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GC-MS ANALYSIS OF METHANOL EXTRACT OF MILLETTIA PACHYCARPA ROOT

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ABSTRACT

The plant *Millettia pachycarpa* belongs to genus Fabaceae which are distributed in the tropical and sub-tropical regions of the world. The present study on Gas Chromatography- Mass Spectrometry (GC-MS) analysis of methanol extract of the root of *Millettia pachycarpa* was carried out to determine qualitatively the presence of phytochemicals. In the GC-MS analysis, phytochemicals from the mass spectra which was matched with the National Institute of Standards and Technology (NIST), revealed the presence of 21 (Twenty one) compounds with their Retention Time. The dominant phytochemicals were Dibutyl phthalate (29.64%), Phthalic acid butyl hex-3-yl-ester (21.92%), Phthalic acid hept-4-yl isobutyl ester (17.31%), Phthalic acid butyl-3-methyl butyl ester (1.55%), Phthalic acid butyl hex-3-yl- ester(1.30%), Phthalic acid hept-4-yl isobutyl ester (1.28%), Phthalic acid pentyl-2-pentyl ester (1.26%), Phthalic acid bis (2-pentyl) ester (1.18%), Phthalic acid butyl hex-3-yl ester (0.84%) and Phthalic acid butyl isohexyl ester (0.81%). The phytochemicals like Ethane peroxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-di-oxolan-2-yl) ethyl] (0.35%); phthalic acid di (2- methyl butyl) ester (0.28%); phthalic acid butyl-2-ethylbutyl ester (0.25%); phthalic acid, 3- methyl butyl pentyl ester (0.18%); 2-Butanone, 3-Chloro-4-hydroxy-1, 4-diphenyl (0.16%); (2-Bromo-1-propenyl), penta fluoro sulphur (0.14%); phthalic acid, 2- pentyl propyl ester (0.10%); phthalic acid, 2-methyl pent-3-yl-pentyl ester (0.08%); 2- cyclohexane-6-carboxylic acid, 3-methyl-1, 1, -(5-oxo, 3,4-di hydro-2H-pyrrolyl)-ethyl ester (0.07%); Tricyclo [4,1,0,0 (2,4) heptane, 5- (phenylthio) – (1a,2a,4a, 5a,

6a) (0.07%); 4-(Hydroxy methyl)-1-phenyl-2- azetidinone (0.06%), Acetophenone, 2-[(p-nitrophenyl) imino] (0.05%); 2-Methyl-2-[(-1E, 3Z - E, 5E) 4 - methyl-6 - (2, 6, 6 - trimethyl - 1 - cyclohexenyl) -1, 3, 5 - hexatrienyl] - 1, 3 - dioxolane (0.05%); Silane, ethyl fluoro dimethyl (0.03%) and phthalic acid hept-1-yl isobutyl ester (0.03%) were found to be minor constituents. Further, these identified phytochemicals possess various biological activities such as antioxidant, antimicrobial, antibacterial, antifungal, anti-inflammatory, anticancer, blood tonics, anti- fertility, pesticides, plasticizer etc. Thus, these phytochemicals in the root of *Millettia pachycarpa* may help in the protection of human being from different kinds of diseases. The present finding highlighted the pharmaceutical importance of the phytochemicals present in the root of *Millettia pachycarpa* and further in depth analysis would be highly recommended for further studies.

KEYWORDS: *Millettia pachycarpa*, GC-MS, Phytochemicals Biological activity, pharmaceutical.

INTRODUCTION

Medicinal plants are used as a medical resource in almost all cultures for the treatment of various ailments since time immemorial. According to the survey of World Health Organisation (WHO) in 2008, more than 80% of the world population depends on traditional medicines for their health care purposes. The medicinal plant contains different biological activities like antimicrobial, antibacterial, antifungal, anti-inflammatory, anticancer, anti-oxidant, antifertility, blood tonics, anthelmintic, pesticides, etc. consequently, due to some other biological activities, on the same time, make excellent leads for new drug development.

The genus *Millettia* belongs to family Fabaceae comprising of more than 200 species which are grown in tropical and subtropical regions of the world (Chen *et al* 2018).[21] Among them, the specie *Millettia pachycarpa* commonly known as Ngamuyai (Local name Manipuri) is a climbing shrub having prickly branches and stems with 30-50cm long, 13 to 17 papery leaflets and lilac coloured flowers as well as leguminous pods containing one to five dark brown coloured reniform seeds. These plants are found in Komlathabi, Purum Khullen, Wangparal and Sarei ST Villages under Chandel District of Manipur State.

In the last few years, Gas Chromatography - Mass Spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species. The present study aims at to investigate the possible

phytocompounds present in *Millettia pachycarpa* root by extracting with methanol followed by separation and identification of the compounds using Gas Chromatography - Mass Spectrometry (GC-MS) analysis and study of their biological activities.

MATERIALS AND METHODS

Collection of plants: The fresh plant roots were collected from the natural habitats from Komlathabi, Purum Khullen, Wangparal and Sarei ST Villages under Chandel District, Manipur. The plant was identified and authenticated by the Department of Botany, South East Manipur College, Komlathabi, Chandel District, Manipur and voucher specimens are deposited at the Department of Botany, South East Manipur College, Komlathabi, Chandel District, Manipur.

Crude extract Preparation and Identification of compounds

The roots of *Millettia pachycarpa* were washed under running tap water, air dried at room temperature and chopped into small pieces and then made into fine powder. 150gm of fine powder was soaked in 1500 ml of methanol (1:10) for 15 days. The methanol extract was filtered, concentrated and the crude extract was preserved for further use (yield 4.47%). The methanol extract was GC-MS analysed on TSQ81712516 and the phytocompounds present were identified by the comparison of their respective Retention Time (RT) and the main spectra fragmentation patterns with those stored in the computer library of National Institute of Standards and Technology (NIST) and published literatures.

RESULT AND DISCUSSION

The Phytocompounds of methanol extract of *Millettia pachycarpa* roots were investigated using GC-MS chromatographic method. The phytocompounds present in the methanol root extract was identified by GC-MS chromatogram (Fig-1)

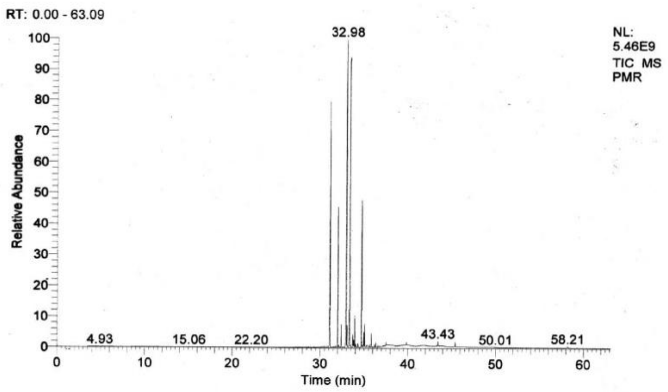
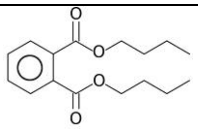
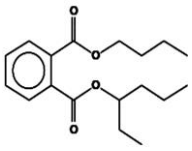
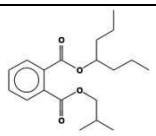
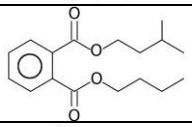
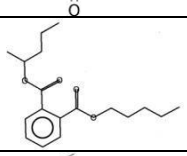
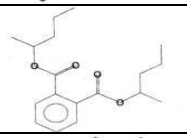
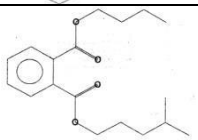
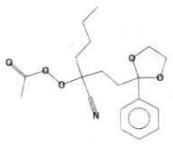
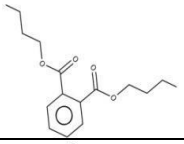
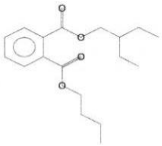
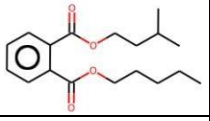
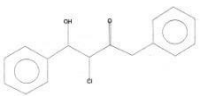
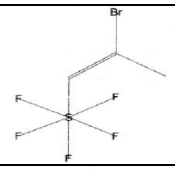
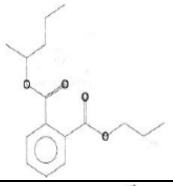
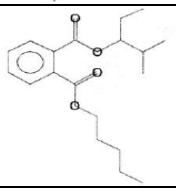
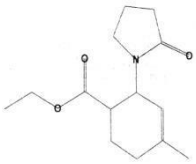
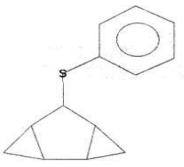
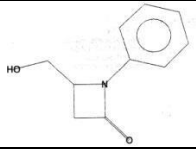
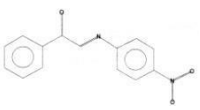


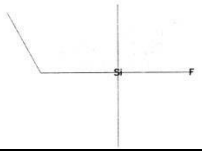
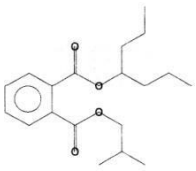
Fig. 1: GC-MS Chromatogram of Methanol extract of Millettia pachycarpa root.

The bioactive phytocompounds with their Retention Time (RT), percentage of concentration, molecular formula, molecular weight, molecular structure and biological activity are displayed in Table -1.

Table 1: Phytochemical compounds present in methanol extract of *millettia pachycarpa* root.

Sl. No	Name of Compounds	RT	% of peak area	Molecular formula	Mole-cular mass	Molecular structure	Biological activity
1	Dibutyl Phthalate	32.98	29.64	$C_{16}H_{22}O_4$	278		Antimicrobial, Antimetabolic, Antibacterial
2	Phthalic acid, butyl hex- 3-yl ester	33.36	21.92	$C_{18}H_{26}O_4$	306		Allelopathic, Phyto-toxic, Antimicrobial Insecticidal Plasticizers,
3	Phthalic acid, hept-4-yl isobutyl ester	31.11	17.31	$C_{19}H_{28}O_4$	320		Antioxidant Anitimicrobial
4	Phthalic acid, butyl-3- methyl butyl ester	33.94	1.55	$C_{17}H_{24}O_4$	292		Allelopathic, Antimicrobial, Insecticidal
5	Phthalic acid, pentyl-2- pentyl ester	35.07	1.26	$C_{18}H_{26}O_4$	306		Anti-oxidant, Plasticizer
6	Phthalic acid, bis (2- pentyl) ester	35.35	1.18	$C_{18}H_{26}O_4$	306		Anti-oxidant, Plasticizer
7	Phthalic acid, butyl iso hexyl ester	35.85	0.81	$C_{18}H_{26}O_4$	306		Allelopathic Antimicrobial
8	Ethane peroxoic acid, 1- cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl) ethyl]	37.54	0.32	$C_{19}H_{25}NO_5$	347		Insecticidal
9	Phthalic acid, di (2- methyl butyl) ester	34.30	0.28	$C_{18}H_{26}O_4$	306		Allelopathic, Antimicrobial, Insecticidal
10	Phthalic acid, butyl-2- ethyl butyl ester	34.04	0.25	$C_{18}H_{26}O_4$	306		Allelopathic, Antimicrobial Insecticidal Plasticizer

11	Phthalic acid, 3-methyl butyl pentyl ester	35.65	0.18	$C_{18}H_{26}O_4$	306		Antioxidant Antimicrobial
12	2-Butanone, 3-Chloro-4-hydroxyl-1,4 -diphenyl	37.65	0.16	$C_{16}H_{15}clO_2$	274		Antibacterial Antifungal
13	(2-Bromo-1-propenyl) penta fluoro sulphur	37.79	0.14	$C_3H_4BrF_5S$	246		Pesticide
14	Phthalic acid, 2-pentyl propyl ester	31.53	0.10	$C_{16}H_{22}O_4$	278		Antimicrobial, Bactericidal, Antibiotic, fungicidal, Anti-inflammatroy
15	Phthalic acid, 2-methyl pent-3-yl-pentyl ester	35.74	0.08	$C_{19}H_{28}O_4$	320		Antimicrobial Plasticizer
16	2-Cyclohexane-6- carboxylic acid, 3- methyl-1-1(5-oxo-3,4-diydro-2H-pyrrolyl)-ethylester	37.94	0.07	$C_{14}H_{21}NO_3$	251		Antioxidant, Antimicrobial, Anitibacterial, Antifungal, Antimalarial, Anticancer, Anti-hypertensive, Anti-inflammatory, Antipsychotic
17	Tricyclo [4,1,0,0(2,4) heptane, 5-(phenylthio)-(1a, 2a, 4a, 5a, 6a)	38.02	0.07	$C_{13}H_{14}S$	202		Antioxidant Antimicrobial Anti-inflammatory, Anti fungal
18	4-(Hydroxy methyl)-1-phenyl-2-azetidinone	36.80	0.06	$C_{10}H_{11}NO_2$	177		Antibacterial, Antifungal, Anticancer Herbicidal
19	Acetophenone, 2-[(P-nitrophenyl) imido]	38.22	0.05	$C_{14}H_{10}N_2O_3$	254		Antioxidant, Anticancer, Antimicrobial

20	Silane, ethyl fluoro dimethyl	31.28	0.03	C4H11FSi	106		Antimicrobial
21	Phthalic acid, hept-1-yl isobutyl ester	32.40	0.03	C19H28NO4	320		Antioxidant, Anticancer, Antibacterial, Antifungal, Anti-infalmmatory, Anti-malarial, Anti-microbial

Twenty one different phytocompounds were identified according to their percentage of concentration in the spectra. Out of these, six phytocompounds were identified as major constituents. They are Dibutyl phthalate (29.64%); Phthalic acid, butyl hex-3-yl ester (21.92%); Phthalic acid, hept- 4 -yl- isobutyl ester (17.31%); Phthalic acid, butyl-3-methyl butyl ester (1.55%); Phthalic acid, pentyl -2- pentyl ester (1.26%) and Phthalic acid, bis (2-pentyl) ester (1.18%). The other remaining fifteen phyto constituents were identified as minor constituents viz Phthalic acid, butyl, isohexyl ester (0.81%); Ethane peroxoic acid, 1-cyano-1- [2-(2-phenyl-1,3-di-oxolan-2-yl) ethyl] (0.32%); Phthalic acid, di (2-methyl butyl) ester (0.28%); Phthalic acid, butyl-2-ethyl butyl ester (0.25%); Phthalic acid, 3-methyl butyl pentyl ester (0.18%); 2-Butanone, 3-Chloro-4-hydroxy-,1, 4-diphenyl (0.16%); (2-Bromo-1-propenyl) pentafluoro sulphur (0.14%); Phthalic acid, 2-pentyl propyl ester (0.10%); Phthalic acid, 2-methylpent-3-yl- pentyl ester (0.08%); 2-cyclohexane-6-carboxylic acid, 3-methyl-1-1-(5-oxo-3,4-dihydro – 2H-pyrrolyl) – ethyl ester (0.07%); Tricyclo [4,1,0,0(2,4)] heptane, 5-(phenyl thio)-(1a,2a,4a,5a,6a) (0.07%), 4-(hydroxymethyl)-1-phenyl-2-azetidinone (0.06%); Acetophenone, 2-[(p-nitro-phenyl) imido] (0.05%); Silane, ethyl fluoro dimethyl (0.03%) and Phthalic acid, hept-1-yl-iso butyl ester (0.03%). The biological activities of these compounds were found to have Antioxidant, Antimicrobial, Antibacterial, Phytotoxic, Anticancer, Allelopathic, Insecticidal, Plasticizer, Pesticide, Anti-fungal, Anti-inflammatory, Antimalarial, Anti-hypertensive, Anti-psychotic, Herbicidal, Antibiotic, etc.

The compound Dibutyl phthalate (29.64%) is the foremost dominant phyto compound which shows the biological activities of anti-microbial, anti- metabolic and anti- bacterial activities. This compound is also one of the constituents present in medicinal plants such as *Mimersops elengi* (Rulkar *et.al.* 2011) and *Ipomoea carnea* (Adsul *et.al* 2012). The 2nd dominant compound Phthalic acid, butyl hex-3-yl ester (21.92%) showed allelopathic, phyto- toxic, antimicrobial, insecticidal and plasticizers activities. This is in accordance with the work

carried out on phytochemical screening and GC-MS analysis of *Gracilaria lemaneiformis, Chactomorpha basiretorsa* and *cladophorafracta*. The third dominant phytocompound present in methanol root extract of *Millettia pachycarpa* is Phthalic acid, hept-4-yl isobutyl ester (17.31%). The biological activities of this compound showed antioxidant and antimicrobial activities. It is also revealed in the GC-MS analysis of *Carlina acaulis* L.

The biological activities of Phthalic acid, butyl hex-3-yl ester (1.55%), the fourth dominant phytocompound present in the methanol extract of *Millettia pachycarpa* root showed allelopathic, antimicorbial and insecticidal activities. This compound is also revealed in the GC-MS analysis of *Andrographis paniculata* (Burm. F)

The compound Phthalic acid, pentyl-2-pentyl ester showed antioxidant and plasticizer activities which have 1.26% of peak area. This is in accordance with the work on *Angelica sinesis* and *Paris polyphylla*.

CONCLUSION

Millettia pachycarpa is a traditionally used medicinal plant which represent rich source of compounds possessing various biological activities. The results of the present investigation on methanol extract of *Millettia pachycarpa* root revealed the presence of twenty one bioactive compounds as identified by GC-MS analysis highlighted pharmaceutical importance of the plant. However, further studies will be required for better understanding of its safety and efficacy.

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