Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating: 3.53
www.plantsjournal.com
JMPS 2021; 9(3): 136-140

Received: 17-03-2021
Accepted: 20-04-2021

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Qualitative analysis of phytochemical in *Eulophia nuda* using LCMS

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Abstract
The beneficial medicinal effects of plant material typically result from combination of secondary products present in plant. The identification and quantification of secondary metabolites plays an important role in exploiting the medicinal potential of plant. Liquid chromatography coupled with mass spectrometry can identify several compounds from plant extract. LC MS analysis of methanolic extract of *E. nuda* confirmed presence of phenolics, flavonoids, and other bioactive compounds such as alkaloids, toxins and antibiotics.

Keywords: *Eulophia nuda*, LC-MS, Bioactive compounds

Introduction
The genus *Eulophia*, commonly known as Amarkand, belonging to most interesting plant family i.e. orchidaceae is well known for its potential in ethnic medicines. *Eulophia* species are used in herbal medicines and food (rhizome) by many different tribes. Out of 30 species distributed throughout India, 6 species namely; *E. nuda*, *E. ochreata*, *E. herbacea*, *E. graminea*, *E. epidendrea* and *E. ramentacea* are reported from Maharashtra. Of these 6 species, *E. nuda* have shown high biological activity as revealed from its TPC, TFC and antioxidant activity [1]. Qualitative phytochemical analysis of *Eulophia nuda* revealed that it stores many phytochemicaly active ingredients like Alkaloids, flavonoids, saponins, Cardiac glycosides, Tepenoids and Steroids [2]. *Eulophia nuda* is exploited as antidote for snake bite, as antihelmintic, against tumors, cases of bronchitis, scrofulous affection of the glands of the neck and in disease of the blood [3]. The plant is also claimed to be useful in tuberculosis [4]. Earlier phytochemical studies showed presence of essential minerals, polyphenols, saponins, alkaloids, phytic acid etc. in *E. ochreata* and *E. nuda*. Nine different phenanthrenes, a rather uncommon class of aromatic metabolites, have been reported from *E. nuda* till date [5].
The present study was carried out in search of bioactive compounds from *E. nuda* with the help of LC-MS.

Materials and methods
Plant material
Eulophia nuda, collected from different parts of Western Ghats of Maharashtra was maintained in Botanical Garden, Dept. of Botany, Shivaji university, Kolhapur (MS), The species was identified by Dr. R. V. Gurav, and herbarium specimen deposited in Dept. of Botany, Shivaji University, Kolhapur (MS), India.

Extraction of plant material
Rhizomes of *Eulophia nuda*, were chopped, shade dried and ground to fine powder with mixer. 500 mg of fine powder of each species extracted with 20 ml methanol at room temperature for 24 hrs. The extract was filtered using Büchner funnel and stored at 4^0C till further use.
The methanolic extract of *E.nuda* was analyzed by reverse phase HPLC on an Agilent 1200 series HPLC system fitted with microchip technology column (Agilent, G1316A). The HPLC conditions were as follows: flow rate, 0.4 µL/min; solvent A,100% water; solvent B, 100% methanol; gradient, 20-100% over 5 min and kept at 100% for 5 min. Then 2 µL of the extract dissolved in methanol-water (80:20, v/v), was analyzed by ESI in positive mode using an Agilent G6540B time-of-flight (TOF) mass spectrophotometer.

Mass spectral data were acquired in the range m/z 100-1700, with an acquisition rate of 1spectra/s, averaging 10,000 transients. The source parameters were adjusted as follows: drying gas temperature 250 ^{0}Cdrying gas flow rate 5 L/min, nebulizer pressure 45 psi, and fragmentor voltage 150 V. Data acquisitions and processing were done using Agilent Mass Hunter Workstation Acquisition v. B.02.00 software.

Results and Discussions

The methanolic extract of *E. nuda* was subjected to liquid chromatography coupled with Mass Spectrometry. The results indicated the presence of number of metabolites including flavonoids, phenolics, toxins and certain antibiotics (Table 1). The presence of antibiotics in extract indicates that may be several endophytic bacteria are symbiotically associated with plant rhizome.

Flavonoids

Flavonoids are polyphenolic plant secondary metabolites. They are synthesized by the polypropanoid pathway with phenylalanine as startup molecule. Flavonoids consist of a large group of polyphenolic compounds having a benzo-γ-pyrone structure and are ubiquitously present in plants. Flavones, Flavonols, Flavanones, Flavanonol, and Isoflavone are subgroups of plant flavonoids. As a dietary component, flavonoids are thought to have health-promoting properties due to their high antioxidant capacity both *in vivo* and *in vitro* systems. Flavonoids have ability to induce human protective enzyme systems. The number of studies has suggested protective effects of flavonoids against many infectious (bacterial and viral diseases) and degenerative diseases such as cardiovascular diseases, cancers and other age-related diseases [6] In present study methanolic extract of *E. nuda* showed presence of six flavononeS namely, 5,6,3‘trimethoxyflavone, 5,3‘dihydroxy 4,5-dimethoxy-6,3,7methylene dioxyisoflavone, 5,7,2‘trihydroxy,3,6,4‘5‘ tetramethoxy flavones, Demethyltorosoflavone D, Dionflavone and Isopongoflavone (Table 1). The study also revealed presence of flavonol; quercetin3-(2‖ galoylrutinoside) and two flavonoids; fulvinervin B and Gancaonin J. Amongst this quercetin was isolated from leaves of *E. epidendrea* [7].

Phenolics

Phenolic compounds are amongst the major compounds of plants‘secondary metabolites, playing an important role in plant defense mechanism. In this study, methanolic extract of *E. nuda* indicated presence of two phenolic compounds namely, 3-ethyl catechol and Orchinol. 3-ethyl catechol is catechol bearing an ethyl substituent at position 3. Catechol is one of the allelochemicals which belong to phenolic compounds synthesized in plants. Orchinol is one of the phytoalexin accumulated in plant system having antimicrobial effect [5]. Orchinol was first isolated from orchid *Orchis militaris* by Gaumann and Kern in 1959 [8] possessing antifungal activity. Orchinol is also reported from several other orchid species such as *Agrostophyllum callosum* [5].

Coumarin

The present investigation shows the presence of 8-Cinnamoyl 3,4 dihydro 5, 7 dihydroxy 4 phenylcoumarin (Table 1) in methanolic extract of *E. nuda*. Coumarins (1, 2-benzopyrones) are ubiquitously found in higher plants where they originate from the phenylpropanoid pathway. They contribute essentially to the persistence of plants being involved in processes such as defense against phytopathogens, response to abiotic stresses, regulation of oxidative stress, and probably hormonal regulation [9].

Alkaloids

Alkaloids are a class of nitrogenous organic compounds of plant origin having immense physiological activities in human. In this study three alkaloids namely protopine, Quadrigemine A and Paclitaxel were detected in methanolic extract of *E. nuda* (Table 1).

Protopine is a benzylisoquinoline alkaloid occurring in opium poppy, *Corydalis* tubers and other plants of the family papaveraceae, like *Fumaria officinalis*. It has been found to inhibit histamine H1 receptors and platelet aggregation, and acts as an analgesic [10]. The cytotoxic activity of Quadrigemine A, on cultured rat hepatoma cells (HTC line) is previously reported [11]. Paclitaxel is an anti-cancer (antineoplastic or cytotoxic) chemotherapy drug, isolated from *Taxus brevifolia* for the first time [12].

Antibiotics

The methanolic extract of *E. nuda* showed presence of five antibiotics as HygromycinA, Penicillin V, Penicillin K, Rifamycin Z and Spiramycin 3, indicating some of the bacteria may be present symbiotically in rhizomes of *E. nuda.* Hygromycin A, an antibiotic produced by *Streptomyces hygroscopicus* is active against grampositive bacteria including mycobacteria and actinomycetes, as well as against endomoeba, leptospira and pleuropneumonia-like organisms. Hygromycin A is a specific inhibitor of the peptide bond formation step of protein synthesis. The action of hygromycin A on peptidyl transfer is similar to that of chloramphenicol, an antibiotic that shares some common structural features with hygromycin A. Both antibiotics inhibit the binding of C-A-C-C-A-Leu to the acceptor site of peptidyl transferase and stimulate that of C-A-C-C-A-LeuAc to the donor site of the enzyme. Moreover, hygromycin A blocks the binding of chloramphenicol to ribosomes, indicating that the binding sites of the antibiotics may be closely related. Hygromycin A is a more potent agent than chloramphenicol and binds quite strongly to ribosomes [13]. Phenoxymethylpenicillin (Penicillin V) and Penicillin K are narrow spectrum antibiotics used to treat mild to moderate infections caused by susceptible bacteria. It is natural penicillin antibiotic that is administered orally. Penicillin V may also be used in some cases as prophylaxis against susceptible organisms (www.drugbank.ca/drugs/DB00417). Rifamycin Z is a member of group rifamycins, a group of antibiotics that are synthesized either naturally by the bacterium *Amycolatopsis mediterranei*. They are a subclass of the larger family of ansamycins. Rifamycins are particularly effective against mycobacteria, and are therefore used to treat tuberculosis, leprosy, and mycobacterium avium complex (MAC) infections [14]. Spiramycin 3 is a macrolide antibiotic and antiparasitic. It is used to treat toxoplasmosis and various other infections of soft tissues.

Toxins

These are toxic secondary metabolites naturally occurring in plants with high chemical diversity. In *E. nuda* four toxins were detected namely, Gonyautoxin 6, Gonyautoxin 8, Phaseolotoxin and vomitoxin. Gonyautoxins (GTX) are a few similar toxic molecules that are naturally produced by algae. They are part of the group of saxitoxins, a large group of neurotoxins along with a molecule that is also referred to as

saxitoxin (STX), neosaxitoxin (NSTX) and decarbamoyl saxitoxin (dcSTX). Currently eight molecules are assigned to the group of gonyautoxins, known as gonyautoxin 1 (GTX-1) to gonyautoxin 8 (GTX-8). Ingestion of gonyautoxins through consumption of mollusks contaminated by toxic algae can cause a human illness called paralytic shellfish poisoning (PSP) [15]. Phaseolotoxin is a modified tripeptide [Nδ-(N′-sulfodiaminophosphinyl)-ornithyl-alanylhomoarginine] produced by certains strains of *Pseudomonas syringae* pv. *phaseolicola*, *Pseudomonas syringae* pv. *actinidiae* and strain *Pseudomonas syringae* pv. *syringae* CFBP 3388 [16]. Phaseolotoxin is a reversible inhibitor of the enzyme ornithine carbamoyltransferase (OCTase; EC 2.1.3.3), which catalyzes the formation of citrulline from ornithine and carbamoylphosphate in the arginine biosynthetic pathway. Phaseolotoxin is an effective inhibitor of OCTase activity from plant, mammalian, and bacterial sources and causes a phenotypic requirement for arginine. Additionally, phaseolotoxin inhibits the enzyme ornithine decarboxylase (EC 4.1.1.17), which is involved in the biosynthesis of polyamines [17]. Vomitoxin also known as deoxynivalenol (DON) is a type B trichothecene, an epoxysesquiterpenoid. This mycotoxin occurs predominantly in grains such as wheat, barley, oats, rye and maize, and less often in rice, sorghum, and triticale. The occurrence of deoxynivalenol is associated primarily with *Fusarium graminearum* (*Gibberella zeae*) and *F. culmorum*, both of which are important plant pathogens which cause fusarium head blight in wheat and gibberella or fusarium ear blight in maize [18]. Besides, two more compounds were detected as Picrasin G and phylloquinone in present study. Phylloquinone is often called vitamin K1. It is a fat-soluble vitamin that is stable to air and moisture but decomposes in sunlight. It is found naturally in a wide variety of green plants.

Phylloquinone is also an antidote for coumatetralyl. Vitamin K is needed for the posttranslational modification of certain proteins, mostly required for blood coagulation (http://www.drugbank.ca/drugs/DB01022). Picrasin G is one of the members of Quassinoids which are known to have various biological activities, including anti-inflammatory, anti-malarial, amoebicidal, antifeedant, insecticidal, and herbicidal [19].

The applications of liquid chromatography along with mass spectrometry (LC–MS) in natural product analysis have been increasing due to improved separation and detection capabilities of LC–MS instruments. Among the methods used for the determination of phytochemical in crude plant extracts, liquid chromatography coupled to mass spectrometry (LC/MS) with atmospheric pressure ionization techniques, i.e., electro spray ionization (ESI) or atmospheric pressure chemical ionization (APCI), has been found to be a powerful tool because of the soft ionization, facilitating the analysis of polar, non-volatile, and thermally labile class of compounds. 60 compounds, including cinnamic and benzoic acid derivatives and flavonoids, were identified from apple residues resulting from the juice industry using LC- MS analysis [20]. Phytochemical from various plant species such as *Potentilla anserine* [21], *Ocimum sanctum* [22] were analyzed qualitatively using LCMS techniques.

The present investigation represents first report of qualitative analysis of phytochemical in *Eulophia nuda* using LCMS. The data obtained can be used for metabolomic study in *Eulophia* species.

Table 1: Phytochemicals in *E. nuda* using LCMS

Sr. no.	Name of Compound	m/z	RT	Mass
	Flavonones			
1	5,6,3'trimethoxyflavone	335.08	12.277	312.096
2	5,3'dihydroxy 4,5-dimethoxy-6,3,7methylene dioxyisoflavone	359.075	10.947	358.068
3	5,7,2'trihydroxy,3,6,4'5' tetramethoxy flavones	413.084	9.937	390.095
4	Demethyltorosoflavone D	357.059	10.235	356.051
5	Dionflavone	645.170	4.901	622.18
6	Isopongoflavone	335.128	4.423	334.121
	Flavonol			
1	Quercetin3-(2"galoylrutinoside)	763.17	14.004	762.167
	Flavonoids			
1	Fulvinervin B	387.159	15.809	386.151
2	Gancaonin J	417.202	15.722	394.214
	Phenolics			
1	3-ethyl catechool	161.056	5.945	138.065
2	Orchinol	147.041	4.021	124.052
	Caumarin			
1	8,Cinnamoyl 3,4dihydro 5,7 dihydroxy 4 phenylcoumarin	409.104	4.383	386.114
	Alkaloids			
1	Protopine	354.134	16.081	353.126
2	Quadrangemine A	691.419	14.313	650.413
3	Paclitaxel	876.317	10.399	853.328
	Antibiotics			
1	Epithienamycin E	393.040	5.067	392.034
2	Hygromcin A	512.174	13.639	511.167
3	Penicillin V	351.101	4.674	350.093
4	Penicillin k	343.169	7.405	342.161
5	Rifamycin Z	674.256	6.589	651.267
6	Spiramycin 3	450.275	14.731	898.528
	Toxins			
1	Gonyautoxin 6	396.094	15.352	395.086
2	Gonyautoxin 8	476.048	12.59	475.042
3	Phaseolotoxin	532.206	15.589	531.196

4	Vomitoxin	319.116	12.282	296.127
	Others			
1	Phylloquinone (vitamine K)	226.183	14.79	450.351
2	Picrasin G (triterpenoid antifeedant)	393.190	15.354	392.186

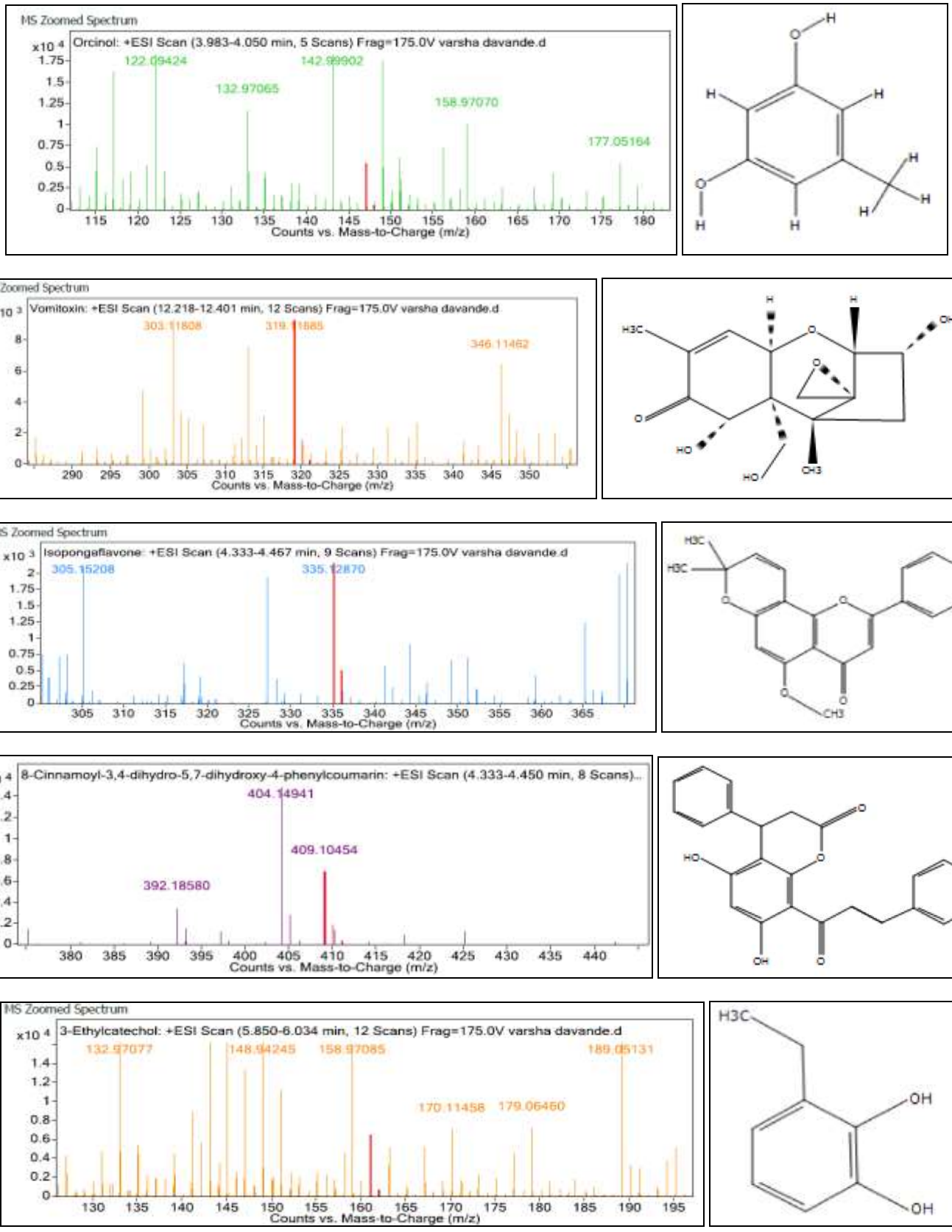

Fig 1: Zoom spectrum and structure of some of compounds identified from methanolic extract of *E nuda* in LCMS analysis

References

1. Dawande V and Gurav R. Total phenolics, flavonoids content and antioxidant activities of some *Eulophia* species. Journal of Medicinal Plants Studies 2017;5(2):106-111.
2. Kurapa S, Upadhyay R, Trivedi R, Upadhyay S, Tiwari S. Qualitative phytochemical analysis of *Eulophia nuda* lind an endangered terrestrial orchid, International

Journal of Pharmaceutical Research and Biosciences 2012;1(5):456-462.
3. Singh S, Duggal S. Medicinal orchids, an overview. *Ethnobotanical leaflets* 2009;13:351-363.
4. Chopra R. Letters to the editor. Glossary of Indian Medicinal Plants 1956;31:112.
5. Kovács A, Vasas A, Hohmann J. Natural phenanthrenes and their biological activity. Phytochemistry 2008;69(5):1084-1110.
6. Kumar S, Pandey AK. Chemistry and Biological Activities of Flavonoids: An Overview The Scientific World Journal 2013. Article ID 162750, http://dx.doi.org/10.1155/2013/162750
7. Maridass M, Ramesh U. Investigation of phytochemical constituents from Eulophia epidendraea, Int. J. of Biol. Technology 2010;1(1):1-7.
8. Rasmussen HN. Terrestrial orchids, from seed to mycotrophic plant. Cambridge: Cambridge University Press 1995.
9. Bourgaud F, Hehn A, Larbat R, Doerper S, Gontier E, Kellner S *et al*. Biosynthesis of coumarins in plants: a major pathway still to be unravelled for cytochrome P450 enzymes, Phytochem Rev 2006;5:293-308.
10. Saeed SA, Gilani AH, Majoo RU, Shah BH. "Anti-thrombotic and anti-inflammatory activities of protopine." Pharmacological research: the official journal of the Italian Pharmacological Society 1997;36(1):1-7.
11. Roth A, Kuballa B, Bounthanh C, Cabalion P, Sévenet J, Beck P *et al*. Cytotoxic Activity of Polyindoline Alkaloids of Psychotria forsteriana (Rubiaceae). Planta medica 1986,450-453.
12. chemocare.com/chemotherapy/drug-info/Paclitaxel.aspx
13. Guerrero MD, Modolell J. Hygromycin A, a novel inhibitor of ribosomal peptidyltransferase Eur J Biochem 1980;107(2):409-14.
14. Floss HG, Yu T. Rifamycin-Mode of Action, Resistance, and Biosynthesis. Chem. Rev 2005;105(2):621-32.
15. Christophersen C.. "Marine Alkaloids". The Alkaloids: Chemistry and Pharmacology 1986,24-26
16. Bender CL, Alarcón-Chaidez F, Gross DC. Pseudomonas syringae phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiology and Molecular Biology Reviews 1999;63:266-292.
17. Bachmann AS, Matile P, Slusarenko AJ. Inhibition of ornithine decarboxylase activity by phaseolotoxin: implications for symptom production in halo blight of French bean. Physiol. Mol. Plant Pathol 1998;53:287–299.
18. Gautam P, Dill-Macky R. Impact of moisture, host genetics and Fusarium graminearum isolates on Fusarium head blight development and trichothecene accumulation in spring wheat. Mycotoxin Research 2012,28(1). doi:10.1007/s12550-011-0115-6 [1]
19. Daido M, Fukamiya N, Okano M, Picrasinol D. a new quassinoid from the stem wood of Picrasma ailanthoides. J. Nat. Prod 1995, 58.
20. Sa´nchez Rabaneda F, Ja´uregui O, Lamuela-Ravento´ SRM, Viladomat F, Bastida J, Codina C. Qualitative analysis of phenolic compounds in apple pomace using liquid chromatography coupled to mass spectrometry in tandem mode. Rapid Communications in Mass Spectrometry 2004;18:553–563.
21. Mari A, Lyon D, Fragner L, Montoro P, Piacente S *et al*. Phytochemical composition of *Potentilla anserina* L. analyzed by an integrative GC-MS and LC-MS metabolomics platform, Metabolomics 2013,9(3):599-607.
22. Devendran, Balasubramanian U. Qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* L. leaves G Asian Journal of Plant Science and Research 2011;1(4):44-48.