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GC-MS INVESTIGATION OF PHYTOCOMPONENTS PRESENT IN ETHANOLIC EXTRACT OF PLANT *ICHNOCARPUS FRUTESCENS* (L.) W. T. AITON AERIAL PART

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Keywords:

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ABSTRACT: *Ichnocarpus frutescens* (Apocynaceae) generally renowned as Kali Sariva in Sanskrit, Krishna Sariva in Hindi which is a large, evergreen, red woody climber, native to India, Java, China, Southeast Asia, Ceylon, Northern Australia and found ascending to an altitude of 4,000 ft. Different tribes of India are used this plant as a substitute of Indian Sarsaparilla (*Hemidesmus indicus*). It has been used conventionally in various diseases and disorders of human being that is a headache, wound, fever, tongue ulcers, cramps, night blindness, stomach pain, bone fracture, skin infection, diabetes, liver disorders, as alterative, tonic, diuretic and diaphoretic. Pharmacologically plant shows different activities include anti-diabetic, anticarcinoma, antiurolithiatic, antiobesity, analgesic, antipyretic, anti-inflammatory, skeletal muscle relaxant, antitumor, hepatoprotective. The present study was aimed to identify the phytocomponents present in *I. frutescens* using GC-MS analysis. The aerial plant part was extracted successively by solvents according to their increasing order of polarity using Soxhlet apparatus. The phytocomponents of ethanolic extract was identified by GC-MS investigation and found 19 number of phytocomponents. The major constituents of the extracts were 3-O-Methyl-d-glucose, 3, 4, 6-Tri-O-methyl-d-glucose, 2-O-Methyl- D-mannopyranose, Myo-In-ositol, 4-C-methyl and Myo-In-ositol, 2-C-methyl. This study is the prime characterization of the phytocomponents of plant data that indicates the ethanolic extract has remarkable activities.

INTRODUCTION: In various traditional systems of medicine, plants are used as a remedy for different ailments. In general, plants are utilized by Vaidya, tribals and local healer as medicine and are improved by time to time for better efficacy. Different species of plant restrain a huge number of phytocomponents which have medicinal values but are a virgin till date.

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Ichnocarpus frutescens (Linn.) W. T. Aiton (Apocynaceae) is well-known as Krishna Sariva in Ayurvedic system of medicine [1]. It is a large, evergreen, laticiferous, woody creeper with rusty red appearance, found almost all over India, ascending to an altitude of 4,000 ft [2] and also found in Ceylon, China, Java and Australia [3]. The plant root is used as an alternative of Indian sarsaparilla (*Hemidesmus indicus*).

Conventionally the plant has been used in headache, wound, fever [2], tongue ulcers, cramps, night blindness, headache [4], bone fracture, skin infection, diabetes and liver disorders [5]. Pharmacologically plant showed different activities include antidiabetic, anticarcinoma, antiurolithiatic,

anti-obesity, analgesic, antipyretic, anti-inflammatory, skeletal muscle relaxant, antitumor, hepatoprotective [6, 7, 8] anticonvulsant activity [9]. The plant may show different activity due to the presence of phytocomponents phenylpropanoids, phenolic acids, coumarins, flavonoids, sterols and pentacyclic triterpenoids, *i.e.* Δ^{12}-dehydrolupanyl-3β-palmitate, lupeol acetate, friedelin, friedelinol, Δ^{12}-dehydrolupeol, oleanolic acid, nonane, 5-hydroxyoctacosan-25-one, dotriacontanoic acid, sitosterol and sitosterol palmitate [10]. With this background, the present study was aimed to identify the phytocomponents from the ethanolic extract of *I. frutescens* aerial part by using GC-MS investigation.

MATERIAL AND METHODS:

Collection and Preparation of Plant Materials: Fresh aerial parts of the plant were collected from an adjoining area of Barpali, Odisha, India and identified by Botanical Survey of India, Kolkata, India, bearing Ref. no: CNH/I-I(5)/2009/Tech. II/35. The aerial parts of the plant are dried under shade and pounded into powder using a mechanical grinder. The powdered material was stored in an airtight container until use.

Preparation of Extract: The powdered material was extracted with petroleum ether (60-80 °C), chloroform, ethyl acetate and ethanol successively in the Soxhlet apparatus. The extracts thus obtained were concentrated in a rotary evaporator and stored in the refrigerator at 4 °C for further use. The extracts were employed for GC-MS analysis.

GC-MS Investigation: The investigation of *I. frutescens* ethanolic extract was performed by using GC-MS instrument Thermo Trace 1300GC coupled with Thermo TSQ 800 Triple Quadrupole MS. The investigational circumstance of GC-MS system was as the following condition; TG 5MS (30 m × 0.25 mm ID × 0.25 µm) column composed of 5% phenyl methylpolysiloxane, helium gas was used as carrier gas at a constant flow rate of 1.0 ml/min and an injection volume of 1.0 µl was employed (split ratio of 10:1, injector temperature operated at 250 °C; ion-source temperature 280 °C and the oven temperature was programmed from 60 °C (isothermal for 2 min.) with an increase of 10°C/ min. to 280 °C (isothermal for 10 min.). Mass spectroscopy (MS TSQ 8000) was taken at 70 eV; a scanning interval of 0.5 sec and fragments from 40 to 550 Da.

Identification of Compounds: The phyto-components were identified by interpretation of mass-spectrum with the library data of National Institute standard and Technology (NIST). The name, molecular formula and molecular weight of the components were established.

RESULTS: The GC - MS chromatogram of the *I. frutescens* ethanolic extract showed the presence of nineteen compounds. The identified phyto-components name, retention time (RT), peak area (%), molecular formula, molecular weight, nature of compound and activities are presented in **Table 1**.

TABLE 1: PHYTOCOMPONENTS IDENTIFIED IN THE ETHANOLIC EXTRACT OF *I. FRUTESCENS* AERIAL PART BY GC-MS

RT	Name of the compound	Molecular formula	Molecular weight	Peak area%	Nature of compound	Activity [11, 12]
7.85	Dodecane	$C_{12}H_{26}$	170.34	4.34	Alkane	Enhances antifungal activity
7.85	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212.421	4.34	Alkane	Antifungal, antibacterial activities
7.85	Undecane	$C_{11}H_{24}$	156.313	4.34	Alkane	Mild sex attractant for various types of moths & cockroaches, ants
9.77	Tetradecane	$C_{14}H_{30}$	198.394	4.62	Alkane	Antimicrobial, cytotoxicity, antipyretic, anthelmintic, tumor, bronchitis, asthma, tuberculosis, dyspepsia, constipation, anemia, throat diseases, elephantiasis, anti-diabetic, anti-inflammatory, anti-diarrhoeal
9.77	Pentadecane	$C_{15}H_{32}$	212.421	4.62	Alkane	Antibacterial activities
12.26	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.183	49.55	Methylated sugar	Preservative
12.26	3,4,6-Tri-O-methyl-d-glucose	$C_9H_{18}O_6$	222.237	49.55	Methylated sugar	Not reported

12.26	2-O-Methyl- D-mannopyranosa	$C_7H_{14}O_6$	194.183	49.55	Methylated sugar	Not reported
12.64	Myo-In-ositol, 2-C-methyl (Isomytilit)	$C_7H_{14}O_6$	194.183	17.83	Inositol	Not reported
12.64	Myo-In-ositol, 4-C-methyl	$C_7H_{14}O_6$	194.183	17.83	Inositol	Not reported
14.31	Hexadecanoic acid, ethyl ester (Ethyl Palmitate)	$C_{18}H_{36}O_2$	284.484	4.05	Palmitic acid ester	Antioxidant, nematicide, pesticide, anti-androgenic, flavor haemolytic, 5alpha reductase inhibitor
14.31	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	270.457	4.05	Fatty acid ethyl ester	Not reported
14.31	Hexadecanoic acid, 2-methyl, methyl ester	$C_{18}H_{36}O_2$	284.477	4.05	Fatty acid methyl ester	Not reported
15.10	Phytol	$C_{20}H_{40}O$	296.539	4.66	Terpene Alcohol	Antimicrobial, anti inflammatory, antinociceptive activity, anticancer, antioxidant, anti-diuretic, immune-stimulatory and anti-diabetic
15.10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.531	4.66	Diterpene	Antimicrobial, anti inflammatory, anticancer, diuretic, antifungal against *S. typhi*, resistant gonorrhea, joint dislocation, hernia headache, stimulant, anti-malarial, anti-diabetic
15.10	1-Hexadecen-3-ol,3,5,11,15-tetramethyl (Isophytol)	$C_{20}H_{40}O$	296.531	4.66	Diterpene	Not reported
19.66	13-Docosenamide,(Z)	$C_{22}H_{43}NO$	337.582	14.95	The amide of docosenoic acid	Reduced mobility, slightly lessened awareness in rats
19.66	9-Octadecenamide,(Z)	$C_{18}H_{35}NO$	281.476	14.95	The amide of octadecenoic acid	Induces physiological sleep
19.66	8-Methyl-6-nonenamide	$C_{10}H_{19}NO$	169.264	14.95	Amide compound	Antimicrobial, anti-inflammatory

The phytocompound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service, USDA. The results showed the presence of dodecane, 2,6,11-trimethyl, undecane, tetradecane, pentadecane, 3-O-Methyl-d-glucose, 3, 4, 6- Tri-O-methyl-d-glucose, 2- O-Methyl- D-mannopyranosa, Myo-In-ositol, 2-C-methyl (Iso-mytilit), Myo-In-ositol, 4-C-methyl, hexadecanoic acid, ethyl ester (Ethyl palmitate), pentadecanoic acid, ethyl ester, hexadecanoic acid, 2-methyl, methyl ester, phytol, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, 1-Hexadecen-3-ol, 3, 5, 11, 15-tetramethyl (Isophytol), 13-Docosenamide,(Z), 9-Octadecenamide, (Z), 8-Methyl-6-nonenamide. The spectrum profile of GC-MS confirmed the presence of 19 components and the chromatogram of the phytocomponents are illustrated in **Fig. 1-7**.

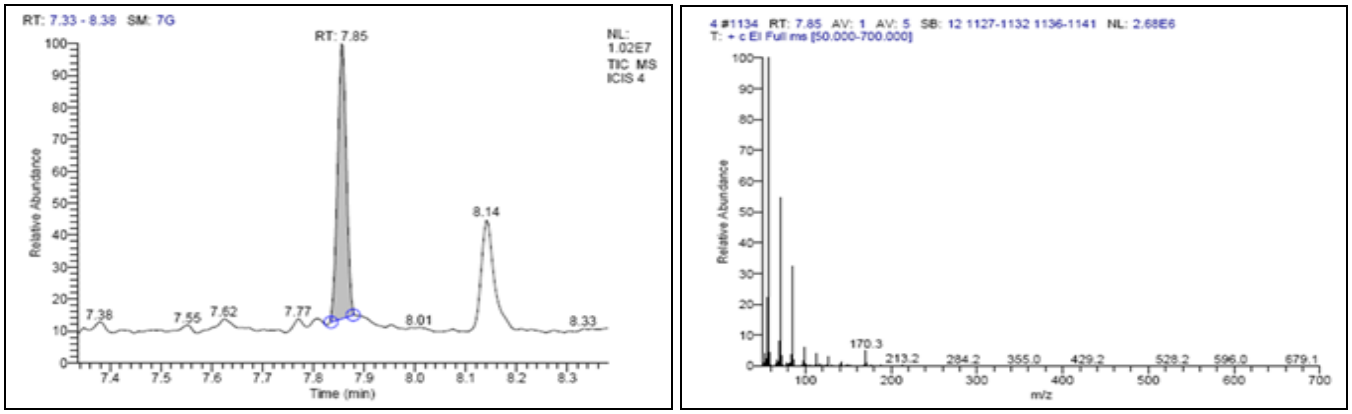

FIG. 1: GC-MS CHROMATOGRAM OF Dodecane, Dodecane, 2, 6, 11-trimethyl and Undecane (RT 7.85)

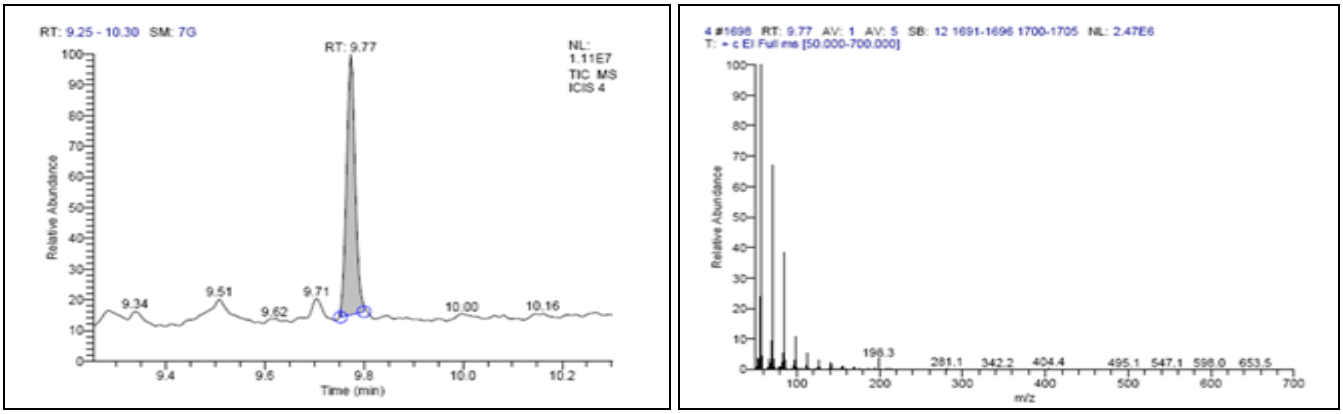

FIG. 2: GC-MS CHROMATOGRAM OF Tetradecane, Pentadecane (RT 9.77)

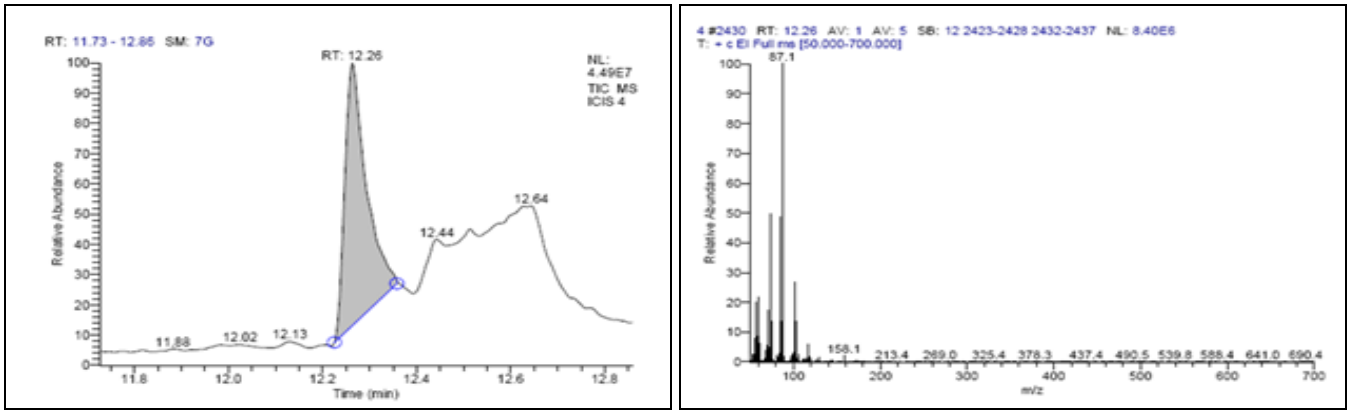

FIG. 3: GC-MS CHROMATOGRAM OF 3-O-Methyl-d-glucose, 3, 4, 6- Tri-O-methyl-d-glucose, 2- O-Methyl- D-mannopyranosa (RT 12.26)

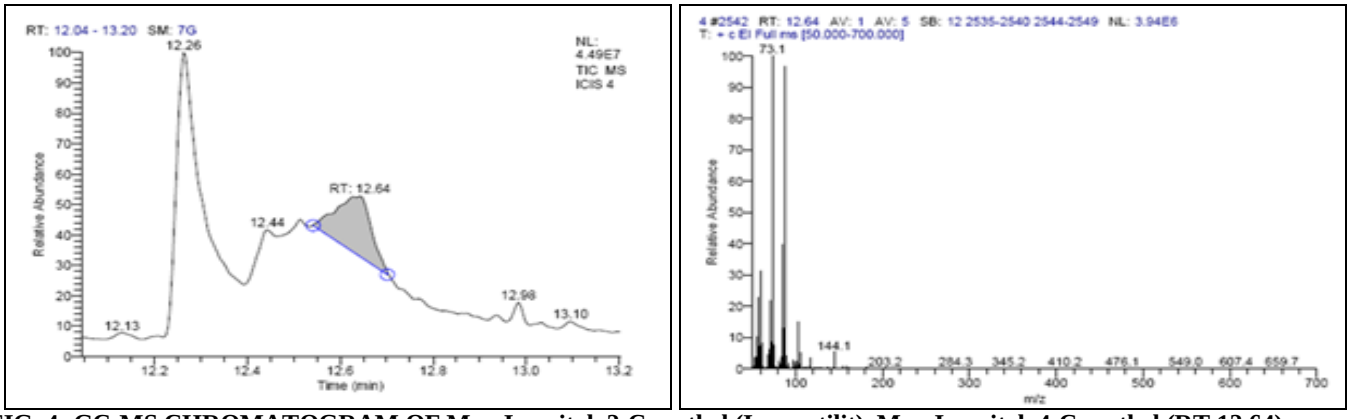

FIG. 4: GC-MS CHROMATOGRAM OF Myo-In-ositol, 2-C-methyl (Iso-mytilit), Myo-In-ositol, 4-C-methyl (RT 12.64)

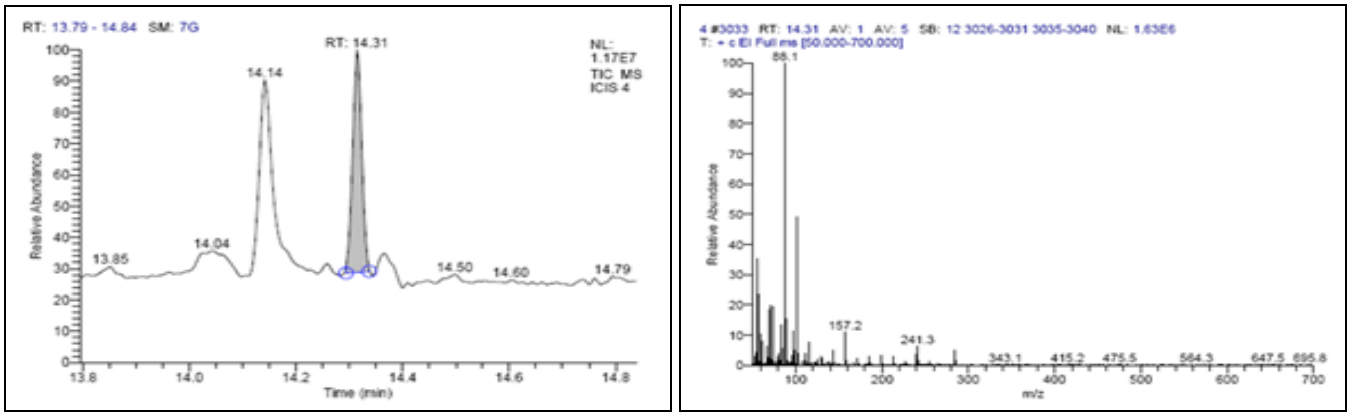

FIG. 5: GC-MS CHROMATOGRAM OF hexadecanoic acid, ethyl ester (Ethyl palmitate), pentadecanoic acid, ethyl ester, hexadecanoic acid, 2-methyl, methyl ester (RT 14.31)

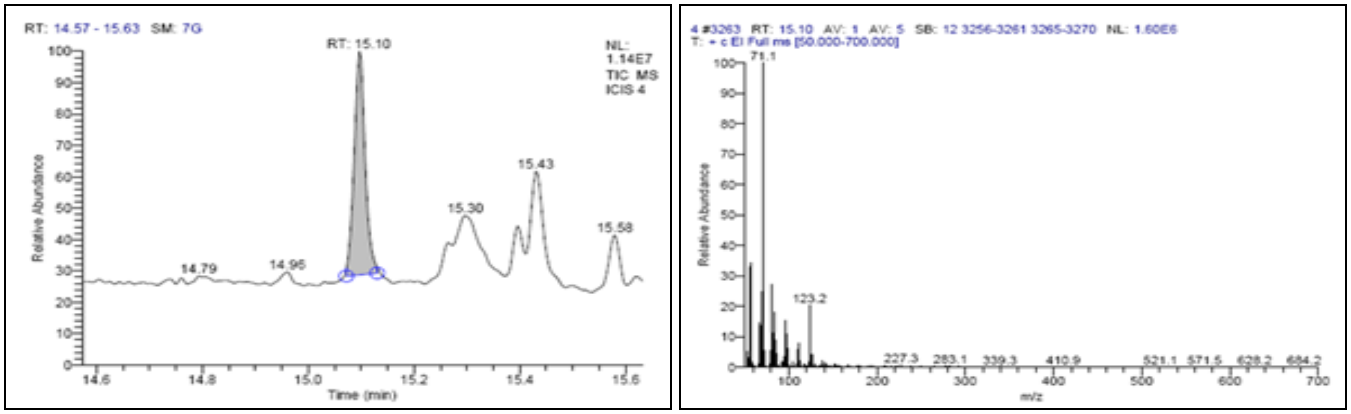

FIG. 6: GC-MS CHROMATOGRAM OF phytol, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, 1-Hexadecen-3-ol, 3, 5, 11, 15-tetramethyl (RT 15.10)

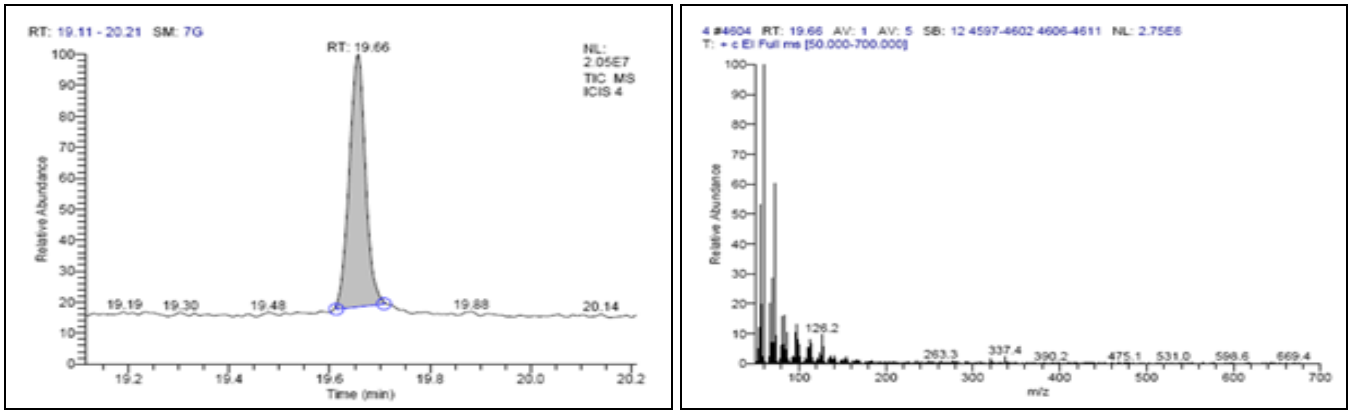

FIG. 7: GC-MS CHROMATOGRAM OF 13-Docosenamide, (Z), 9-Octadecenamide, (Z), 8-Methyl-6-nonenamide (RT 19.66)

DISCUSSION: In the present study, the GC-MS investigation of the ethanolic extract of *Ichnocarpus frutescens* showed the presence of nineteen compounds. In terms of percentage quantity, 3-O-Methyl-d-glucose, 3, 4, 6-Tri-O-methyl-d-glucose & 2-O-Methyl-D-mannopyranosa were found to be a prime percentage in the extract. The major phytocompound 3-O-Methyl-d-glucose has preservative activity. Antimicrobial, anti-inflammatory, anticancer and antidiabetic activities are shown by tetradecane, phytol and 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol similarly 8-Methyl-6-nonenamide showed antimicrobial and anti-inflammatory activities. The other identified phytocomponents are responsible for various activities except 3, 4, 6-Tri-O-methyl-d-glucose, 2-O-Methyl- D-mannopyranosa, Myo-In-osito- l, 2-C-methyl (Isomytilit), Myo-In-osito-l, 4-C-methyl, pentadecanoic acid, ethyl ester and hexadecanoic acid, 2-methyl, methyl ester.

CONCLUSION: The present investigation concluded that the ethanolic extract has a number of bio-active phytocomponents responsible for many biological activities and justify the use of the plant for different diseases and disorders of a human being by Vaidya, tribals and local healer or traditional practitioners as medicine. So, further separation, isolation and characterization of individual phyto-components from the plant, may be undertaken to discover novel drugs and their therapeutic actions to treat various ailments.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

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