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GC-MS analysis of bioactive compounds in methanolic extract of tubers of *Pueraria tuberosa* (Roxb. ex Willd.) DC. - Fabaceae

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Abstract—*The present experiment was designed to determine the bioactive constituents from tuber extracts of Pueraria tuberosa (Roxb. ex Willd.) DC. of the family Fabaceae. The medicinal value of a plant species is dependent upon its various phytochemical constituents. The chemical compositions of the methanolic extract of tubers of P. tuberosa were investigated using Gas chromatography-Mass spectrometry and about nineteen bioactive phytochemical compounds were identified. The prevailing compounds were 2, 3-Dimethylaziridine; 2-Cyclopenten-1-one, 2-hydroxy-; 2-Hydroxy-gamma-butyrolactone; 3-Methyl-1,2-cyclopentanedione; 2,5- Dimethyl-4-hydroxy-3 (2H) – furanone; Butane 2-methyl; Oxetane; Maltol; 1, 5-Anhydro-6-deoxyhexo-2,3-diulose; 2, 3-Dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-One; 5-Hydroxymethylfurfural, Phenol,2,6-dimethoxy; Dodecanoic Acid; Guanosine; Tetradecanoic acid; Myo-inositol; Hexadecanoic Acid; 9, 12-Octadecadienoic acid, methyl ester and Cis-vaccenic acid. This was the first report on identification of bioactive compounds from methanolic extract of tubers of P. tuberosa.*

Keywords— Pueraria tuberosa, methanolic extract, GC-MS analysis, bioactive compounds.

I. INTRODUCTION

From ancient times plants are best sources of bioactive compounds having interesting biological activities. Literature studies represent the medicinal plants as reservoir of effective chemotherapeutants, play a principal role in the maintenance of human health. Knowledge on the phytoconstituents of plants is highly desirable for disclosing the actual significance of folkloric remedies, Milne, (1993). The secondary metabolites of plants have a variety of structural arrangements and properties, De-Fatima *et al*., (2006). Phytochemicals of natural drugs have overlapping and complementary mechanism of action; hence thorough validation of natural drugs was prioritized and emphasized. Mass Spectrometry coupled with Gas Chromatography is normally used for the direct analysis of chemical constituents present in plant based medicine. GC-MS analysis is a highly commended analysis for non-polar components, fatty acids, volatile essential oil, lipids, Jie and Choi, (1991) and alkaloids, Betz *et al*., (1997).

The tubers of *P.tuberosa* are widely used in ayurveda and in ethanomedicine. It has been recommended for the treatment of menopausal syndrome, sexual debility, cardiovascular diseases, fertility disorders, hepatosplenomegaly and spermatorrhoea and has been used as antiaging, spermatogenic and immune booster, Amal *et al*., (2014). There has been tremendous progress in medicinal plant research which involve the isolation and identification of secondary metabolites of plants and their use as active principles in therapeutics, Mary *et al*., (2013). Literature studies indicate that no reports on GC-MS analysis *P. tuberosa* has so far been undertaken to provide enough data in favour of its traditional uses. As part of the endeavor for the study of therapeutic properties of *P. tuberosa* we herein reported the GC-MS analysis of methanol extract of the tubers.

II. MATERIALS AND METHODS

2.1 Collection of plant sample

The tubers (Plate 1) of the *P. tuberosa* (Roxb. ex Willd.) DC. were collected from Nelliyampathy forests of Palakkad district, Kerala state. The tubers were authenticated by Dr. P.S. Udayan, Sree Krishna College, Guruvayur and the voucher specimens were preserved for further reference.

2.2 Preparation of powder and extract

Collected tubers were thoroughly washed in running tap water for 10 minutes. These were cut into pieces and were air dried in shade so as to prevent

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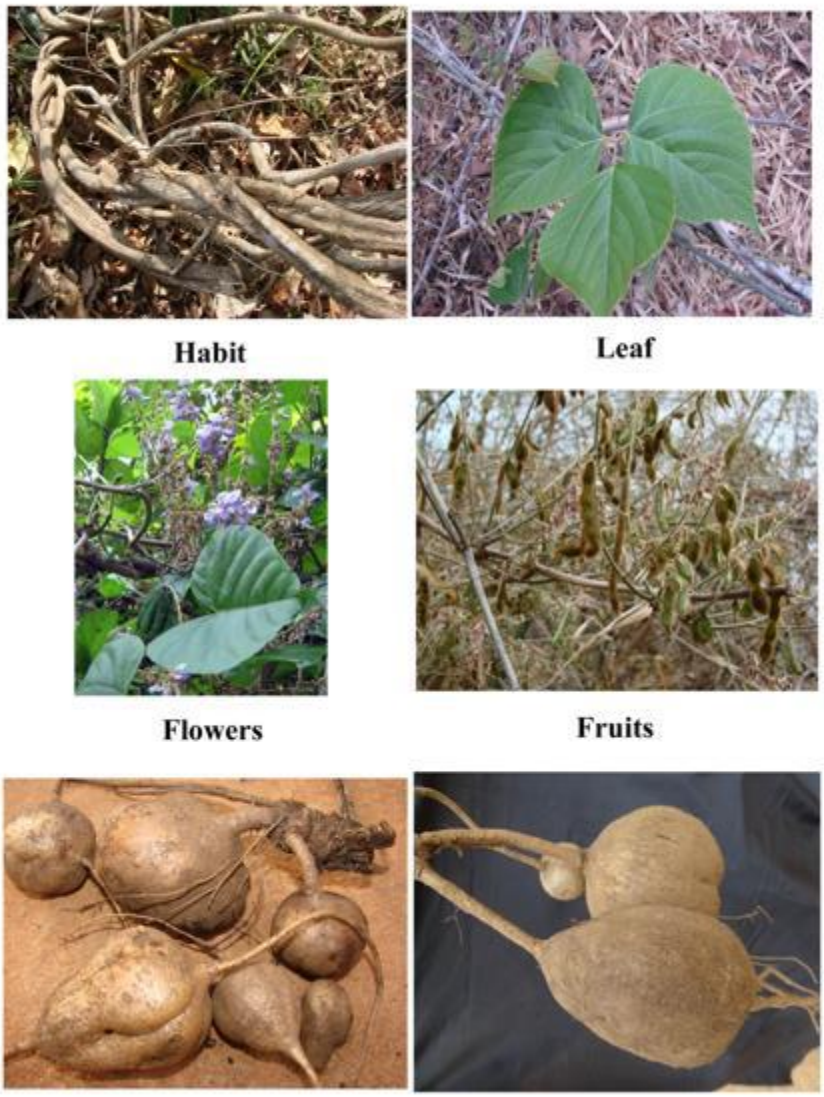
decomposition of active principle and made fine powder by using mechanical grinder. Then the powder was extracted using methanol as a solvent. Twenty gram of dried powder was weighed and subjected to extract successively with 200 ml methanol in soxhlet extractor. The extract was condensed and preserved in refrigerator in air tight bottles.

2.3 GC-MS analysis of bioactive compounds

The methanolic extracts obtained were subjected to GC-MS analysis for the determination of various bioactive volatile compounds in *P. tuberosa*. The analysis was carried out using Shimadzu Make QP-2010 with nonpolar 60 M RTX MS column, operating in electron impact mode at 70 eV. Helium was used as the carrier gas and an injection volume of 0.5µl was employed in split less mode at injection temperature 260ºC; ion-source temperature 200ºC. The oven temperature programming was set with a rate of 10 ºC with an initial oven temperature at 60º C and final temperature at 280º C, held for 8 minutes. The total running time for the sample was 25 minutes. The chemical constituents of the methanolic tuber extracts of plant samples were identified by comparing the retention times of peaks using NIST Library to relative retention indices. The relative percentage of each of the component in the extract was calculated by comparing its average peak area to the total areas.

III. RESULTS

PLATE 1
Pueraria tuberosa (Roxb. ex Willd.) DC.



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The results pertaining to GC-MS analysis leads to the identification of number of chemical constituents from the GC fractions of methanolic extract of tubers of *P. tuberosa*. Nineteen bioactive compounds were identified and their retention time (RT), % of peak area, molecular formula, molecular weight and biological activities are presented in Table 1 & 2 and Fig. 1. The prevailing compounds were 2, 3- Dimethylaziridine (1.96%); 2-Cyclopenten-1-one, 2-hydroxy-(2.84%);2-Hydroxy-gamma-butyrolactone(12.16%);3-Methyl-1,2-Cyclopentanedione (1.78%); 2, 5-Dimethyl-4-hydroxy-3 (2H)-furanone (3.24%); Butane 2-methyl (0.83%); Oxetane (12.37%); Maltol (7.50%); 1, 5-Anhydro-6-deoxyhexo-2, 3-diulose (3.28%); 2, 3-Dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-One (2.94%); 5-Hydroxymethylfurfural (19.82%); Phenol, 2,6-dimethoxy (0.92%); Dodecanoic Acid (3.39%); Guanosine (12.11%); Tetradecanoic acid (1.05%); Myo-inositol (3.36%); Hexadecanoic Acid (6.23%); 9, 12-Octadecadienoic acid, methyl ester (1.58%) and Cis-vaccenic acid (2.64%). Most abundant bioactive components among these were 5-Hydroxymethylfurfural (19.82%); Oxetane(12.37%); 2-Hydroxy-gamma-butyrolactone (12.16%) and Guanosine (12.11%).

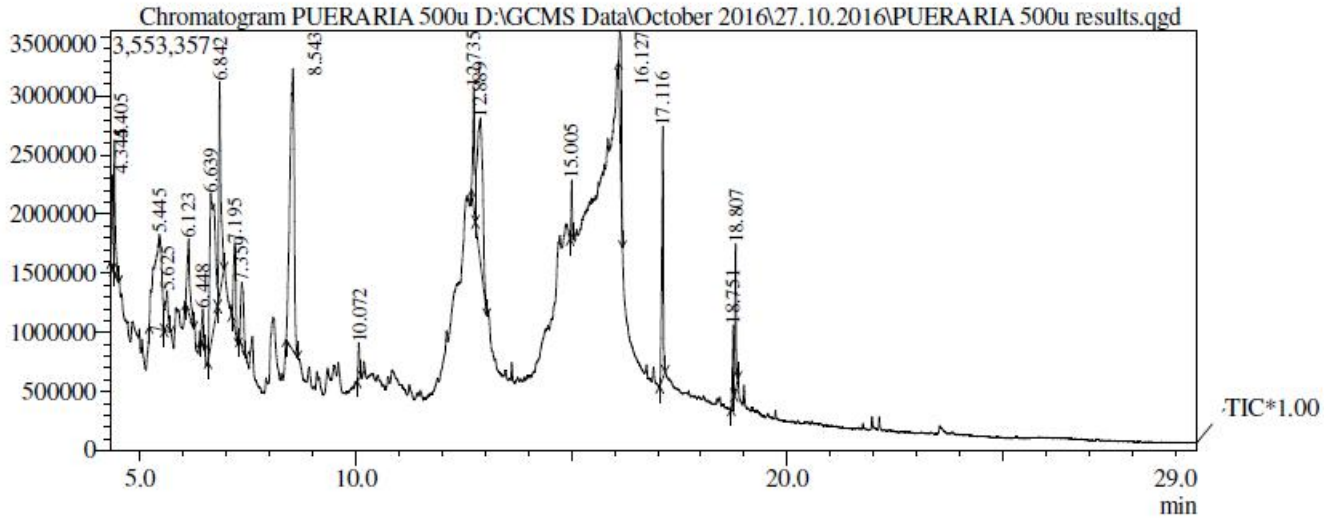

Fig.1: GC-MS Chromatogram of methanolic extract of tuber of P. tuberosa.

Table.1: Bioactive compounds detected from methanol extract of tubers of P. tuberosa.

Peak	Retention time	% of peak area	Compounds analysed	Nature of compounds	Molecular formula	Molecular weight
1	4.345	1.96	2,3-Dimethylaziridine	Heterocyclic compound	C_4H_9N	71.12
2	4.405	2.84	2- Cyclopenten-1-one, 2-hydroxy-	Organic compound	$C_6H_6O_2$	98.10
3	5.445	12.16	2-Hydroxy-gamma-butyrolactone	Lactone	$C_4H_6O_3$	102.09
4	5.625	1.78	3-Methyl-1,2-cyclopentanedione	Lactone	$C_6H_8O_2$	112.13
5	6.123	3.24	2,5- Dimethyl-4-hydroxy-3(2H)-furanone	Lactone	$C_6H_8O_3$	128.13
6	6.448	0.83	Butane 2-methyl	Alkane	C_5H_{12}	72.15
7	6.639	12.37	Oxetane	Heterocyclic	$C_{10}H_{16}O_3$	184.24
8	6.842	7.50	Maltol	Heterocyclic	$C_6H_6O_3$	126.11
9	7.195	3.28	1,5-Anhydro-6-deoxyhexo-2,3-diulose	Glucoside	$C_6H_8O_4$	144.0

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10	7.359	2.94	2,3-Dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-One	Unsaturated cyclic compound	$C_6H_8O_4$	144.13
11	8.543	19.82	5-Hydroxymethylfurfural	Heterocyclic compound	$C_6H_6O_3$	126.11
12	10.072	0.92	Phenol,2,6-dimethoxy	Organic compound	$C_9H_{12}O_3$	168.19
13	12.735	3.39	Dodecanoic Acid	Saturated Fatty acids	$C_{12}H_{24}O_2$	200.32
14	12.889	12.11	Guanosine	Purine nucleoside	$C_{10}H_{13}N_5O_5$	283.24
15	15.005	1.05	Tetradecanoic acid	Saturated Fatty acid	$C_{14}H_{28}O_2$	228.37
16	16.127	3.36	Myo- inositol	Vitamin like substance	$C_6H_{12}O_6$	180.16
17	17.116	6.23	Hexadecanoic Acid	Saturated Fatty acid	$C_{12}H_{32}O_2$	257.42
18	18.751	1.58	9,12-Octadecadienoic acid, methyl ester	Unsaturated fatty acid	$C_{19}H_{34}O_2$	294.47
19	18.807	2.64	Cis-vaccenic acid	Fatty acid	$C_{18}H_{34}O_2$	282.46

Table.2: Activity of Bioactive compounds identified in the methanol extract of tubers of P. tuberosa.

S. N.	Chemical constituents	Biological Activities	Literature cited
1	2,3-Dimethylaziridine	Antimicrobial and anticancer	Aleksandra *et al*., 2017
2	2- Cyclopenten-1-one, 2-hydroxy	Inducer of Heat Shock Protein 70 with Antiviral Activity	Antonio *et al*., 1996
3	2-Hydroxy-gamma-butyrolactone	Epigoitrin from4-hydroxy-γ-Butyrolactone presents antithyroid and antivirus activities	Wei and Chunhua, 2013
4	3-Methyl-1,2-cyclopentanedione	An effective anti-inflammatory agent	Jae *et al*., 2007
5	2,5- Dimethyl-4-hydroxy-3(2H)-furanone	Broad spectrum antimicrobial activities	Sung *et al*., 2007
6	Maltol	Flavor compound	Bhesh *et al*., 2013
7	1,5-Anhydro-6-deoxyhexo-2,3-diulose	Preservative	Prabu *et al*., 2013
8	5-Hydroxymethylfurfural	Antioxidant,Antiproliferative activity	Zhao *et al*., 2013
9	Phenol,2,6-dimethoxy	Antimicrobial, Antioxidant, Anti inflammatory	Salem *et al*., 2018
10	Dodecanoic Acid	Both antibacterial and antifungal	Belakhdar *et al*., 2015
11	Tetradecanoic acid	Both antibacterial and antifungal	Belakhdar *et al*., 2015
12	Hexadecanoic Acid	Antioxidant	Belakhdar *et al*., 2015

IV. DISCUSSION

Now a day, the study of bioactive components from medicinal plants and their activity has increased. The combination of GC (best separation technique) with MS (best identification technique) made GC-MS one of the ideal techniques for quantitative analysis of volatile and semi-volatile compounds, Grover and Patni, (2013). The identified compounds with more percentage like 5-Hydroxymethylfurfural (19.82%), Hexadecanoic acid, ethyl ester (Palmitic acid ester) (6.23%), 2-Hydroxy-gamma-butyrolactone (12.16%) showed a wide range of potent bioactivity. These phytochemicals are responsible for various pharmacological actions like antioxidants and antimicrobial activities, Tapiero *et al*., (2002). Among the nineteen compounds identified 7 showed Anti-microbial activity, 2 showed Anti-inflammatory, 1 showed Anti-cancer and 3 showed anti-oxidant and also showed activities such as antithyroid , inducer of heat shock protein 70, up-regulator of immunoglobulin synthesis, Bickerstaffe and Annison, (1970). The GC-MS analysis of methanolic extract of tuber of *Plectranthus rotundifolius* Spreng. showed the presence of forty different phytochemical compounds , among these Cis-Vaccenic acid was identified as an active phytocomponent, Manikandan *et al*., (2016).

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Sweetness enhancer maltol increases the creaminess and decreases the bitterness of food, Bhesh, (2013). Different kinds of dietary fat modify the risks of many chronic and acute inflammatory diseases by the differential regulation of gene expression and activation of macrophages Joo *et al*., (2001). Unsaturated fatty acids like 9, 12-Octadecadienoic acid, methyl ester, known as an omega-6 fatty acid are important for normal cell growth, to lower cholesterol levels of the blood, Igwe and Okwu, (2013) and to support the lubricating quality of skin, Okwu and Morah, (2006).

It has been reported that tuberosin, one of the active principles of *P. tuberosa* inhibits lipopolysaccharide (LPS) induced inflammatory changes in macrophages and directly scavenges various species of free radicals, Panday and Tripati, (2010). *P.tuberosa* showed significant dose dependent ulcer protective effect due to its antioxidant activities and it was comparable to the reference drug OMP, Sumalatha *et al*., (2010). In the present experiment different compounds displayed similar activity and the presence of various radical scavenging and anti-inflammatory compounds in the methanolic extract of *P.tuberosa* may be the responsible for its antioxidant properties.

V. CONCLUSION

The GC-MS studies carried out on methanol extract of tubers of *P. tuberosa* showed the presence of chemical components responsible for its potent medicinal activity. Further work regarding specific activity of various identified compound will provide more insight about the use of the tuber.

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