*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

# LC-MS Profiling of methanolic extract of *Pueraria tuberosa* (Roxb. ex Willd.) DC. tubers

Bindu T.K. and P.S. Udayan

PG Department of Botany and Research Centre, Sree Krishna College, Guruvayur, Ariyannur P.O., Thrissur District, Kerala, India - 680102.

***Abstract*****—** *LC-MS profiling has been developed for the characterization of chemical constituents present in the methanolic extract of Pueraria tuberosa tubers. As a result, 61 compounds were detected using m/z value. Swietenine, Vigabatrin, Barbituric acid, Rhoifolin, Cetrimonium bromide, Octanoic acid, Caprylic acid and 4Z-Decenedioic acid were some of the important phytoconstituents with interesting biological activities. Among the peaks in the chromatogram, 7 unknown compounds were also identified.*

***Keywords— LC-MS profiling, methanolic extract, Pueraria tuberosa, chemical constituents.***

## I. INTRODUCTION

It has been of great interest to characterize plant products for quality control and pharmaceutical production. More than half of the world population in Africa, Asia and Latin America use plant based medicines (Asharf *et al*., 2015; Tahir *et al*., 2016). Species variation, time of harvesting, environment conditions, storage and processing are the factors that influence the quality of herbs. Moreover, plant extract may be falsified with other plants devoid of active constituents. For these reasons, the quality control of the standardized plant extracts is an important step in drug industry. LC-MS technologies are appropriate for characterization and quantification of herbal medicines because full characterization of plant product is a desirable goal (Wang *et al*., 1999). As the LC/MS profiling of tubers of *P.tuberosa* (Roxb. ex Willd.) DC. of the family Fabaceae, is not reported in literature, the purpose of the present study was to explore the composition of various constituents present in the methanolic extract of its tubers. As a result suitable analytical assays could be developed for pharmaceutical production and quality control of plant products. High sensitivity, identification of components of herbal extract and detection of unknown and unexpected compounds are the important advantages of LC-MS/MS analysis (Villas-Boas *et al*., 2005; Krug *et al*., 2008).The tuberous roots of Vidari (*P. tuberosa*) are used for wide variety of ailments like rheumatism and posses pharmacological activities like antihepatotoxic activity (Hsu *et al*., 2003) and antifertility effects in rats (Gupta *et al*., 2004). Vidari is used as demulcent, refrigerant and galactogogue (Chopra *et al*., 1992).

## II. MATERIALS AND METHODS

### 2.1 Collection of plant sample

The tubers of the *P. tuberosa* (Roxb. ex Willd.) DC. (Fabaceae) were collected from Nelliyampathy forests of Palakkad district, Kerala state. The tubers were authenticated by Dr. P.S. Udayan, Sree Krishna College, Guruvayur.

### 2.2 Preparation of powder and extraction

Collected tubers were thoroughly washed in running tap water for 15 minutes. These were cut into pieces and were air dried in shade and powdered using a mechanical grinder. Then, the powder was extracted using methanol as a solvent. Twenty five gram of powder was weighed and subjected to organic extraction successively with 200 ml methanol using a metallic stirrer. The extract was condensed and kept in refrigerator in air tight bottles until further use.

### 2.3 LC- MS Technique

The LC-MS method was performed using TOF/Q-TOF Mass Spectrometer system in IIT, Mumbai equipped with a Dual AJS ESI (electro spray ionization) source. The gradient elution at a flow rate of 0.300 ml/min was operated for 30.00 min stop time. The full-scan mass spectra were obtained within a range of m/z, amu 103-1,000 at 1.00 scan rate. Solvent composition in channel A and B was water (95%) and acetonitrile (5%) respectively. Value switch time 1 was enabled with 5.00 µl injection volume. The analytical data were optimized using the Analyst Version: 1.4.2 software system with a background subtraction technique of chromatography. The principle of this technique is to reduce the background, such as fault peaks and noise from the methanolic extracts of the tubers of *P. tuberosa* (Figure 1). Along with more ions present in the processed mass spectra, data containing more real m/z were observed in subsequent optimization for LC-MS. The LC-MS data were then manually sorted (Table 1) to list

*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

information as m/z values for $[M+H]^+$ from base peak chromatogram. Each compound was then identified from reference compounds by calculating their molecular weight of the structures, already known to be present in the tubers of *P. tuberosa*.

## III. RESULTS

On the basis of the LC-MS, the known compounds identified were 3-Quinolinecarboxylic acid,7-amino-1-etyl-6-fluoro-1,4-dihydro-4-oxo; 2-Furoic acid; 2-Naphthaleneacetic acid 6- hydroxyl; Choline; Aspargine ; 3, 5-Pyridinedinedicarboxylic acid; Trp His Glu; Betaine; Diaminopimelic acid; 1,4 Dideoxy-1,4imino-DArabinitol; Deoxypodophyllotoxin; Meso-erythritol; Vigabatrin; Capriloglycine; Sulfinpyrazone sulfone; Rhoifolin; N-Carboxyethyl-gamma-aminobutyric acid; 7-Dehydrologanintetraacetate; Cosmosiin; Octopine; Recepinephrine; 7-Dehydrologanin tetraacetate; Chrysophanol 8-O-beta-D glucoside; Naringenin-7-O-glucoside; Beta-nonylenic acid; Citrinin; Naringin; Zopiclone N-oxide; 4-Hydroxyfenoprofen-glucuronide; Irigenin, di benzyl ether; Propanoic acid, 2-hydroxy-3-[(4-hydroxy1-naphthalenyl)oxy]-; Aspartame; Sebasic acid; Ethosuximide; Barbituric acid, 5- ethyl 5- (2-hydroxy ethyl)-; Diaziquone; Avocadene acetate; Disopyramide; C16 Spinganine; Pytosphingosine; Swietenine; Dihydroergocornine; N-Desmethyltamoxifen; Dihydrosphingosine; Sulfamenthazine; N-Succinyl-L-diamino pimelic acid; Idebenone metabolite; Cusohygrine; Trans-3-Hydroxycotinine; Cetylpyridinium; 4 methyl-decanoic acid; (E)-2-Methylglutaconic acid; 2-Hydroxy-3-(4-methoxy ethyl phenoxy)-Propanoic acid; 4,7-dioxo-octanoic acid; Centrimonium;5 beta-Chola-3,8(14),11-trien-24-oic acid; Anandamide; 13-hydroxy-tridecanoic acid; 4Z-decenedioic acid; 3 beta, 6 alpha,7 alpha beta-cholan-24 oic acid; Ecgonine- methyl ester. Structures of identified known compounds are shown in Figure 2.

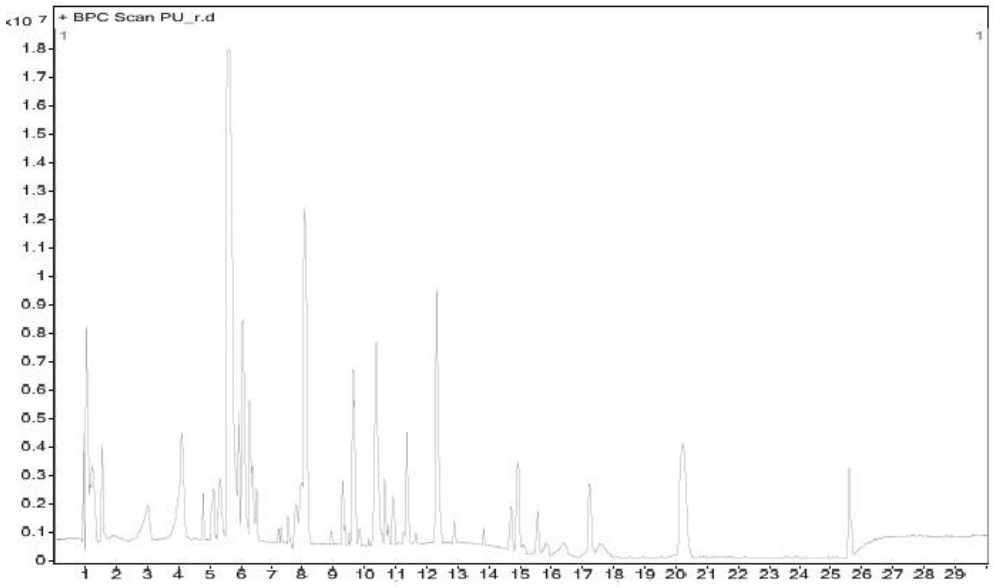



*Fig.1: LC MS Chromatogram of methanolic extract of Pueraria tuberosa tubers.*

*Table.1: Accurate mass data for the bioactive compounds present in the methanolic extract of tuber of Pueraria tuberosa*

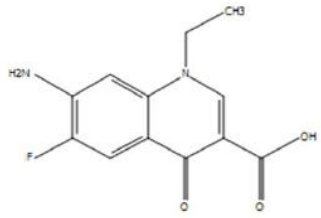
| Sl No | RT | Name of compound | Molecular Formula | Molecular weight g/mol | m/z |
|---|---|---|---|---|---|
| 1 | 0.96 | 3-Quinolinecarboxylic acid,7-amino-1-etyl-6-fluoro-1,4-dihydro-4-oxo | $C_{12}H_{11}FN_2O_3$ | 250.08 | 233.08 |
| 2 | 0.96 | 2-Furoic acid | $C_5H_4O_3$ | 112.01 | 116.99 |
| 3 | 0.963 | 2-Naphthalene acetic acid 6- hydroxy | $C_{12}H_{16}O_3$ | 202.06 | 185.06 |
| 4 | 1.047 | Choline | $C_5H_{14}NO$ | 104.11 | 104.11 |
| 5 | 1.074 | Aspargine | $C_4H_8N_2O_3$ | 132.05 | 133.06 |
| 6 | 1.087 | 3, 5-Pyridinedicarboxylic acid | $C_{17}H_{16}N_2O_8$ | 376.09 | 381.07 |
| 7 | 1.09 | Try His Glu | $C_{22}H_{26}N_6O_6$ | 470.19 | 475.17 |
| 8 | 1.092 | Betaine | $C_5H_{12}NO_2$ | 118.09 | 118.08 |
| 9 | 1.101 | Diaminopimelic acid | $C_7H_{14}N_2O_4$ | 190.09 | 173.09 |
| 10 | 1.111 | 1,4 Dideoxy-14imino-D arabinitol | $C_5H_{11}NO_3$ | 133.08 | 138.05 |

*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

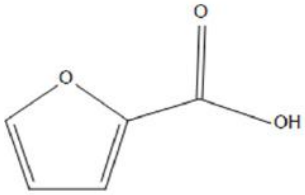
| | | | | | |
|---|---|---|---|---|---|
| 11 | 1.164 | Deoxypodophyllotoxin | $C_{22}H_{22}O_7$ | 398.13 | 421.12 |
| 12 | 1.17 | Meso-erythritol | $C_4H_{10}O_4$ | 122.06 | 127.03 |
| 13 | 1.551 | Vigabatrin | $C_6H_{11}NO_2$ | 129.08 | 130.08 |
| 14 | 1.552 | Capryloglycine | $C_{10}H_{19}NO_3$ | 201.13 | 224.12 |
| 15 | 4.107 | Sulfinpyrazone sulfone | $C_{23}H_{20}N_2O_4S$ | 420.12 | 443.11 |
| 16 | 4.8 | Rhoifolin | $C_{27}H_{30}O_{14}$ | 578.16 | 579.16 |
| 17 | 5.075 | N-Carboxyethyl-gamma-aminobutyric acid | $C_7H_{13}NO_4$ | 175.08 | 176.09 |
| 18 | 5.12 | 7-Dehydrologanintetraacetate | $C_{25}H_{32}O_{14}$ | 556.17 | 579.16 |
| 19 | 5.21 | Cosmosiin | $C_{21}H_{20}O_{10}$ | 432.10 | 433.11 |
| 20 | 5.34 | Octopine | $C_9H_{18}N_4O_4$ | 246.13 | 247.14 |
| 21 | 5.34 | Recepinephrine | $C_9H_{13}NO_3$ | 183.09 | 188.07 |
| 22 | 5.46 | 7-Dehydrologanin tetraacetate | $C_{25}H_{32}O_{14}$ | 556.17 | 579.16 |
| 23 | 5.63 | Chrysophanol 8-O -beta-D glucoside | $C_{21}H_{20}O_9$ | 416.10 | 399.10 |
| 24 | 5.63 | Naringenin-7-O-glucoside | $C_{21}H_{22}O_{10}$ | 434.12 | 417.11 |
| 25 | 5.81 | Beta-nonylenic acid | $C_9H_{16}O_2$ | 156.11 | 139.11 |
| 26 | 5.94 | Citrinin | $C_{13}H_{14}O_5$ | 250.08 | 255.06 |
| 27 | 5.94 | Naringin | $C_{27}H_{32}O_{14}$ | 580.17 | 563.17 |
| 28 | 6.01 | Zopiclone N-oxide | $C_{17}H_{17}ClN_6O_4$ | 404.11 | 387.10 |
| 29 | 6.08 | 4-Hydroxyfenoprofen-glucuronide | $C_{21}H_{22}O_{10}$ | 434.11 | 417.11 |
| 30 | 6.11 | Irigenin,di benzyl ether | $C_{32}H_{28}O_8$ | 540.18 | 563.17 |
| 31 | 6.49 | Propanoic acid, 2-hydroxy-3-[(4-hydroxy 1-naphthalenyl)oxy]- | $C_{13}H_{12}O_5$ | 248.07 | 271.06 |
| 32 | 7.23 | Aspartame | $C_{14}H_{18}N_2O_5$ | 294.13 | 299.11 |
| 33 | 7.55 | Sebasic acid | $C_{10}H_{18}O_4$ | 202.12 | 207.10 |
| 34 | 7.60 | Ethosuximide | $C_7H_{11}NO_2$ | 141.08 | 146.06 |
| 35 | 7.83 | Barbituric acid, 5- ethyl 5- (2-hydroxy ethyl)- | $C_8H_{12}N_2O_4$ | 200.08 | 188.08 |
| 36 | 8.30 | Diaziquone | $C_{18}H_{22}N_2O_6$ | 362.15 | 385.14 |
| 37 | 8.94 | Avocadene acetate | $C_{19}H_{36}O_4$ | 328.27 | 311.27 |
| 38 | 9.27 | Disopyramide | $C_{21}H_{29}N_3O$ | 339.23 | 344.21 |
| 39 | 9.29 | C16 Spinganine | $C_{16}H_{35}NO_2$ | 273.26 | 274.27 |
| 40 | 9.36 | Pytosphingosine | $C_{18}H_{39}NO_3$ | 317.29 | 318.30 |
| 41 | 9.61 | Swietenine | $C_{32}H_{40}O_9$ | 568.27 | 573.24 |
| 42 | 9.62 | Dihydroergocornine | $C_{31}H_{41}N_5O_5$ | 563.31 | 568.28 |
| 43 | 9.73 | N-Desmethyltamoxifen | $C_{25}H_{27}NO$ | 357.22 | 358.22 |
| 44 | 10.25 | Dihydrosphingosine | $C_{18}H_{39}NO_2$ | 301.29 | 302.30 |
| 45 | 10.30 | Sulfamenthazine | $C_{12}H_{14}N_4O_2S$ | 278.08 | 279.09 |
| 46 | 10.37 | N-Succinyl-L-diamino pimelic acid | $C_{11}H_{18}N_2O_7$ | 290.11 | 291.12 |
| 47 | 10.37 | Idebenone metabolite | $C_{13}H_{16}O_6$ | 268.09 | 251.09 |
| 48 | 10.65 | Cusohygrine | $C_{13}H_{24}N_2O$ | 224.19 | 225.19 |
| 49 | 10.75 | Trans-3-hydroxycotinine | $C_{16}H_{20}N_2O_8$ | 368.12 | 351.11 |
| 50 | 10.93 | Cetylpyridinium | $C_{21}H_{38}N$ | 304.29 | 304.30 |
| 51 | 11.27 | 4 methyl-decanoic acid | $C_{11}H_{22}O_2$ | 186.16 | 209.15 |
| 52 | 11.36 | (E)-2-Methylglutaconic acid | $C_6H_8O_4$ | 144.04 | 149.02 |
| 53 | 11.36 | 2-Hydroxy-3-(4-methoxy ethyl phenoxy)-propanoic acid | $C_{12}H_{16}O_5$ | 240.10 | 245.07 |
| 54 | 11.36 | 4,7-dioxo-octanoic acid | $C_8H_{12}O_4$ | 172.07 | 177.05 |
| 55 | 11.87 | Centrimonium | $C_{19}H_{42}N$ | 284.33 | 284.33 |
| 56 | 12.89 | 5 beta-Chola-3,8(14),11-trien-24-oic acid | $C_{24}H_{34}O_2$ | 354.26 | 259.24 |
| 57 | 12.89 | Anandamide | $C_{24}H_{37}NO_2$ | 371.28 | 254.28 |
| 58 | 13.83 | 13-hydroxy-tridecanoic acid | $C_{13}H_{26}O_3$ | 230.19 | 235.17 |
| 59 | 14.71 | 4Z-Decenedioic acid | $C_{10}H_{16}O_4$ | 200.10 | 205.08 |
| 60 | 20.23 | 3 beta,6 alpha,7 alpha-trihydroxy-5 beta-cholan-24 oic | $C_{24}H_{40}O_5$ | 408.28 | 413.26 |

*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

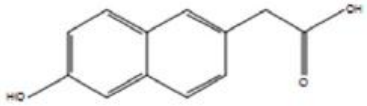
| | | | | | |
|---|---|---|---|---|---|
| | | acid | | | |
| 61 | 25.59 | Ecgonine- methyl ester | $C_{10}H_{17}NO_3$ | 199.12 | 222.11 |
| 62 | 7.77 | Unknown | - | 678.22 | 679.23 |
| 63 | 9.66 | Unknown | - | 942.51 | 943.51 |
| 64 | 9.83 | Unknown | - | 186.12 | 187.12 |
| 65 | 10.56 | Unknown | - | 157.14 | 158.15 |
| 66 | 10.92 | Unknown | - | 322.12 | 323.12 |
| 67 | 12.33 | Unknown | - | 325.37 | 326.37 |
| 68 | 12.85 | Unknown | - | 311.35 | 312.36 |



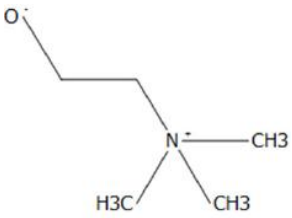
(1)



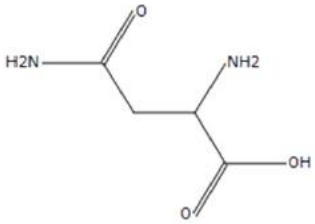
(2)



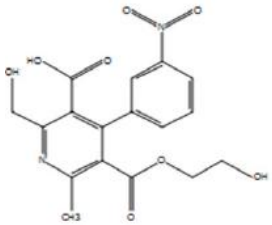
(3)



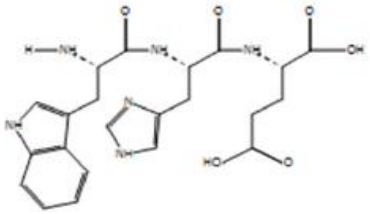
(4)



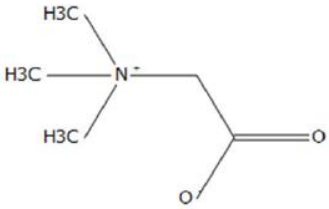
(5)



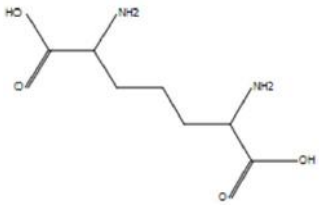
(6)



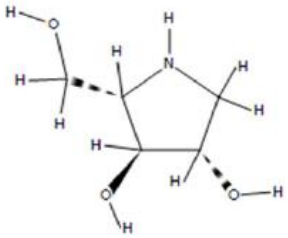
(7)



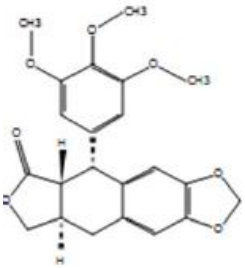
(8)



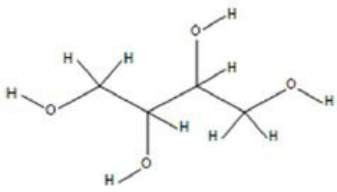
(9)



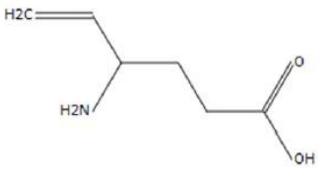
(10)



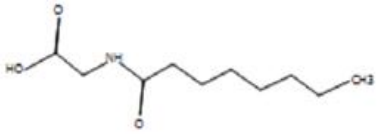
(11)



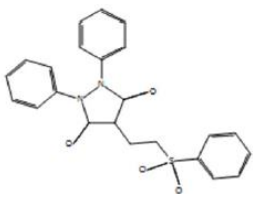
(12)



(13)

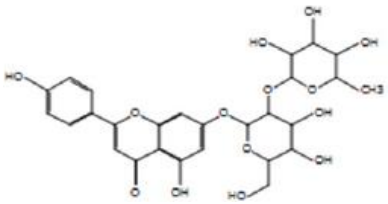


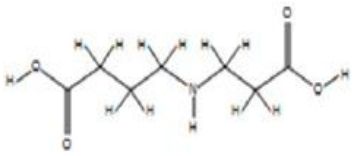
(14)

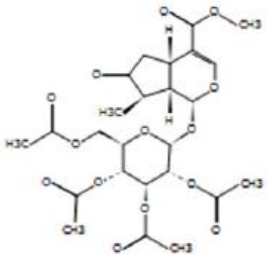


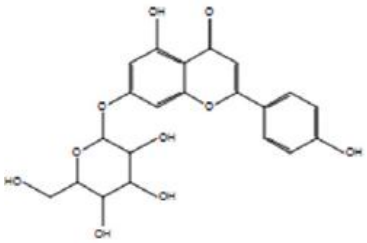
(15)

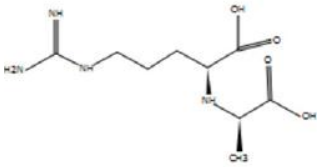
*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

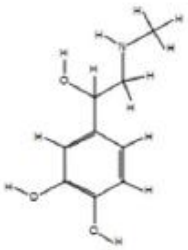

(16)

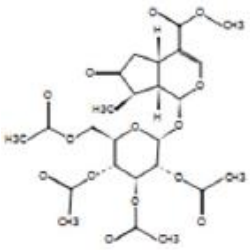

(17)

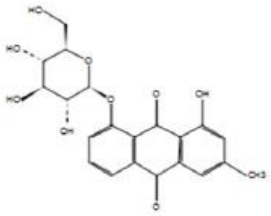

(18)

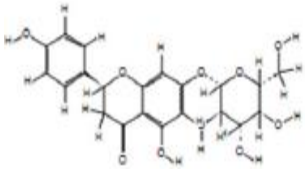

(19)

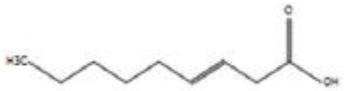

(20)

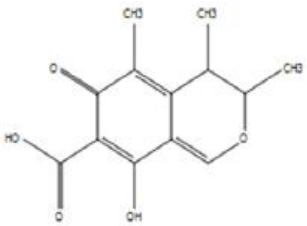

(21)

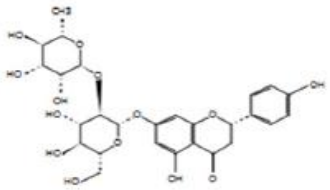

(22)

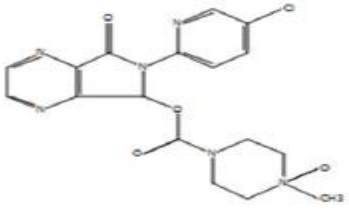

(23)

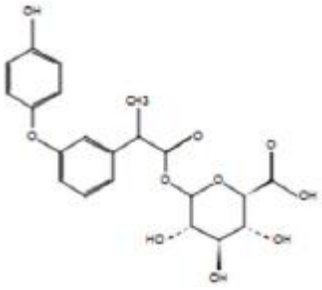

(24)

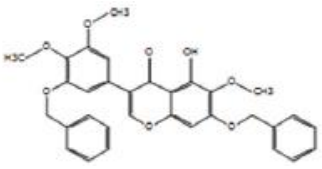

(25)


(26)

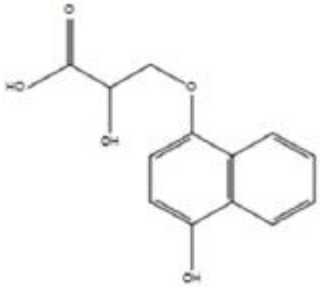

(27)

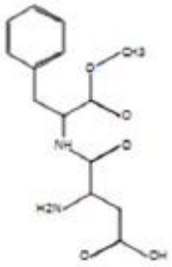

(28)

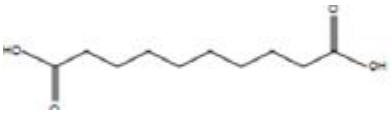

(29)

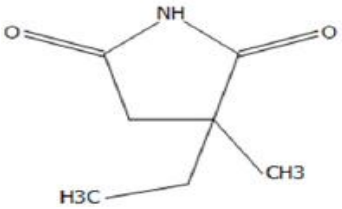

(30)

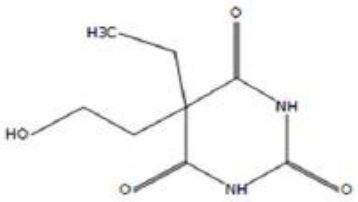
*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

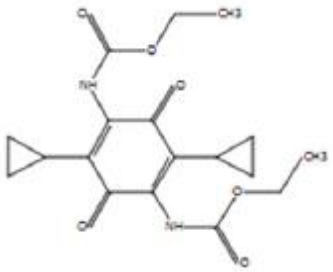

(31)

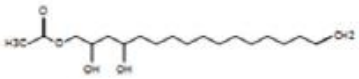

(32)

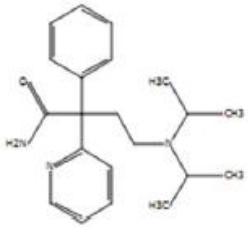

(33)

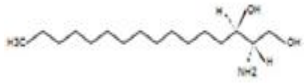

(34)

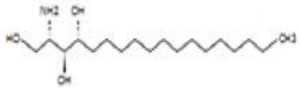

(35)

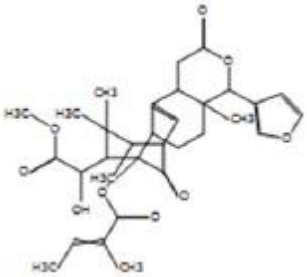

(36)

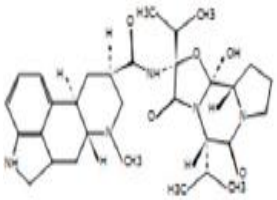

(37)

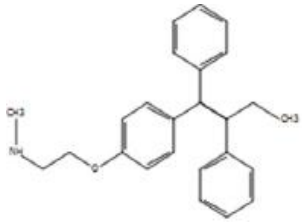

(38)

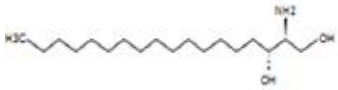

(39)

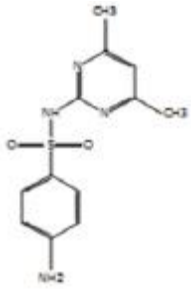

(40)


(41)

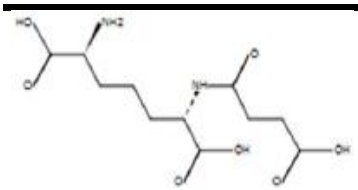

(42)


(43)

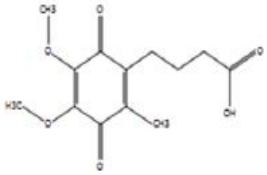

(44)


(45)

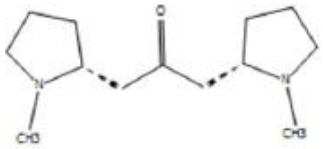
*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*



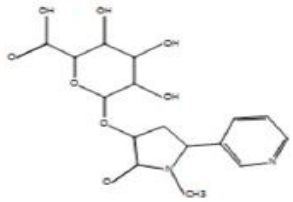
(46)



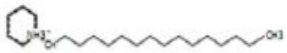
(47)



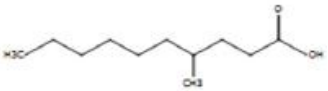
(48)



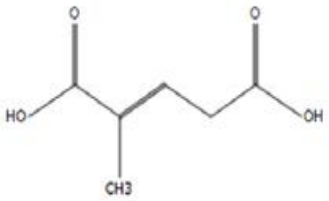
(49)



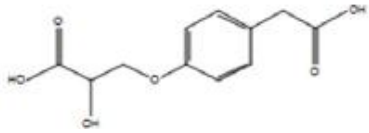
(50)



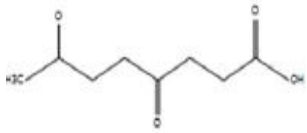
(51)



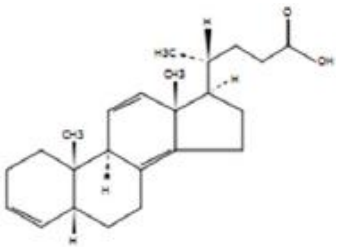
(52)



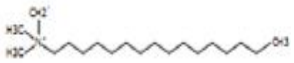
(53)



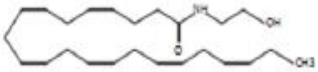
(54)



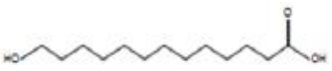
(56)



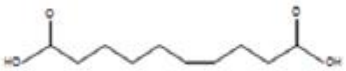
(55)



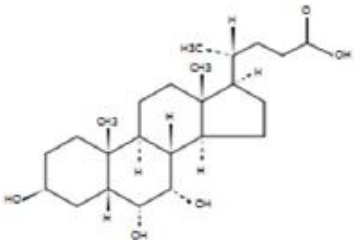
(57)



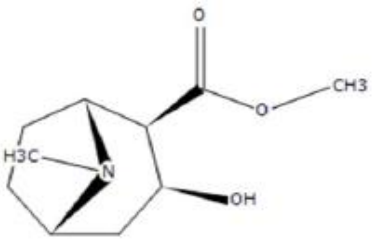
(58)



(59)



(60)



(61)

*Fig.2: Structure of compounds in the methanolic tuber extracts of Pueraria tuberosa*

*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

## IV. DISCUSSION

The LC-MS analysis of methanolic tuber extracts of *P.tuberosa* showed the presence of various phytoconstituents that showed interesting biological activity. A tetranotriterpenoid, swietenine was reported to posses significant hypolipidemic and hypoglycemic activity in type 2 diabetic rat (Dewanjee *et al*., 2009). Antiepileptic vigabatrin is a selective and irreversible GABA-transaminase inhibitor that greatly increases whole-brain levels of GABA, (Angehagen *et al*., 2003). Barbituric and thiobarbituric acid derivatives were found to be effective against non-alcoholic fatty liver disease (Ma *et al*., 2011). An important flavonoid rhoifolin, is used extensively in phytomedicine to treat a wide range of diseases. Antioxidant, antimicrobial, anti-inflammatory, anticancer and hepatoprotective effects are the significant biological activities of rhoifolin (Refaat *et al*., 2015). It has been reported that cetrimonium bromide is a potential therapeutic agent for human head and neck cancer (Ito *et al*., 2009). Octanoic acid decreases *Campylobacter jejuni* colonization in market-aged broiler chickens (Santos *et al*., 2009). Both caprylic acid (octanoic acid) and monocaprylin have antibacterial effect on major bacterial mastitis pathogens (Nair *et al*., 2005). The nutrient, 4Z-decenedioic acid act as a membrane stabilizer and energy source.

## V. Conclusion

As shown in the present study, LC-MS based diverse bioactive compound profiling of methanolic extract of *P. tuberosa* tubers appear to be useful for distinguishing between known to unknown compounds. Accurate mass assignment at high resolution provides opportunities for the interpretation of major components from methanolic extract of tubers of *P. tuberosa*. This method can be used for the routine quality control of crude drug and also for screening the novel compounds 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. LC-MS based chemical screening of diverse bioactive compounds in the tuber extract of *P. tuberosa* would appear to be an effective approach for discovering the unknown compounds.

**Acknowledgement**

The authors are thankful to IIT Mumbai, for providing LC-MS chromatogram.

## REFERENCES

[1] Angehagen M, Ben-Menachem E, Rönnbäck L and Hansson E. (2003). Novel Mechanisms of Action of Three Antiepileptic Drugs, Vigabatrin, Tiagabine, and Topiramate. *Neurochemical Research*. 28 (2): 333–340.

[2] Asharf A, Sarfraz R. A, Mahmood A, Din M. U. (2015). Chemical composition and *in vitro* antioxidant and antitumor activities of *Eucalyptus camaldulensis* Dehn. leaves. *Industrial Crops and Products*.74: 241–248.

[3] Chopra R. N, Nayar S. L and Chopra I. C. 1992. Glossary of Indian Medicinal Plant Council for Scientific and Industrial Research, Government of India, New Delhi, India.

[4] Dewanjee S, Maiti A, Das A. K, Mandal S. C and Dey S. P. (2009). Swietenine: a potential oral hypoglycemic from *Swietenia macrophylla* seed. *Fitoterapia*. 80 (4): 249-51.

[5] Gupta R. S, Sharma R, and Sharma A. (2004). "Antifertility effects of *Pueraria tuberosa* root extract in male rats," *Pharmaceutical Biology*. 42 (8): 603–609.

[6] Hsu F.L , Liu I.M , Kuo D.H, Chen W.C , Su H.C and Cheng J.T. (2003). Antihyperglycemic effect of puerarin in streptozotocin-induced diabetic rats. *Journal of Natural Products*. 66 (6): 788-792.

[7] Ito E , Yip K. W, Katz D , Fonseca S. B , Hedley D .W, Chow S , Xu G. W , Wood T. E , Bastianutto C , Schimmer A. D , Kelley S. O and Liu F. F. (2009). Potential Use of Cetrimonium Bromide as an Apoptosis-Promoting Anticancer Agent for Head and Neck Cancer. *Molecular Pharmacology*. 76 (5): 969-983.

[8] Krug D, Zurek G, Revermann O, Vos M, Velicer G. J and Muller R. (2008). Discovery the hidden secondary metabollome of *Myxoccus xanthus*: A study of intraspecific diversity. *Applied and Environmental Microbiology*. 74 (10): 3058-68.

[9] Ma L, Li S, Zheng H, Chen J, Lin L, Ye X, Chen Z, Xu Q, Chen T, Yang J, Qiu N, Wang G, Peng A, Ding Y, Wei Y and Chen L. (2011). Synthesis and biological activity of novel barbituric and thiobarbituric acid derivatives against non-alcoholic fatty liver disease. *European Journal of Medicinal Chemistry*. 46 (6): doi: 10.1016/j.ejmech.2011.02.033.

[10] Nair M. K, Joy J, Vasudevan P, Hinckley L, Hoagland T. A and Venkitanarayanan K. S (2005). Antibacterial effect of caprylic acid and monocaprylin on major bacterial mastitis pathogens. *Journal of Dairy Science*. 88**:** 3488-3495.

[11] Refaat. J, Yehia S. Y , Ramadan M. A and Kamel M. S. (2015). Rhoifolin: a review of sources and biological activities. *International Journal of Pharmacognosy*. 2(3): 102-109.

[12] Santos F. S, Donoghue A. M, Venkitanarayanan K, Metcalf J. H, Reyes-Herrera I, Dirain M. L, Aguiar V. F, Blore P. J and Donoghue D. J. (2009). The

*https://dx.doi.org/10.22161/ijhaf.2.5.4* *ISSN: 2456-8635*

natural feed additive caprylic acid decreases *Campylobacter jejuni* colonization in market-aged broiler chickens. *Poultry science*. 88: 61-64.

[13] Tahir H. U, Sarfraz A. R, Asharf A and Adil S. (2016). Chemical composition and anti-diabetic activity of essential oils obtained from two spices (*Syzygium aromaticum* and *Cuminum cyminum*). *International Journal of Food Properties*. 19: 2156–2164.

[14] Villas-Boas S.G , Mas S, Akesson M, Smedsgaard J and Nielson J. (2005). Mass spectrometry in metabolom analysis. *Mass Spectrometry Reviews*. 24: 613-646.

[15] Wang X, Sakuma T, Asafu-Adjaye E and Shiu G. K. (1999). Determination of ginsenosides in plant extracts from *Panax ginseng* and *Panax quinquefolius* L. by LC/MS/MS. *Analytical Chemistry*.71: 1579-1584