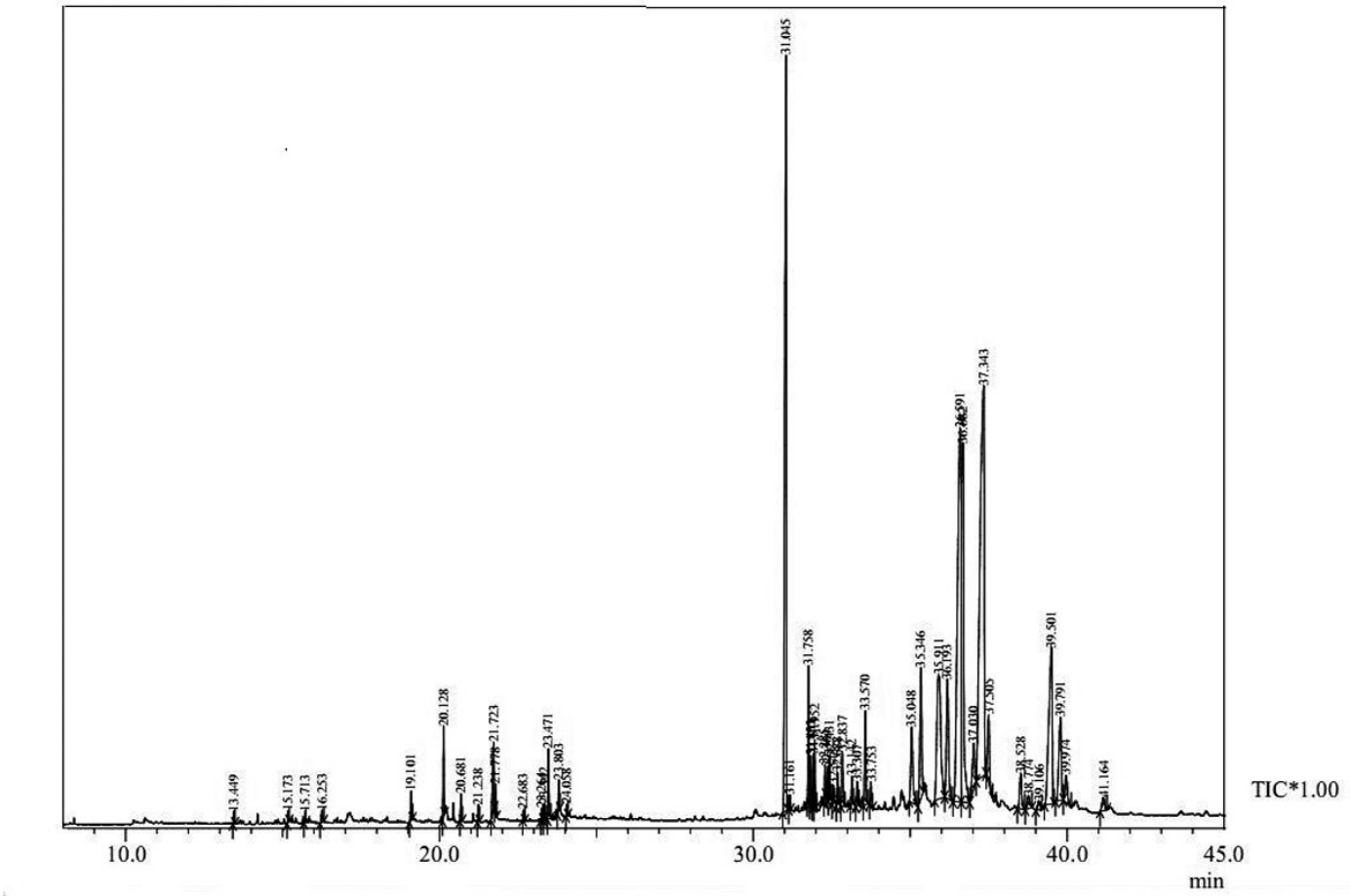
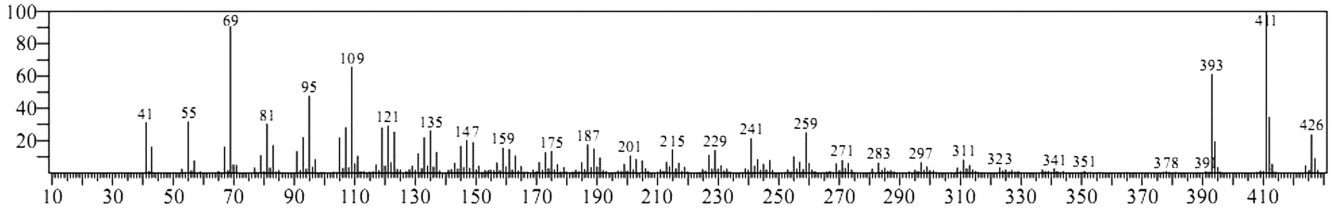
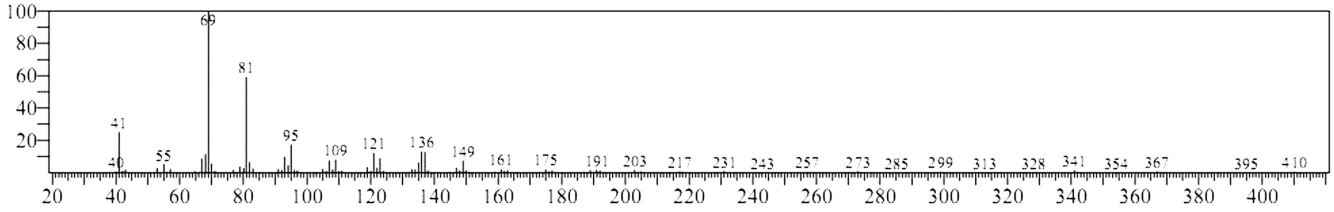


Figure 1. GC-MS peak of *Garcinia xanthochymus.*

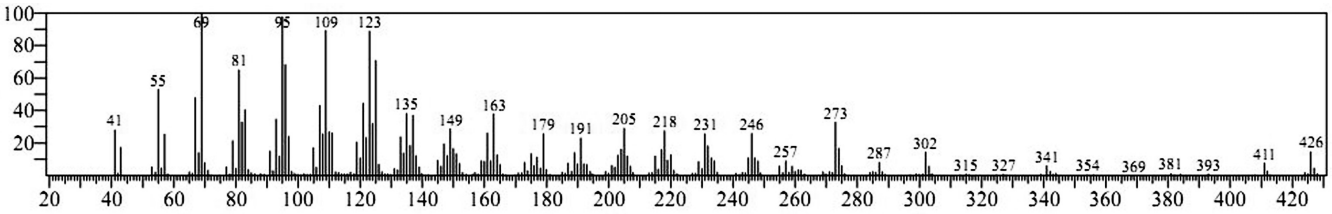
Figure 2. Habitat of *Garcinia xanthochymus.*



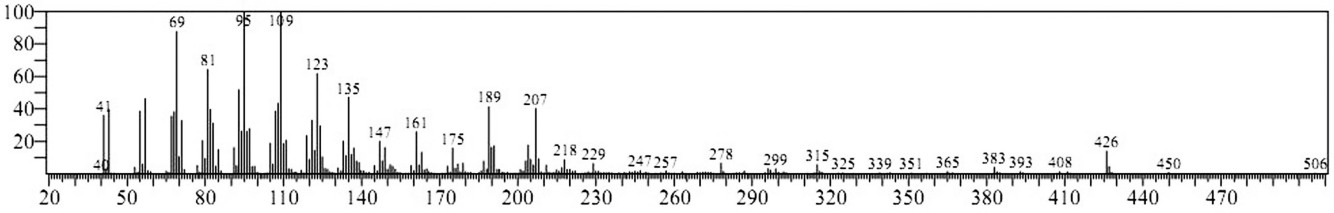
3. A. Lanosterol



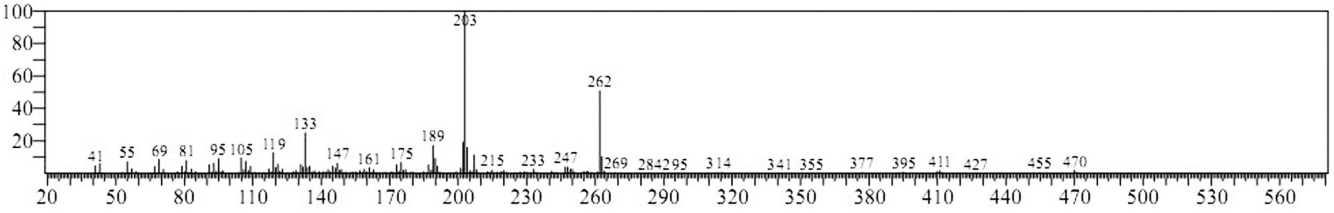
3. B. Squalene



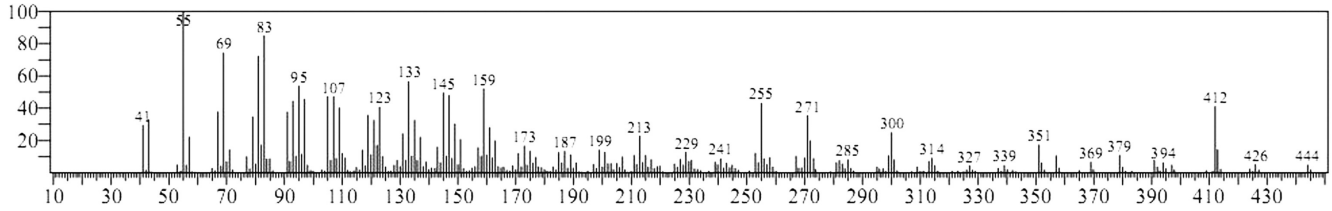
3. C. 4,4a,6b, 8a,11,11,12b,14a-octamethyl-eicosahydo- picen-3-one



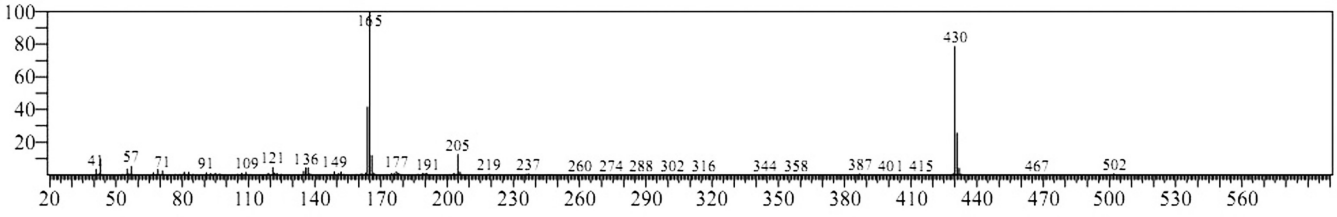
3. D. Lupeol



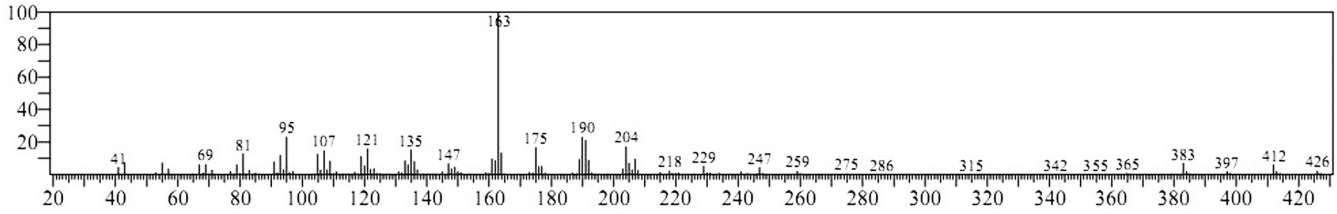
3. E. Olean-12-en-28-oic acid, 2. β 3. β .,23-trihydroxy-,methyl ester



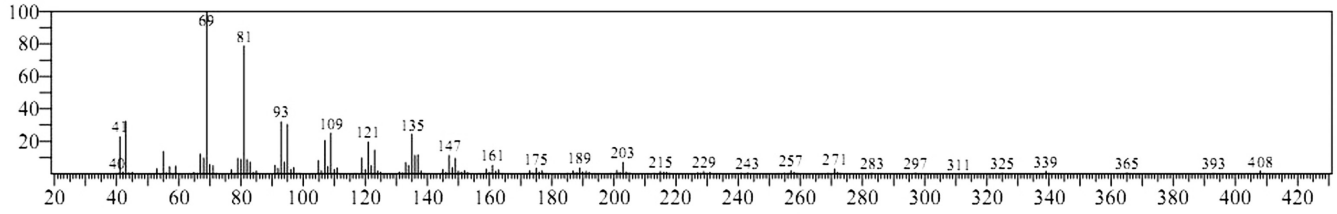
3. F. Stigmasterol



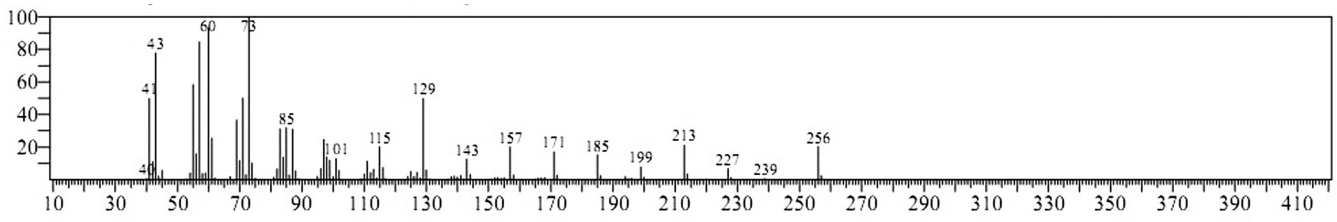
3. G. Vitamin E



3. H. Tricyclo [5.4.3.0(1,8)] tetradecan-3-ol-9-one(1.38%),



3. I. Solanesol



3. J. n-Hexadecanoic acid.

Fig. 3. Individual compounds GC-MS chromatogram of *G. xanthochymus* leaf extract.

Results

Phytochemical compounds identified by GC–MS analysis

The National Institute of Standards and Technology (NIST), Dr. Duke's phytochemical ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA and earlier literature studies were used for the identification of compounds. The GC–MS chromatogram is shown in Figure 1. A total of 35 compounds have been identified and a total of 10 major peaks are obtained showing phyto-constituents that are most abundant. The retention time, molecular formula and relative peak area percent of components in leaf extract of *G. xanthochymus*is represented in Table 2. The nature and biological activity of some of the major identified compounds of the leaves of *G. xanthochymus* is shown in Table 3. Individual major compounds spectra is shown in Fig. 3.

The major compounds found were Lanosterol (19.23%), Squalene (14.20%), 4,4a,6b, 8a,11,11,12b,14a-octamethyl-eicosahyd (6.14%), Lupeol (3.13%), Olean-12-en-28-oic acid, 2-β-3-β-23-trih (2.33%), Stigmasterol (1.97%), Vitamin E (1.54%), Tricyclo [5.4.3.0(1,8)]tetradecan-3-ol-9-one,4-E (1.38%), Solanesol (1.34%) and n-hexadecanoic acid (1.17%). Lanosterol was found to be the most abundant among the compounds found in the leaves of *G. xanthochymus.* Some

minor phytoconstituents viz. Neophytadiene (0.89%), γ-tocopherol (0.82%), Humulane-1,6-dien-3-ol (0.80%), 2-Hexadecan-1-ol 3,7,11,15-tetramethyl R-[R](0.79%), Isolongifolol heptafluorobutyrate (0.71%) were also reported.

Discussion

Phytoconstituents of medicinal plants have a variety of roles in giving a plant defence against different environmental stress and harmful and invasive pathogens. Their medicinal value is due a diverse range of different classes of secondary metabolites viz. alkaloids, carbohydrates, glycosides, phenols, flavonoids, saponins, steroids, and tannins compounds. These phytochemicals have great potentialities in drug discovery for curing a wide range of diseases (Rajshekharan, 2002). Different secondary metabolites of the target plant can be determined by using sophisticated chromatographic techniques like HPLC, UPHPLC, LCMS etc. GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids, esters etc. Besides GC/MS has other advantages like better chromatographic resolution and non-selective nature.

The GC-MS analysis of *G. xanthochymus* leaves revealed the presence of a total of 35 compounds (phytochemical constituents) that could contribute to the medicinal potential of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. GC-MS studies revealed that the leaves of *G. xanthochymus* are a rich source of lanosterol that have roles in aggregation of proteins in a ordered interactive macro-structure form in the crystalline proteins of the human lens (Yeagle *et al*., 1977; Zhao *et al*., 2015). So it could be possible that this plant might play a role in formulation of some herbal medicine that could potentially target certain genes that disrupt this ordered aggregation of the proteins. The present findings points to a novel strategy for cataract prevention and treatment. Moreover certain other phytoconstituents such as squalene have roles as emollient and antioxidant modulation of carcinogen activation (Huang *et al*., 2009; Desai *et al*., 1996). Olean-12-en-28-oic acid, 2β-3-3β-2,3-trihydroxy-,methyl esterhave reportedly shown antibacterial activity (Marwani *et al*., 1997) of Lupeol that has anticancer, antiprotozoal, anti-inflammatory, chemopreventive activities (Gallo *et al*., 2009) and solanesol which besides having antibacterial, antifungal, antiviral, anticancer, antioxidant, anti-inflammatory, and anti-ulcer activitiesis also widely used in the pharmaceutical industry as an intermediate for the synthesis of ubiquinone drugs, such as coenzyme Q10 and vitamin K2. Solanesol derivatives can also be used for the treatment of cardiovascular disease, osteoporosis, acquired immune deficiency syndrome, and wound healing (Yan *et al*., 2015). Some of the other major compounds viz. 4, 4a, 6b, 8a, 11, 11, 12b, 14a-octamethyl-eicosahydo- picen-3-one and tricyclo [5.4.3.0(1,8)] tetradecan-3-ol-9-one were also found that have no reported biological activity till date.

Conclusion

The present study is thus first of its kind to assess the phytoconstituent of this plant from Arunachal Himalayan region. The study provides a baseline for further isolation and purification of individual compounds of this species so as to understand the mechanisms and pathways that those compounds follow to give the desired pharmacological effects. This will help in formulation of future herbal medicines and may also lead to new drug discoveries having minimal side effects.

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