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Research Article

Phyto-chemical Characterization of Aeschynanthus sikkimensis (Clarke) Stapf. (Gesneriaceae) using GC-MS

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ABSTRACT
This study was implement to actuate the chemical components of Aeschynanthus sikkimensis (Clarke) Stapf. whole plant using Gas Chromatography–Mass Spectrometry, our results of GC-MS compounds in the extract was relevant to the National Institute of Standards and Technology (NIST) library. GC-MS analysis of methanol extract of Aeschynanthus sikkimensis (Clarke) Stapf leaves confess the presence pyridine, tetradecane, 2,4-Di-tert-butylphenol, hexadecane, Tridecanoic acid, 12-methyl-, methyl ester, octadecane, 13-methyltetradec-9-enoic acid methyl ester, neophytadiene, 2-Pentadecanone - 6,10,14-trimethyl, 9,12-Octadecadienoic acid (Z,Z)-, hexadecanoic acid, methyl ester, n-Hexadecanoic acid, eicosane, hexadecanoic acid, 14-methyl-, methyl ester, palmitic Acid, TMS derivative, methyl 10-trans,12-cis-octadecadienoate, 13-Octadecenoic acid, methyl ester, 11,13-Dimethyl-12-tetradecen-1-ol acetate, phytol (Figure 11), methyl stearate and oleic acid. This study result will make a way for the production of herbal medicines for various ailments by using Aeschynanthus sikkimensis (Clarke) Stapf.

Keywords: Bioactive compounds, Aeschynanthus sikkimensis (Clarke) Stapf, GC-MS, Phytol

INTRODUCTION
The most expensive gift nature has given to human are herbal plant. Secondary products combination is distinct in a particular plant species, making plant unique to its medicinal action. The phyto chemical presents in plant extract target the bio chemical pathways making it a safer medicine. With increasing side effect of the synthetic drugs, the plant based herbal drugs are becoming more promising. In recent decades, studies on phytochemical constituents of medicinal plant and its pharmacological activities have received wide attention [1].

Many modern drugs are the synthetic analogues of natural compound found in plant and about 20% to 30% of modern drugs are derived from plant sources [2]. India with 45,000 plant species is one of the 12 mega biodiversity centres in the world due to 16 different agro-climatic zones, 10 vegetative zones and 15 biotic provinces. It is mentioned in the ancient text book that around 1500 plants are having medicinal uses and 800 plants have been used as traditional medicine [3].

Aeschynanthus sikkimensis (Clarke) Stapf. is an epiphytic under shrub in the Gesneriaceae family that grows in Himalaya at an altitude of 5000-7000ft above sea level in north east India, Nepal and Bhutan. Rootstock leaves and flowers are widely used traditionally for fever, throat pain and tonsillitis [4]. A detailed literature review on the plant under investigation has shown that so far, there are no published reports on the chemical components of Aeschynanthus sikkimensis (Clarke) Stapf. GC-MS studies were undertaken to explore the phytochemical constituents present in Aeschynanthus sikkimensis (Clarke) Stapf.

MATERIAL AND METHOD
Plant Material
The plant Aeschynanthus sikkimensis (Clarke) Stapf. was collected from the hill region of Sikkim, India. The authenticity of the plant species was identified by Botanical Survey of India, Gangtok, Sikkim by referring the deposited specimen. The voucher number of the specimen is SHRC-5/02/2012-Tech 88. Voucher specimen has been deposited at the Department of Pharmaceutical

Technology, Jadavpur University, Kolkata, India for further reference.

Preparation of Crude Extract

The whole plant was washed properly and shade-dried at room temperature (24–26 °C) with occasional shifting and then powdered mechanically into coarse powder. The powdered plant material (200 g) was macerated with methanol (450ml) at room temperature (24–26°C) for 4 days with occasional shaking, followed by re-maceration for 3 days. After filtration, the filtrate was evaporated at 30 °C under reduced pressure in a rotary evaporator (Buchi R-210). The extract was re dissolved with methanol for GC-MS analysis.

GC – MS Analysis

The Gas Chromatography – Mass Spectroscopy (GC - MS) analysis of methanol extract was performed by using GC-MS (Agilent 7890 B Models 5977A; Agilent). Extract were mixed with methanol, sonicated in a bath sonicator and chromatographic separation was achieved on the fused-silica capillary column HP-5MS (30m x 0.25mm diameter and film thickness of 0.25μm). Flow rate of mobile phase (Carrier gas: helium) was set at 0.8ml/min in a constant flow rate mode. The injector temperature was 270^{0}C and the injector was operated in split mode with a 10:1 ratio. The oven temperature was maintained at 80^{0}C. For 1minute; programmed at 10^{0}C per minute to 200^{0}C and 20^{0}C per minute to 270^{0}C.

Identification of Compound

Interpretation on GC mass spectrum was conducted using the database of National Institute Standard and Technology (NIST) having more than 62, 000 patterns. 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.

RESULT

The numerous interesting compounds with different retention times were found through gas chromatograms in Aeschynanthus sikkimensis (Clarke) Stapf. The identification of the compounds was done through mass spectrometry attached with GC. The GC-MS study depicted the presence of 21 compounds. Among of the 21 compounds presence in the methanol extract of Aeschynanthus sikkimensis (Clarke) Stapf. 11 compounds have been reported earlier with their biological activities. The compounds identified through GC-MS along with their molecular formula, molecular weight, retention time, biological activities and the nature of the compound in the methanol extract of Aeschynanthus sikkimensis (Clarke) Stapf. are given in Table 1.

The prevailing compounds in methanol extract were pyridine, tetradecane (Figure 2), 2,4-Di-tert-butylphenol (Figure 3), hexadecane (Figure 4), Tridecanoic acid, 12-methyl-, methyl ester, octadecane, 13-methyltetradec-9-enoic acid methyl ester, neophytadiene (Figure 5), 2-Pentadecanone - 6,10,14-trimethyl (Figure 6), 9,12-Octadecadienoic acid (Z,Z)- (Figure 7), hexadecanoic acid, methyl ester (Figure 8), n-Hexadecanoic acid (Figure 9), eicosane (Figure 10), hexadecanoic acid, 14-methyl-, methyl ester, palmitic Acid, TMS derivative, methyl 10-trans,12-cis-octadecadienoate, 13-Octadecenoic acid, methyl ester, 11,13-Dimethyl-12-tetradecen-1-ol acetate, phytol (Figure 11), methyl stearate and oleic acid (Figure 12).

Table 1: Bioactive compounds identified in the methanol extract of *Aeschynanthus sikkimensis (Clarke) Stapf.*

SL. NO	Name of the compound	Molecular formula	Molecular Weight (g/mol)	Retention Time	Compound nature	Activity
1	Pyridine	C_5H_5N	79.1	3.131	Heterocyclic	
2	Tetradecane	$C_{14}H_{30}$	198.39	9.643	Alkane hydrocarbon	Antifungal and Antibacterial[5]
3	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32	10.588	Lipophilic Phenol	Antioxidant activity, active Antibacterial against Pseudomonas aeruginosa

						and Staphylococcus aureus, prevention the fungal mycelial growth of Aspergillus niger, Fusarium oxysporum and Penicillium chrysogenumon[6-8]
4	Hexadecane	$C_{16}H_{34}$	226.44	11.326	Alkane hydrocarbon	Antifungal, Antibacterial, Antioxidant activity[9]
5	Tridecanoic acid, 12-methyl-, methyl ester	$C_{15}H_{30}O_2$	242.4	12.277		No activity reported
6	Octadecane	$C_{18}H_{38}$	254.5	12.84	Hydrocarbon	
7	13-Methyltetradec-9-enoic acid methyl ester	$C_{16}H_{30}O_2$	254.41	12.896		
8	Neophytadiene	$C_{20}H_{38}$	278.5	13.103	Sesquiterpenoids	Antimicrobial, Analgesic, Antipyretic, Anti inflammatory[10-11]
9	2-Pentadecanone, 6,10,14-trimethyl	$C_{18}H_{36}O$	268.5	13.146		Antibacterial activity against Gram +ve and Gram-ve bacteria[12]
10	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.4	13.409	Linoleic acid	Antioxidant[13], Antiarthritic and Anti inflammatory[14] Anti-inflammatory and antia
11	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	13.703	Fatty acid methyl ester	Antibacterial and Antifungal[15] Antioxidant, hypocholesterolemic[17]
12	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	13.922	Palmitic acid	Antioxidant, Pesticide, Flavor, 5-Alpha reductase-inhibitor, Antifibrinolytic, Hemolytic, Lubricant, Nematicide and Antialopecic[16] Anti inflammatory[17] and Anti cancer[18]
13	Eicosane	$C_{20}H_{42}$	282.5	14.21	Alkane	Antioxidant and Antidiabetic[19]
14	Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	284.5	14.366	-	
15	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	328.6	14.472	Fatty acid	
16	Methyl 10-trans,12-cis-octadecadienoate	$C_{19}H_{34}O_2$	294.5	14.848		
17	13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.5	14.892		No activity reported
18	11,13-Dimethyl-12-tetradecen-1-ol acetate	$C_{18}H_{34}O_2$	282.5	14.967	Acetate compound	No activity reported
19	Phytol	$C_{20}H_{40}O$	296.5	14.973	Diterpene	Deceases the autoimmune response and to ameliorate both acute and chronic phases of arthritis[20] Antioxidant[21]
20	Methyl stearate	$C_{19}H_{38}O_2$	298.5	15.067	Fatty acid	
21	Oleic Acid	$C_{18}H_{34}O_2$	282.5	15.148	Fatty acid	Increase insulin production and to reverse the inhibitory insulin effect of TNF-α.[22]

Figure 1 GC-MS chromatogram of the methanol extract of *Aeschynanthus sikkimensis (Clarke) Stapf.*

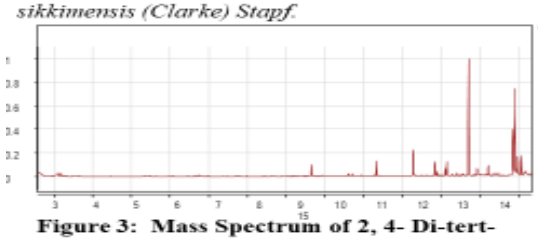


Figure 2: Mass Spectrum of Tetradecane

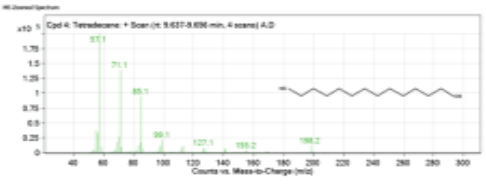


Figure 3: Mass Spectrum of 2, 4- Di-tert-butylphenol

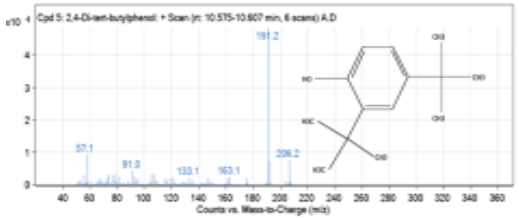


Figure 4: Mass Spectrum of Hexadecane

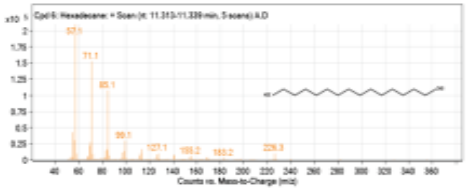


Figure 5: Mass Spectrum of Neophytadiene

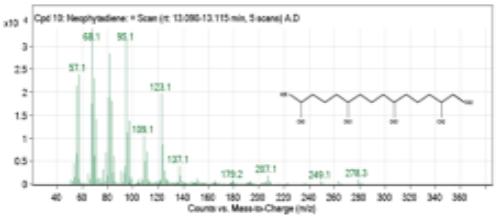


Figure 6: Mass Spectrum of 2-Pentadecanone, 6,10,14-trimethyl-

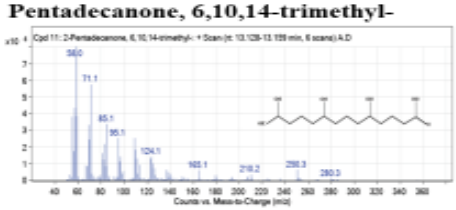


Figure 7: Mass Spectrum of 9, 12-Octadecadienoic acid (Z,Z)-

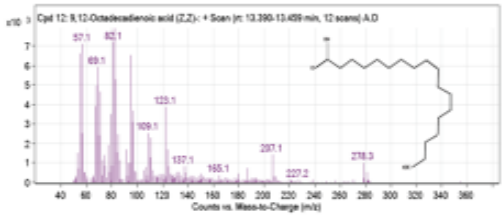


Figure 8: Mass Spectrum of Hexadecanoic acid, methyl eater

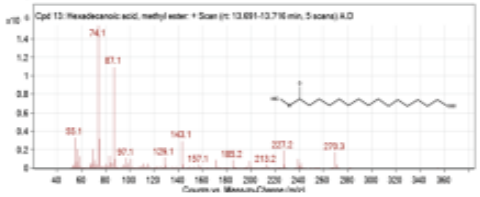


Figure 9: Mass Spectrum of n-Hexadecanoic acid

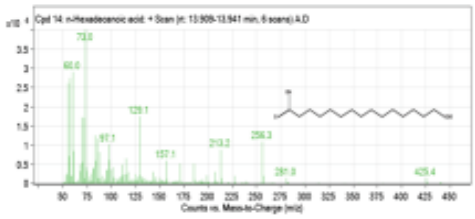


Figure 10: Mass Spectrum of eicosane

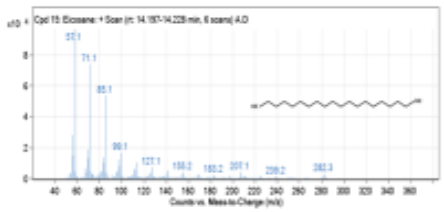


Figure 11: Mass Spectrum of Phytol

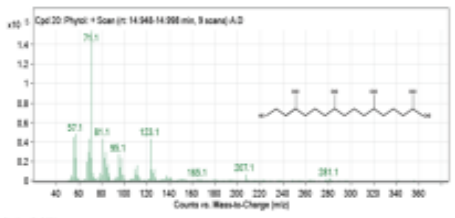
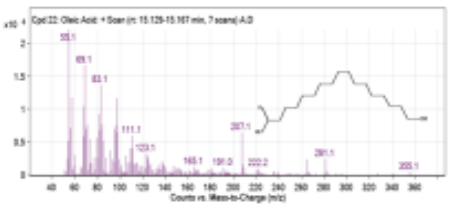


Figure 12: Mass Spectrum of Oleic acid



DISCUSSION

Among the identified phytochemical lipophilic phenol compound, 2, 4-Di-tert-butylphenol is reported to have antioxidant activity, active antibacterial [5-8]. Alkane hydrocarbon, hexadecane is reported to have antifungal, antibacterial and antioxidant activity [9]. Sesquiterpenoids, neophytadiene has antimicrobial, analgesic, antipyretic and anti-inflammatory properties [10-11]. 2-Pentadecanone, 6, 10, 14-trimethyl posses antibacterial activity [12]. The linoleic acid present in the extract 9, 12-Octadecadienoic acid (Z,Z)- has been reported to have antioxidant [13], Antiarthritic and anti inflammatory activities [14]. The other linoleic acid ester hexadecanoic acid, methyl ester are reported to have hypocholesterolemic property [15]. Palmitic acid, n-Hexadecanoic acid has been reported for antialopecic [16], anti inflammatory [17] and anti cancer [18]. Eicosane is reported to have antioxidant and antidiabetic activity [19]. Phytol ameliorates both acute and chronic phases of arthritis [20]. Increase insulin production and reverse the inhibitory insulin effect of TNF- α by oleic acid [21-22].

CONCLUSION

GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of Aeschynanthus sikkimensis (Clarke) Stapf for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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