IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
Vol. 1, Issue 3, Aug 2013, 49-58

PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOL EXTRACT OF *CEROPEGIA PUSILLA*

K. KALIMUTHU & R. PRABAKARAN

Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India

ABSTRACT

Phytochemical constituents are responsible for medicinal activity of plant species. Hence in the present study preliminary phytochemical screening of *Ceropegia pusilla* a medicinal plant was carried out. Qualitative phytochemical analysis of these plants confirm the presence of various secondary metabolites like saponins, triterpenoids, steroids, tannins, lignins, alkaloids, glycosides, flavonoids and phenols. The results suggest that the phytochemical properties for curing various ailments and possess potential anti-inflammatory, antimicrobial and antioxidant and leads to the isolation of new and novel compounds. GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *Ceropegia pusilla* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

KEYWORDS: *Ceropegia pusilla* , Indian Medicinal Plants, Phytochemical Screening

INTRODUCTION

Medicinal plants are the richest bio-resources of folk medicines and traditional systems of medicine; and food supplements, nutraceuticals, pharmaceutical industries and chemical entities for synthetic drugs (Ncube *et al.* 2008). Modern medicine has evolved from folk medicine and traditional system only after through chemical and pharmaceutical screening (Boopathi and Sivakumar, 2011). India is the birth place of renewed system of indigenous medicine such as Siddha, Ayurvedha and Unani. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug (Vinoth *et al.* 2011; Savithramma *et al.* 2010). There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity. Turger and Usta (2008) screening active compounds from plants has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer (Sheeja and Kuttan 2007) and Alzhemir's diseases (Muherjee and Kumar 2007).

Phytochemicals are responsible for medicinal activity of plants (Savithramma *et al.* 2011). These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that is primary and secondary metabolites based on the function in plant metabolism. Primary metabolites are comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on (Jigna and Sumitra, 2007; Kumar *et al.* 2009). Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs (Savithramma *et al.* 2011).

The genus *Ceropegia* L. is the largest genus of the tribe Ceropegiaeae with about 200 species distributed only in tropical and sub tropical regions of the Old World, ranging from the Spanish Canary Islands in the west, through Central, Southern, and Northern Africa, Madagascar, Arabia, India, South Asia to Northern Australia in the East (Good, 1952; Anonymous, 1992; Bruyns, 2003). The maximum diversity of *Ceropegia* occurs in South Africa followed by Kenya and Madagascar. Its species diversity eastwards diminishes in Arabia where only 10 species were recorded and only one species in Pakistan. The species of *Ceropegia* as a whole are under threat, owing to either destructive collection or habitat degradation. They are not only genetically depleted but also are scarcely available. Ansari (1984) revised the Indian *Ceropegia* and reported 44 species, of which 28 are said to be endemic to India. Many species of the genus *Ceropegia* have now been added to the list of Indian endangered plants (BSI, 2002). These species are placed under the categories of rare, endangered, vulnerable, extinct, and threatened plants (Nayar and Sastry, 1987; Goyal and Bhadauria, 2006; Madhav Gadgil, 2004)

Economic Importance of the Genus *Ceropegia*

The sweet-sour leaves are edible and are considered to be tonic and digestive. (Kirtikar and Basu 1935) It is used as an Antidote for snake bite (Duraisamy Suresh and Paulsamy 2010**)**.The tubers are edible (Mabberly, 1987) and contain starch, sugars, gum, albuminoids, carbohydrates, fats, crude fibre, and the medicinally important alkaloid Ceropegin (Kirtikar and Basu, 1935; Nadkarni, 1976; Anonymous, 1980; Jain and Defillips, 1991). The root tubers also contain starch, sugars, gum, albuminoids, fats, crude fiber and valuable constituents in many traditional Indian Ayurvedic drug preparations that are active against ulcers, inflammation etc., (Adibatti *et al.* 1991). The boiled or roosted tubers are edible and a rich source of carbohydrates (Nikam and Savanth, 2007). These species are of economic importance (Jagtap and Singh, 1999) due to their starchy edible tubers with medicinal value. The fresh tubers of these species are usually boiled before they are eaten to remove the bitterness. The active compound of tuberous roots is the alkaloid Ceropegin which is active against diarrhea and dysentery inflammation of gums and delirious fevers of parturition (Nadkarni, 1976). The tubers then again are a kind of energy source, among other things they are used to suppress fatigue. Furthermore the sticks with which the Toda stamp milk to butter (http://www.biodiversitylibrary.org). The alkaloid Ceropegin from the tubers of *C. bulbosa* was used in Bihar to cure cold, sneezing and eye diseases (Kirtikar and Basu, 1935). The crude extracts of *Ceropegia tuberose* were active against different bacterial strains (Vijayakumar *et al*. 2013)

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant material was collected in from Nilgiri hills, Tamilnadu, (India). Plants with tuber and follicle removed from soil and deposited in polythene bag. The proper identification of the plant is the most important aspect of any research programme. The plant material collected was identified and authenticated by Botanical Survey of India (**BSI/ SRC/ 5/23/2012-13/ tech 1268**) Coimbatore.

Sampling of Plant Material

Fresh plant tuber free from diseases was collected during the month of June, 2012. The tubers were washed thoroughly 2-3 times with running tap water, tuber was cut into small pieces then air dried under shade. The plant tuber was grinded and powders were kept in air tight plastic bags with paper labeling for future uses.

Phytochemical Studies

Preparation of plant extracts for preliminary phytochemical studies: The grinded tuber materials of 5g weighed separately using an electronic balance and were crushed in 25 ml of sterile water, boiled at 50-60^{0}C for 30 minutes on

water bath and it was filtered through Whatman No.1 filter paper. Then filtrate was centrifuged at 2500 rpm for 15 minutes and filtrate was stored in sterile bottles at 5^0C for further use (Harbone, 1973).

Preliminary Phytochemical Studies

The extract was subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material was tested. Condensed extracts were used for preliminary screening of phytochemicals such as alkaloids, steroids, and phenols (Gibbs, 1974); glycosides, triterpenoids and saponins (Ayoola *et al.* 2008); tannins, (Treare and Evan, 1985); flavonoids (Peach and Tracey, 1956).

Gas Chromatography-Mass Spectrometry Analysis

The GC – MS analysis was carried out using a Clarus 500 Perkin – Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 μm DF of capillary column. The instrument was set to an initial temperature of 110ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280ºC, at the rate of an increase of 5ºC /min, and maintained for 9 min. Injection port temperature was ensured as 200 ºC and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Version –Year 2011 were used MS data library and comparing the spectrum obtained through GC – MS compounds present in the plants sample were identified.

RESULTS AND DISCUSSIONS

Phytochemical Screening Medicinal Plants

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species. Hence in the present study preliminary phytochemical screening of *Ceropegia pusilla* a medicinal plants was carried out, qualitative phytochemical analysis of this plant confirm the presence of various secondary metabolites like alkaloids, glycosides, tannins, saponin, flavonoids, steroid, triterpenes and phenol. The results suggest that the phytochemical properties for curing various ailments and possess potential anti-inflammatory, antimicrobial and antioxidant and leads to the isolation of new and novel compounds.

Alkaloids have a bitter taste while many to toxic to other organisms (Gupta *et al.* 2010). Flavonoids are a group of polyphenolic compounds which influence the radical scavenging, inhibition of hydrolytic and oxidative enzymes and also act as anti-inflammatory agent (Frankel, 1995). The flavonoids show antioxidant activity and their effects on human nutrition and health is considerable. The mechanism of action of flavonoids are through scavenging or chelating process (Kessler *et al.* 2003; Cook and Samman, 1996), they also inhibit microbes which are resistant to antibiotics (Linuma *et al.* 1994). Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity (Salah *et al.* 1995). As antioxidants flavonoids provide anti-inflammatory actions (Okwu, 2001A; Okwu, 2001B). Glycoside compounds are containing a carbohydrate and non-carbohydrates residue (moiety) in the same molecule. In these compounds, the carbohydrate moiety is attached by an acetyl linkage carbon-I to the non-carbohydrate residue (aglycone). They all contain steroid as aglycone component in combination with sugar molecules. They are important in medicine because of their action on heart and are used in cardic insufficiency

(Balch and Balch, 2000). Thus, cardiac glycosides are drugs and can be used in the treatment of congestive heart failure and cardiac arrhythmia. They work by inhibiting the Na^+/ Na^+ pump, resulting in an increase in the levels of sodium ions in the myocytes, which then leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca^{2+} ions available for concentration of the heart muscle, improves cardiac output and reduces distention of the heart (Bertorello *et al.* 1990; Clausen and Nielsan, 1994; Beltowski *et al.* 1998). However, same glycosides such as ovarian are toxic as it inhibits active transport of Na^+ in cardiae muscle (Sodium pump inhibitor), resulting in inhibition of translocases during electron transport chain and leading to death (Beltowski *et al.* 1998).

Primarily phenolic compounds are of great importance as cellular support material because they form the integral part of cell wall structure by polymeric phenolics (Gupta *et al.* 2010), bioactive polyphenols have attracted special attention because they can protect the human body from the oxidative stress which may cause many diseases, including cancer, cardiovascular problems and ageing (Robards *et al.* 1999). The phenolic compounds are one of the largest and most ubiquitous group of plant metabolites. A number of studies have focused on the biological properties such as antiapoptosis, anti-ageing, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection and improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity (Han *et al.* 2007). Phenolic compounds have been extensively used in disinfections and remain the standards with which other bacteriocides are compared (Okwu, 2001).

Tannins has astringent properties, hastens the healing of wounds and inflamed mucous membrane. Tannins contribute property of astringency i.e. fasten the healing of wounds and inflamed mucous membrane and have received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant, antimicrobial and anti-inflammatory properties. Tannins are complex moieties produced by majority of plants as protective substances, they have wide pharmacological activities. They have been used since past as tanning agents and they posses astringent, anti-inflammatory, antidiarrhoeal, antioxidant and antimicrobial activities (Killedar and More, 2010).

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the methanolic extract of *C.pusilla*. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *C.pusilla* using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *C.pusilla* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

CONCLUSIONS

The medicinal plants appear to be rich in secondary metabolites, widely used in traditional medicine to combat and cure various ailments. The anti-inflammatory, antispasmodic, analgesic and diuretic can be attributed to their high alkaloids, phenols, tannins and flavonoids. Exploitation of these pharmacological properties involves further investigation

of these active ingredients by implementation of techniques like extraction, purification, separation, crystallization and identification.

REFERENCES

1. Adibatti NA, Thirugnanasambantham P and Kuilothngan C .A pyridine alkaloid from *Ceropegia juncea.* Phytochemistry (1991) 30:2449-2450.
2. Anonymous (1992). *Ceropegia* Linn. (Asclepiadaceae). In: The wealth of India.. New Delhi: CSIR: 3: 448-449.
3. Anonymous The Wealth of India: A dictionary of Indian raw materials and Industrial products: first supplement series (Raw materials): Vol. A-Ci. New Delhi, (1980). pp. 283.
4. Ansari MY Asclepiadaceae: Genus *Ceropegia* - Fascicles of Flora of India. Botanical Survey of India, Calcutta (1984). 16: 1-34.
5. Ayoola GA, Coker HAB, Adesegun SA, Adepoju–Bello AA, Obaweya K, Ezennia EC and Atangbayila TO, Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in South Western Nigeria. Trop. J. Pharm. Res., (2008); 7: 1019-1024.
6. Balch, JF and Balch, PA. Prescription for Nutritional Healing. New York. A very,penguin Putnam Inc. (2000); 267-270.
7. Beltowski J, Gorny D and Maeciniak A. The mechanism of Na+ - K+ - ATPase inhibition by artiral nattruiretic factor in rat and medulla. J Physio Pharma, (1998); 49: 271-283.
8. Bertorello A and Aperra A. Short term of Na+ - K+ - ATPase activity by dopamine. Ameri J Hyper, (1990); 3: 515-545.
9. Boopathi AC and Sivakumar R. Phytochemical screening studies on the leaves and stem of *Andrographis neesiana* wight – An endemic medicinal plant from India. World App Sci J, (2011); 12(3): 307-311.
10. Bruyns PV Three new succulent species of Apocynaceae (Asclepiadoideae) from southern Africa. Kew Bull (2003) 58:427–435.
11. BSI (2002). Studies on Rare and Endangered Species http://www.envfor.nic.in/bsi/research.html.
12. Clausen T and Nielsan O.B. The Na+ - K+ - ATPase and muscle contractivity. Acta Physio Scander. (1994); 152: 365-375.
13. Cook NC and Samman S, flavonoids chemistry, metabolism, cardioprotective effects and dietary sources. Nutr Bioche, (1996); 7: 66-76.
14. Duraisamy Suresh and Subramaniam Paulsamy Phenological observation and population dynamics of six uncommon medicinal plants in the grasslands of Nilgiris, Western Ghats, India *Maejo Int. J. Sci. Technol.* (2010) *4* (02), 185-192.
15. Frankel E, Nutritional benefits of flavonoids, International conference on food factors: chemistry and cancer prevention, Hamamatsu, Japan, Abstracts, C-2, (1995).
16. Gibbs RD, Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen’s University Press, Montreal and London, (1974).

17. Good R (1952) An atlas of the Asclepiadaceae. New Phytol 51:198–209.

18. Goyal D, Bhadauria S (2006). *In vitro* propagation of *Ceropegia bulbosa* using nodal segments. Indian J Biotechnol., 5: 565-567.

19. Gupta VK, Singh GD, Singh S and Kaul A, Medicinal Plants: Phytochemistry, Pharmacolgoy and Therapeutics, Daya Publishing House, Delhi. (2010).

20. Han X, Shen T and Lou H, Dietary polyphenols and their biological significance. Int J Mol Sci, (2007); 8: 950-988.

21. Harbone JB, Phytochemicals methods. London. Chapman and Hill, (1973).

22. Jagtap A, Singh NP (. Fasc. Flora of India 24, Botanical Survey of India, Kolkata, 1999) pp. 211-241.

23. Jain SK, Defillips RA (1991). Asclepiadaceae. In: Medicinal plants of India. Algonac, India 1:89-94.

24. Kessler M. Ubeand G and Jung L, Anti and prooxidant activity of rutin and quercetin derivatives, J. Pharm and Pharmacol, (2003); 55: 131-142.

25. Killedar SG and More HN, Estimation of tannins in different parts of *Memecylon umbellatum* Burm., J Phar Res, (2010); 3(3): 554-556.

26. Kirtikar KR, Basu BD (1935). Indian Medicinal Plants Vol.3 Bishen Singh Mahendra, New Delhi, India.

27. Kumar A, Ilavarasan R, Jayachandran T, Decaraman M, Aravindhan P, Padmanaban N and Krishna MRV. Phytochemical investigation on a tropical plants. Pak J Nutri, (2009); 8: 83-85.

28. Linuma M, Tsuchiya H, Salo M, Yokoyama J, Ohyama M, Ohkawa Y et al., Flavanones with potent antibacterial activity against methicillin-resistant staphylococcus aureus. J. Pharm. Pharmacol, (1994b); 46(11): 892-895.

29. Mabberly DJ (1987). The plant book. Cambridge University Press, Cambridge, pp.114-115.

30. Madhav Gadgil (2004). ENVIS Technical Report No. 16, Environmental Information, Bangalore, http://www.ces.iisc.ernet.in/hpg/envis pp. 96-98.

31. Mukherjee PK, Kumar V, Houghton PJ. Screening of Indian medicinal plants for acetyl cholinesterase inhibitory activity. Phytother Res (2007); 21: 1142-1145.

32. Nadkarni KM) Indian MateriaMedica. Popular Prakasha, Bombay, India, ISBN: 81- 7154-144-5, (1976) pp.: 303-304.

33. Nayar MP, Sastry ARK Red Data Book of Indian plants Vol. 1 Botanical Survey of India, Calcutta. pp. (1987). 170.

34. Ncube NS, Afolayan AJ and Okoh AI, Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotechnol., (2008); 7: 1797-1806.

35. Nikam TD, Savanth RS Callus culture and micropropagation of *Ceropegia sahyadrica* Ans. et Kulk: An edible starchy tuberous rare asclepiad. Indian J. Plant Physiol., 12: (2007). 108-114.

36. Okwu, D.E. Evaluation of Chemical composition of indigenous species and flavouring agents. Global J. Pure Appl. Sci., (2001): 458-459.

37. Okwu, D.E. Evaluation of the chemical composition of indigenous species and flavouring agents. Global J. Pure Appl, Sci, (2001B); 8; 455-459.

38. Okwu, D.E. Improving the nutrition value of cassava tapioca meal with local species. Nutracentical, Functional and medicinal food, (2001A); 3: 43-51.

39. Parekh Jigna and Chanda V Sumitra. *In vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal plants. Turk J Biol, (2007); 31: 53-58.

40. Peach K and Tracey MV, Modern methods of plant analysis. Vol.3, Springer Verlag, Berlin, (1956).

41. Robards K, Prernzler PD, Tucker G, Swatsitang P and Glover W, Phenolic compounds and their role in oxidative processes in fruits. Food Chem., (1999); 66: 401-36.

42. Salah, W., N. Miller, G. Pagauga, G. Tybury, E. Bolwell, E. Rice and C. Evans, Polyphenolic flavonoids as scavenger of aqueous phase radicals and chain breaking antioxidants. Arch. Biochem., (1995). 2: 239-346.

43. Savithramma N, Linga Rao M and Beenaprabha, Phytochemical studies of *Dysophylla myosuroides* (Roth.) Benth. In. wall. and *Talinum cuneifolium* (Vahl.) Willd. Res J Phyto, (2011); 5(3): 163-169.

44. Savithramma N, Venkateswarlu P, Suhrulatha D, Basha SKM and Venkataramanadevi CH, Studies of *Boswellia ovalifoliolata* Bal. and Herny – An endemic and endangered medicinal plant. The Biosc., (2010); 5: 359-362.

45. Sheeja K, Kuttan G. Activation of cytotoxic T lymphocyte responses and attenuation of tumor growth in vivo by Andrographis paniculata extract and andrographolide. Immunopharmacol Immunotoxicol (2007); 29: 81-93

46. Treare GE and Evans WC, Pharmacognosy 17th edn, Bahive Tinal, London, (1985): 149.

47. Turker AU, Usta C. Biological screening of some Turlish medicinal plants for antimicrobial and toxicity studies. Nat Prod (2008); 22: 136-146.

48. Vijayakumar S, Chandrasekar S & Prabhu S Screening of ethnomedicinal plants for antibacterial activity International Journal of Medicine and Pharmaceutical Sciences (2013) Vol. 3, Issue 2, , 11-20.

49. Vinoth S, Rajesh Kanna P, Gurusaravanan P and Jayabalan N, Evaluation of phytochemical, antimicrobial and GC-MS analysis of extracts of *Indigofera trita* L.F. spp. Subulata (Vahl ex poir). Int J Agric Res. (2011); 6(4): 358-367.

APPENDICES

Table 1: Preliminary Phytochemical Analysis of Ethanolic Extract of *C.pusilla*

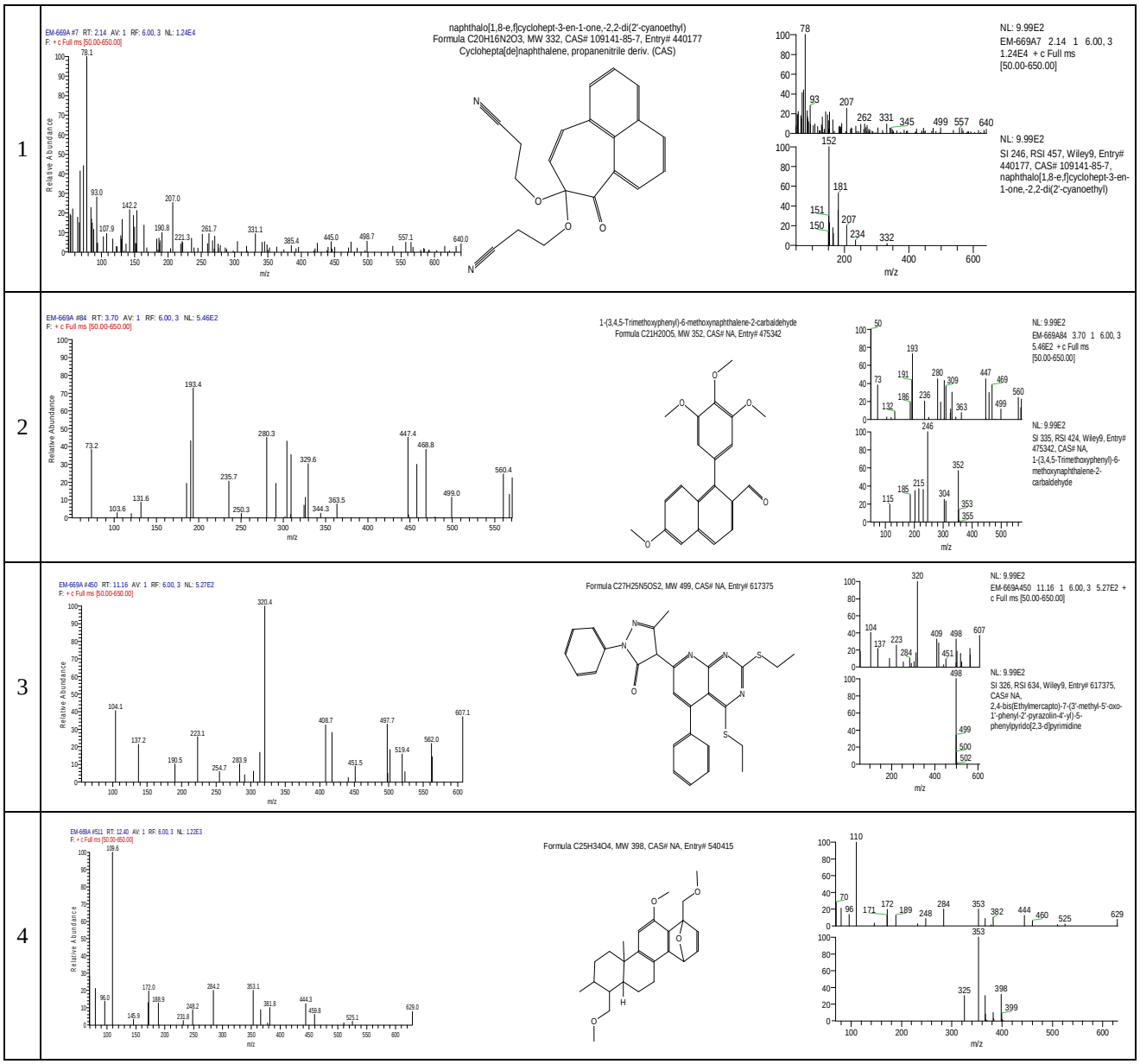
S. No	Secondary Metabolites	Ethanol Extract
1	Alkaloids	+
2	Glycosides	+
3	Tannins	+
4	Saponins	+
5	Flavonoids	+
6	Steroid	+
7	Triterpenes	+
8	Phenol	+

Table 2: Activity of Phytocomponents Identified in *C.pusilla* by GC-MS

S. No	RT	Compound Name	Molecular Formula	MW	Peak Area
1	2.14	naphthalo[1,8-e,f]cyclohept-3-en-1-one,-2,2-di(2'-cyanoethyl)	$C_{20}H_{16}N_2O_3$	332	4.53
2	2.65	à-[Tris(trimethylsilyl)methyl]silyl-4-methoxybenzyl propanoate	$C_{21}H_{42}O_3Si_4$	454	3.81
3	3.70	1-(3,4,5-Trimethoxyphenyl)-6-methoxynaphthalene-2-carbaldehyde	$C_{21}H_{20}O_5$	352	4.01
4	4.87	2,6-Dibromo-3,5-dimethoxy-4-(1,2,2,2-tetraffluoro-1-trifluoromethylethyl) pyridine	$C_{10}H_6Br_2F_7NO_2$	463	3.03
5	5.17	6-methyl-2-(4-methylphenyl)-7-(2,4,5-trimethylbenzyl) indolizine	$C_{26}H_{27}N$	353	2.21
6	9.35	7-Bromo-1-(2'-methylprop-2'-en-1'-yl)-4,6-dimethoxy-2,3-diphenylindole	$C_{26}H_{24}BrNO$	461	2.42
7	9.65	GRAYANOTOXIN 111 3,6-DIPROPINATE	$C_{26}H_{42}O_8$	482	2.99
8	11.16	2,4-bis(Ethylmercapto)-7-(3'-methyl-5'-oxo-1'-phenyl-2'-pyrazolin-4'-yl)-5-phenylpyrido[2,3-d]pyrimidine	$C_{27}H_{25}N_5OS_2$	499	5.10
9	12.40	[6aR-(1a,4a,6ab,7a,10aa)]-1,4-epoxy-12-methoxy-1,7-bis(methoxymethyl)-7,10a-dimethyl-1,4,5,6,6a,7,8,9,10,10a-decahydrochrysene and [6aR-(1a,4a,6aa,7b,10ab)]-1,4-epoxy-12-methoxy-1,7-bis(methoxymethyl)-7,10a-dimethyl-1,4,5,6,6a,7,8,9,10,10a-decahydrochrysene	$C_{25}H_{34}O_4$	398	4.33
10	14.60	1-(4'-Nitrophenyl)-4-(4"-N,N-dimethylaminophenyl)imino-3-phenyl-4,5-dihydro-5-oxopyrazole	$C_{23}H_{19}N_5O_3$	413	2.34
11	18.56	Dimethyl 6,7-(methylenedioxy)-4-(3,4-methylenedioxyphenyl) naphthalene-2,3-dicarboxylate	$C_{22}H_{16}O_8$	408	2.04
12	19.01	1-[(2-Hydroxyethoxy)methyl]-5-n-propyl-6-(1-naphthylmethyl) uracil	$C_{21}H_{24}N_2O_4$	368	4.45
13	19.29	5,8-Dihydroxy-3,4',7-trimethoxyflavone	$C_{18}H_{16}O_7$	344	2.14
14	20.17	2,4-Dimethoxy[carboxy-13C]benzoic acid	$C_9H_{10}O_4$	182	2.79
15	20.59	13-di-O-acetyl-19,10-thiogibberellin A(1) methyl ester	$C_{24}H_{30}O_7S$	462	6.91
16	20.86	4-Diallylaminomethyl-3-ethoxymethyl-2-methyl-3H-imidazole-4-carboxylic acid ethyl ester	$C_{17}H_{27}N_3O_3$	321	3.29
17	23.06	Ethyl ester of 2-[6-(ethylamino)-3-(ethylimino)-2,7-dimethyl-3H-xathen-9-yl]-benzoic acid	$C_{28}H_{30}N_2O_3$	442	2.60
18	24.47	16á,28-O,O-ETHYLIDENELUP-20(29)-ENE-3á,16á,28-TRIOL	$C_{32}H_{52}O_3$	484	4.90
19	25.36	2-[Diacetylamino]-6-(3'-methyl-5'-oxo-1'-phenyl-2'-pyrazolin-4'-yl)-4-phenylpyridine-3-carbonitrile	$C_{26}H_{21}N_5O_3$	451	4.93
20	27.85	Ethylene ketal of Methyl trans-(6-Methoxy-2-naphthyl)-1-methyl-5-oxocyclopentane-r-1-acetate	$C_{22}H_{26}O_5$	370	3.81
21	29.44	EXO-1,3-DIMETHYL-2,9-DIOXA-BICYCLO(3.3.1) NONANE	$C_9H_{16}O_2$	312	3.44

Table 2: Contd.,

22	31.11	7-à-Acetoxy-(1Hà,6Hà)-bicyclo[4.4.1]undeca-2,4,8-trien-11-one	$C_{13}H_{14}O_3$	218	2.18
23	31.33	Tetramethyl ester of 9-benzyl-9-azabicyclo-[4.2.1]nona-4,7-dien-1,4,7,8-tetroic acid	$C_{23}H_{25}NO_8$	443	2.08
24	35.61	6à,7á-DIACETOXYDIHYDRODRIMENIN	$C_{19}H_{28}O_6$	352	1.85
25	39.73	2-Phenyl-4,7-dihydro-4,7-methano-2H-isoindole	$C_{15}H_{13}N$	207	2.74
26	40,70	2,6-Diacetoxyandrosta-1,4,6-trine-3,17-dione	$C_{23}H_{26}O_6$	398	4.80
27	41.23	methyl elaiate	$C_{19}H_{36}O_2$	296	2.48
28	41.93	ERIOCALYXIN C	$C_{22}H_{28}O_7$	404	1.82



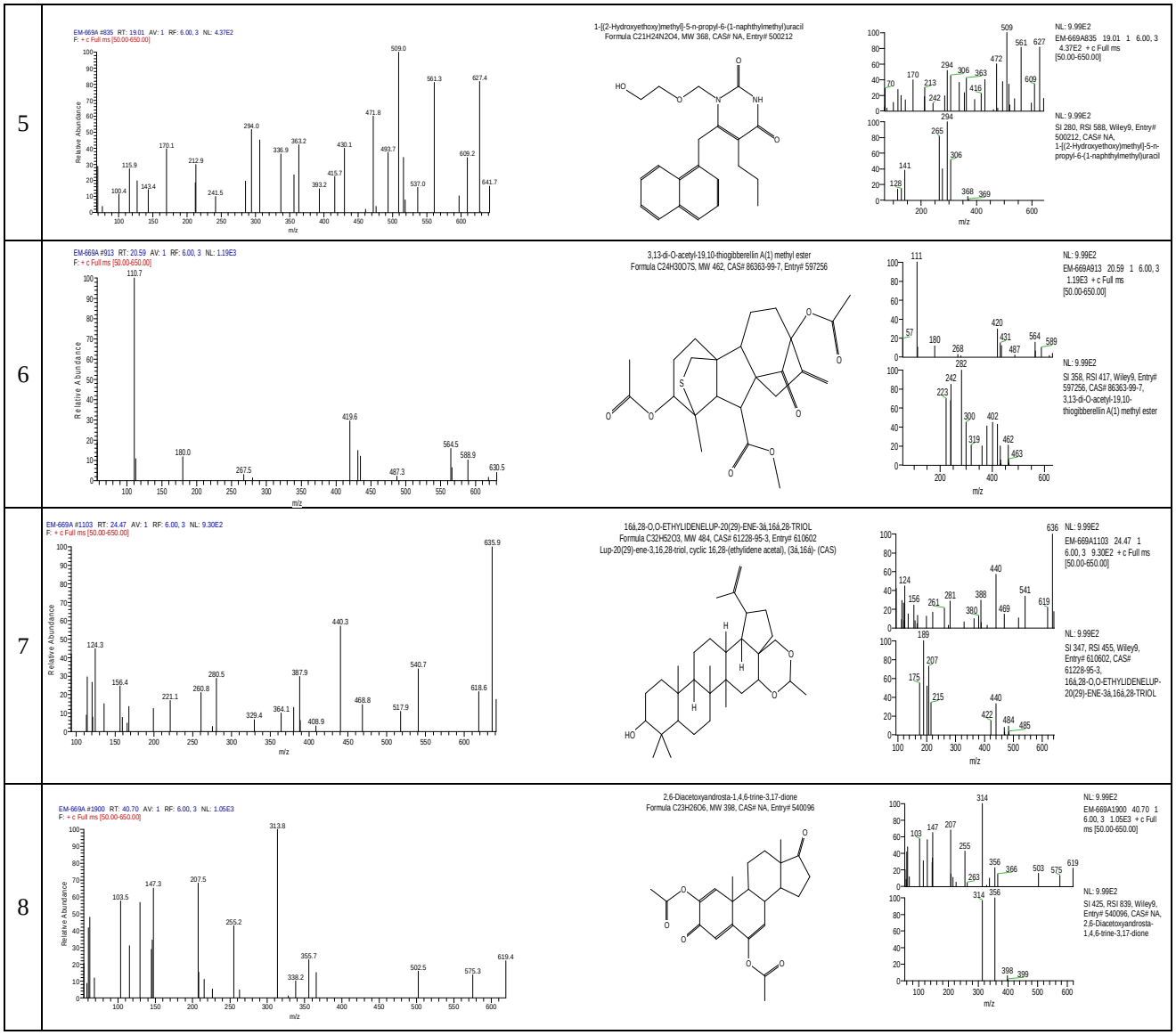


Figure 1: GC-MS Chromatogram of Methanolic Extract of Stem of *C.pusilla*