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Determination of bioactive components of *Enicostema axillare* (Lam.) Raynal ssp. *littoralis* (Blume) Raynal through gas chromatography-mass spectrometry analysis

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Abstract
Enicostema axillare (Lam.) Raynal ssp. *littoralis* (Blume) Raynal is one of the medicinally important herbs belonging to the family Gentianaceae, commonly known as vellaruk. The present study deals with the GC-MS analysis of methanolic extract of *E. axillare*. Forty phytochemical constituents have been identified by comparing the chromatogram, peak value of the unknown compound with entries in NIST database. The prevalent compounds detected are 2—Phenyl—5—methoxy-oxadiazol-1,3,4 (12.15%), n-Hexadecanoic acid (6.47%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (5.46%), beta-sitosterol (5.09%), stigmasterol (4.97%), 3-Undecen-5-yne, (E)- (4.45%), campesterol (4.10%), 1,5-Decadiyne (3.75%), Methoxy-4-vinylphenol (2.77%) and erythrocentaurin (2.58%). The result of this study justifies the use of this plant in folk and herbal medicine.

Keywords: *Enicostema axillare*, GC-MS, herbal medicine, phytoconstituents

Introduction
The usage of herbs for healing is the method of medicine as old as humankind itself [1]. Historical context of using herbs traditionally depict that various medicinal plants were in use from several centuries by many civilizations and cultures like Chinese, Ayurvedic and Unani. Medicinal plants play an appealing role in modulation of several human disorders [2]. Plants synthesize variety of low-molecular-weight organic compounds with unique and complex structures. Presently, 100,000 such compounds have been isolated from higher plants [3]. These plant metabolites possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors, and fragrances [4]. Plant products are usually safe, efficient and rarely have side effects. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for the synthesis of complex chemical substances by increasing the awareness about possible new therapeutic sources [5].
Enicostema axillare (Lam) Raynal ssp. *littoralis* (Blume) Raynal is a perennial herb belonging to family Gentianaceae. It is naturally distributed throughout India up to an altitude of 450 m but more common in the coastal areas [6]. Leaves and tender parts are very bitter similar to Indian bitter drug *Swertia chirayata*, hence the plant is also known as chota chirayata. It is used as a folk medicine for treatment of diabetes, fever, stomach-ache, malaria and dyspepsia [7]. The plant is used in folk medicine to treat diabetes mellitus, rheumatism, abdominal ulcers, hernia, swelling, itching and insect poisoning [8]. In recent years, this plant is reported to possess anti-inflammatory [9], hypolipidemic, antioxidant, antidiabetic [10], hepatoprotective [11], analgesic [12] and antimicrobial properties [13]. The present study was attempted to identify the chemical constituents of the plant to ascertain its medicinal properties by using gas chromatography-mass spectrometry approach.

Materials and Method
Plant Specimen
Fresh plants of *E. axillare* were collected from the natural habitats of Vattakottai, Tamil Nadu, India. They were identified and authenticated in the Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. The plant samples were thoroughly washed in running tap water to remove soil particles and other debris. The whole plants were shade dried and ground into fine powder by mechanical grinder. The powdered material was stored in air tight polythene bag until use.

Extraction

About 10 g of the powdered sample was weighed and subjected to Soxhlet extraction for 6-7 hrs using 200 ml methanol. The extract was concentrated using rotary evaporator (Superfit rotavap) under reduced pressure. The concentrated extract was then transferred to air-tight containers, corked and stored in the refrigerator at 4 °C until required for the analysis. Two microliters of the extract was employed in GC–MS analysis, for the identification of different compounds.

GC-MS Analysis

The analysis of the extract was performed using GC–MS (Model: GC MS-QP 2010, Shimadzu, Japan) equipped with elite-1 fused silica capillary column of 30 m length, 0.25 mm diameter and 0.25µm film thickness composed of 100% dimethyl poly siloxane. For GC–MS detection, electron ionization system with ionization energy of 70eV was used. Helium (99.999%) was used as carrier gas at a constant flow rate of 1.51 ml/min. Injector and mass transfer line temperature were set to 250 °C and 200 °C respectively. The oven temperature was programmed from 70 °C for 2 minutes and raised to 300 °C for 7 minutes at the rate of 10 °C/min. Two microliters of the sample was injected in a split mode with a scan range of 40-1000 m/z and scan interval of 0.5 seconds. The total running time of GC–MS was 35 min.

Identification of the components

Interpretation of mass spectrum obtained from GC-MS was conducted using the database of National Institute Standard and technology (NIST). The spectrum of the unknown components was compared with that of the known components stored in the NIST library. The name, molecular weight and molecular mass of the identified compounds were further confirmed by comparing their retention indices with that of literature data.

Results and Discussion

The GC- MS analysis of *Enicostema axillare* revealed the presence of 40 compounds. The GC-MS chromatogram of the methanol extract of whole plant of *E. axillare* is presented in Fig. 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in are presented in Table 1. 2—Phenyl—5—methoxy-oxadiazol-1,3,4 showed highest peak (12.15%) which is the dominant component followed by n-Hexadecanoic acid (6.47%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z) (5.46%), beta-sitosterol (5.09%), stigmasterol (4.97%), 3-Undecen-5-yne, (E)- (4.45%), campesterol (4.10%), 1,5-Decadiyne (3.75%), Methoxy-4-vinylphenol (2.77%) and erythrocentaurin (2.58%).

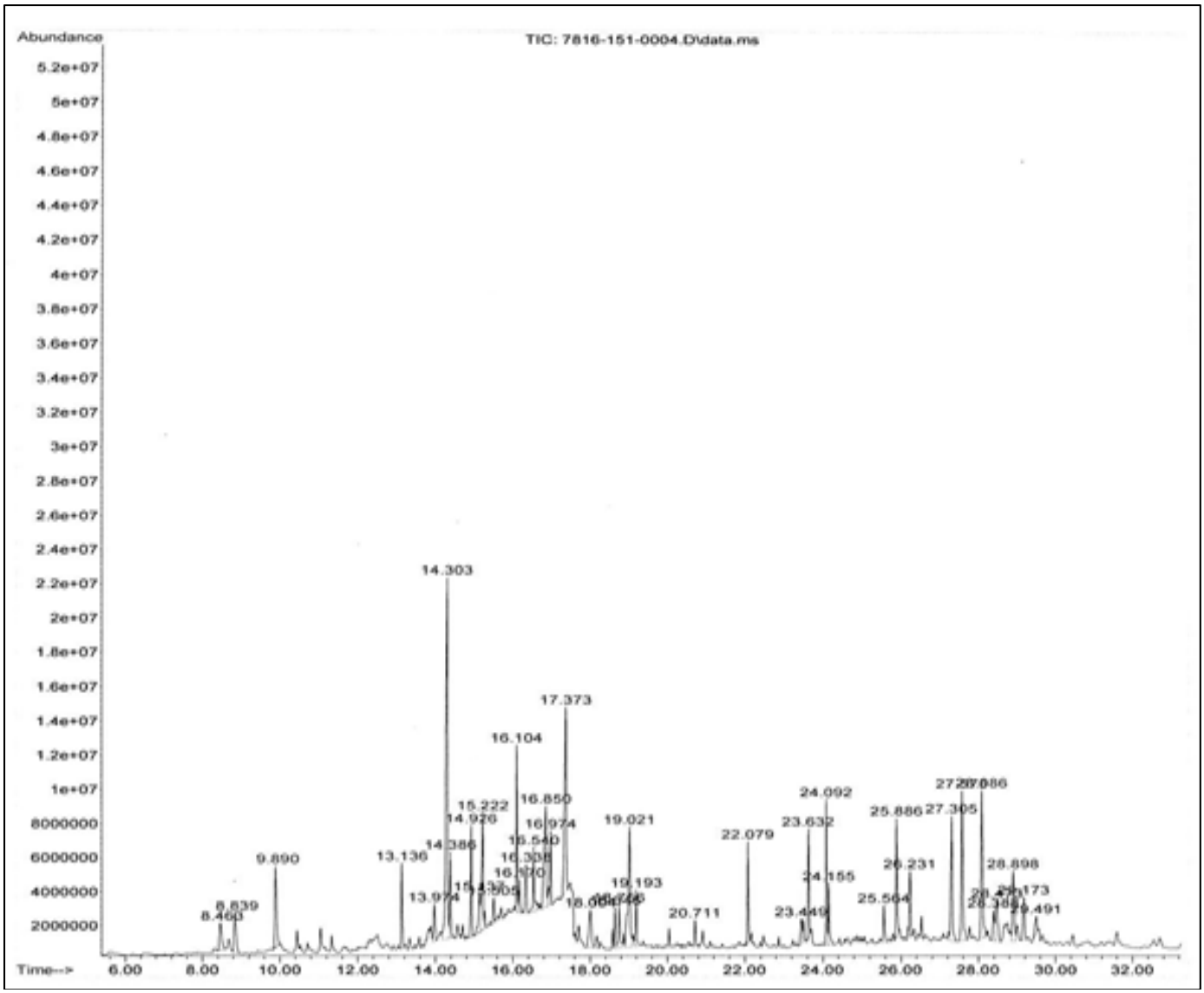

Fig 1: GC-MS chromatogram for whole plant methanolic extract of *Enicostema axillare* (Lam.) Raynal ssp. *littoralis* (Blume) Raynal

Table 1: Phytocomponents identified in the methanolic extract of *Enicostema axillare* (Lam.) Raynal ssp. *littoralis* (Blume) Raynal by GC-MS analysis

S. No	Retension Time	Peak area	Compound name	Molecular formula	Molecular mass (g/mol)
1.	8.462	1.46	Benzene, propyl—	C_9H_{12}	120.195
2.	8.841	1.71	Cinnamaldehyde, (E)—	C_9H_8O	132.159
3.	9.889	2.77	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.177
4.	13.135	1.94	2,3,5,6~Tetrafluoroanisole	$C_7H_4F_4O$	180.102
5.	13.974	1.10	Phenylethanolamine	$C_8H_{11}NO$	137.182
6.	14.301	12.15	2—Phenyl—5—methoxy-oxadiazol-l,3,4	$C_9H_8N_2O_2$	176.175
7.	14.383	1.80	2(3H)-Benzofuranone, 3—methy1—	$C_9H_8O_2$	148.159
8.	14.925	2.58	Erythrocentaurin	$C_{10}H_8O_3$	176.171
9.	15.133	1.50	Coniferyl alcohol	$C_{10}H_{12}O_3$	180.203
10.	15.222	3.75	1,5-Decadiyne	$C_{10}H_{14}$	134.222
11.	15.505	0.62	Benzenepropanoic acid, alpha.-(hydroxyimino)-	$C_9H_9NO_3$	179.175
12	16.106	3.27	Phytol, acetate	$C_{22}H_{42}O_2$	338.576
13	16.173	0.73	Decanoic acid, 3—methy1-	$C_{11}H_{22}O_2$	186.295
14	16.337	1.61	alpha.-D-Glucopyranoside,	$C_{18}H_{32}O_6$	344.443
			4,6-O-(undec-10-enylidene)-methyl-		
15	16.537	1.60	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	224.388
16.	16.849	4.57	3-Undecen-5-yne, (E)-	$C_{11}H_{18}$	150.261
17.	16.975	1.42	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.457
18.	17.369	6.47	Hexadecanoic acid	$C_{14}H_{28}O_2$	228.376
19.	18.001	1.87	Esculetin	$C_9H_6O_4$	178.143
20.	18.647	0.88	cis—13—Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.495
21	18.758	1.14	Phytol	$C_{20}H_{40}O$	296.539
22.	19.018	5.46	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{19}H_{32}O_2$	292.463
23	19.197	1.35	Octadecanoic acid	$C_{18}H_{36}O2$	284.484
24	20.712	0.64	Bicyclo[11.3.0]hexadecane—2,l4—dione	$C_{16}H_{26}O_2$	250.382
25.	22.079	2.11	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358.555
26.	23.446	0.89	2,3—Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356.547
27.	23.632	2.22	Octadecanoic acid, 2-hydroxy-1-	$C_{21}H_{42}O_4$	358.555
			(hydroxymethyl)ethyl ester		
28.	24.092	3.33	13-Docosenamide, (Z)—	$C_{22}H_{43}NO$	337.592
29.	24.152	1.54	1,19-Eicosadiene	$C_{20}H_{38}$	278.524
30.	25.563	1.00	1,21—Docosadiene	$C_{22}H_{42}$	306.578
31	25.882	2.79	7-(iodomethyl)-6,7-dimethyl-3a-	$C_{15}H_{25}I$	332.269
			(prop-1-en-2-yl)octahydro-1H-indene		
32	26.232	1.74	Isolongifolen—5—one	$C_{15}H_{22}O$	218.340
33	27.309	4.10	Campesterol	$C_{28}H_{48}O$	400.680
34	27.576	4.97	Stigmasterol	$C_{29}H_{48}O$	412.690
35.	28.089	5.09	beta.-Sitosterol	$C_{29}H_{50}O$	414.706
36.	28.386	1.00	Olean-13(18)-ene	$C_{30}H_{50}$	410.730
37.	28.475	1.95	2,4-Chloro—5—nitrobenzotrifluoride	$C_7H_2Cl_2F_3NO_2$	259.993
38.	28.898	2.65	beta.—Amyrin	$C_{30}H_{50}O$	426.729
39.	29.493	0.69	dl-a-Tocopherol	$C_{29}H_{50}O_2$	430.717
40.	29.493	0.69	alpha-Selinene	$C_{15}H_{24}$	204.357

Among the identified compounds, hexadecanoic acid is reported to have antioxidant [14] and anti- inflammatory properties [15]. β-sitosterol is a waxy substance which is white in colour which possessing antimicrobial, antioxidant, antiinflammatory, antiasthma, antiarthritic and hepato protective activities [16]. β- sitosterol is reported to lower down the cholesterol level in blood [17]. Stigmasterol, a precursor in manufacturing of semisynthetic progesterone and vitamin D3, also an intermediate in biosynthesis of androgens, estrogens and corticoids [18, 19]. Campesterol is known to inhibit intestinal absorption of cholesterol [20].

Erythrocentaurin is a relatively simple natural product present among the members of Gentianaceae [21]. It is a metabolite of swertiamarin which is a secoiridoid glycoside and is mostly employed as a bitter tonic. Natural occurrence of erythrocentaurin has been reported earlier in *Enicostema hyssopifolium* and *Swertia lawii* [22]. Erythrocentaurin derivatives act as hepatitis B virus inhibitors [23]. Beta-amyrin is a pentacyclic triterpene which possess anxiolytic, antidepressant, analgesic, anti-inflammatory properties [24]. Esculetin, 6,7-dihydroxycoumarin, is a coumarin derivative which possesses various biological and pharmaceutical properties including anti-edema, anti-inflammatory and anti-tumour effects [25, 26, 27], and as a result, it is also used to synthesize drugs [28].

Amethyl/ethyl linoleate and linolenate have a depigmenting activity and may be introduced as a possible therapeutic agent for hyperpigmentation or as a cosmetic lightening agent [29]. Vitamin E (α tocopherol) is the most important lipid-soluble antioxidants, and that it protects cell membranes from oxidation, thus stabilizing them and maintaining their permeability [30, 31]. Phytol is a diterpene with antimicrobial properties, significantly against many bacterial strains [32]. Phytol is used as a precursor for the manufacture of synthetic forms of vitamin E [33]. Cinnamaldehyde is an antidiabetic agent, possesses hypoglycemic and hypolipidemic effects in STZ-induced diabetic rats [34]. It is also reported to possess antimicrobial, anticancer properties [35, 36, 37]. Due to the presence of above mentioned compounds, the whole plant

methanolic extract of *Enicostema axillare* may be used in various pharmaceutical applications.

Conclusion

In the present study, 40 compounds were identified from methanol extract of the whole plant of *Enicostema axillare* (Lam.) Raynal ssp. *littoralis* (Blume) Raynal by Gas Chromatography – Mass Spectrometry (GC-MS) analysis. These findings support the traditional use of *E. axillare* in the treatment of various disorders. Further studies are needed to isolate active principle of the extract as well as to elucidate their exact mechanism of action in various disorders.

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References

1. Barber P, Parkes J, Blundell D. Further essentials of pharmacology for nurses. McGraw-Hill Education (UK), 2012, 198.
2. Majid M, Khan MR, Shah NA, Haq IU, Farooq MA, Ullah S *et al*. Studies on phytochemical, antioxidant, anti-inflammatory and analgesic activities of *Euphorbia dracunculoides*. BMC complementary and alternative medicine. 2015; 15(1):349.
3. Verpoorte R, van der Heijden R, Memelink J. Engineering the plant cell factory for secondary metabolite production. Transgenic research. 2000; 9(4-5):323-343.
4. Goossens A, Hakkinen ST, Laakso I, Seppanen-Laakso T, Biondi S, De Sutter V *et al*. A functional genomics approach toward the understanding of secondary metabolism in plant cells. Proceedings of the National Academy of Sciences. 2003; 100(14):8595-8600.
5. Jigna P, Sumitra C. Phytochemical screening of some plants from western region of India. Plant Archives. 2008; 8(2):657-662.
6. Abirami P, Gomathinayagam M, Panneerselvam R. Preliminary study on the antimicrobial activity of *Enicostemma littorale* using different solvents. Asian Pacific journal of tropical medicine. 2012; 5(7):552-555.
7. Vishwakarma SL, Rakesh D, Rajani M, Goyal RK. Evaluation of effect of aqueous extract of *Enicostemma littorale* Blume in streptozotocin-induced type 1 diabetic rats. Indian Journal of Experimental Biology. 2010; 48:26-30.
8. Kirtikar KR, Basu BD. Indian Medicinal Plants, Bishen Sing, 2 nd edition. Mahendra Pal Sing publication, Dehradun, 1999, 1655-1656.
9. Leelaprakash G, Dass SM. *In vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. International Journal of Drug Development and Research. 2011; 3(3):189-196.
10. Thirumalai T, Viviyan TS, Elumalai EK, David E. Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume. Asian Pacific journal of tropical biomedicine. 2011; 1(5):381.
11. Jaishree V, Badami S, Krishnamurthy PT. Antioxidant and hepatoprotective effect of the ethyl acetate extract of *Enicostemma axillare* (Lam). Raynal against CCl_4-induced liver injury in rats. Indian Journal of Experimental Biology. 2010; 48:896-904.
12. Shabi MM, Uthrapathy S, Raj CD, Krishnamoorthy G, Ravindhran D, Joseph J, Rajamanickam VG. Analgesic and Anti-Arthritic Effect of *Enicostemma littorale* Blume. Advances in Bioscience and Biotechnology. 2014; 5(13):1018.
13. Deore SL, Khadabadi SS, Bhagure L, Ghorpade DS. *In vitro* antimicrobial and antioxidant studies on *Enicostemma axillare* (Lam.) Raynal. leaves. Natural product radiance. 2008; 7(5):409-412.
14. Kala SM, Balasubramanian T, Soris PT, Mohan VR. GC-MS determination of bioactive components of *Eugenia singampattiana* Bedd. International Journal of Chem Tech Research. 2011; 3(3):1534-1537.
15. Elizabeth T, Aneesh TP, Della GT, Anandan R. GC-MS analysis of phytochemical compounds present in the rhizomes of *Nervilia aragoana*. Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(3):68-74.
16. Sujila SR, Rajadurai M, Shairibha SR. Hepatoprotective effect of β sitosterol on lipid peroxidation and antioxidant status in ethanol-induced hepatotoxic rats. International Journal of Integrative Sciences, Innovation and Technology (IJIIT). 2014; 3(4):1-11.
17. Scholz B, Guth S, Engel KH, Steinberg P. Phytosterol oxidation products in enriched foods: Occurrence, exposure, and biological effects. Molecular nutrition & food research. 2015; 59(7):1339-1352.
18. Sundararaman P, Djerassi C. A convenient synthesis of progesterone from stigmasterol. The Journal of organic chemistry. 1977; 42(22):3633-3634.
19. Kametani T, Furuyama H. Synthesis of vitamin D3 and related compounds. Medicinal research reviews. 1987; 7(2):147-171.
20. Ikeda I, Tanaka K, Sugano M, Vahouny GV, Gallo LL. Inhibition of cholesterol absorption in rats by plant sterols. Journal of Lipid Research. 1988; 29(12):1573-1582.
21. Hassan N, Ahamad J, Amin S, Mujeeb M, Mir SR. Rapid preparative isolation of erythrocentaurin from *Enicostemma littorale* by medium-pressure liquid chromatography, its estimation by high-pressure thin-layer chromatography, and its α-amylase inhibitory activity. Journal of separation science. 2015; 38(4):592-598.
22. Ghosal S, Singh AK, Sharma PV, Chaudhuri RK. Chemical constituents of gentianaceae IX: Natural occurrence of erythrocentaurin in *Enicostemma hyssopifolium* and *Swertia lawii*. Journal of pharmaceutical sciences. 1974; 63(6):944-945.
23. Geng CA, Huang XY, Ma YB, Zhang XM, Chen JJ. Synthesis of erythrocentaurin derivatives as a new class of hepatitis B virus inhibitors. Bioorganic & medicinal chemistry letters. 2015; 25(7):1568-1571.
24. Pinto SH, Pinto LM, Cunha GM, Chaves MH, Santos FA, Rao VS. Anti-inflammatory effect of α, β-Amyrin, a pentacyclic triterpene from *Protium heptaphyllum* in rat model of acute periodontitis. Inflammopharmacology. 2008; 16(1):48-52.
25. Paya M, Halliwell B, Hoult JR. Interactions of a series of coumarins with reactive oxygen species: scavenging of superoxide, hypochlorous acid and hydroxyl radicals. Biochemical pharmacology. 1992; 44(2):205-214.
26. Huang HC, Lai MW, Wang HR, Chung YL, Hsieh LM, Chen CC. Antiproliferative effect of esculetin on vascular smooth muscle cells: possible roles of signal transduction

pathways. European journal of pharmacology. 1993; 237(1):39-44.
27. Cho JH, Shin JC, Cho JJ, Choi YH, Shim JH, Chae JI. Esculetin (6, 7-dihydroxycoumarin): a potential cancer chemopreventive agent through suppression of Sp1 in oral squamous cancer cells. International journal of oncology. 2015; 46(1):265-271.
28. Kostova I. Synthetic and natural coumarins as cytotoxic agents. Current Medicinal Chemistry-Anti-Cancer Agents. 2005; 5(1):29-46.
29. Huh S, Kim YS, Jung E, Lim J, Jung KS, Kim MO *et al*. Melanogenesis inhibitory effect of fatty acid alkyl esters isolated from *Oxalis triangularis*. Biological and Pharmaceutical Bulletin. 2010; 33(7):1242-1245.
30. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. Journal of physiology and biochemistry. 2001; 57(1):43-56.
31. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. Free radical biology and medicine. 2007; 43(1):4-15.
32. Bharathy V, Sumathy BM, Uthayakumari F. Determination of phytocomponents by GC-MS in leaves of *Jatropha gossypifolia* L. Science Research Reporter. 2012; 2(3):286-290.
33. Byju K, Vasundhara G, Anuradha V, Nair SM, Kumar NC. Presence of Phytol, a Precursor of Vitamin E in *Chaetomorpha antinnina*. Mapana-Journal of Sciences. 2013; 12(2):57-65.
34. Babu PS, Prabuseenivasan S, Ignacimuthu S. Cinnamaldehyde—a potential antidiabetic agent. Phytomedicine. 2007; 14(1):15-22.
35. Ka H, Park HJ, Jung HJ, Choi JW, Cho KS, Ha J, Lee KT. Cinnamaldehyde induces apoptosis by ROS-mediated mitochondrial permeability transition in human promyelocytic leukemia HL-60 cells. Cancer letters. 2003; 196(2):143-152.
36. Gill AO, Holley RA. Mechanisms of bactericidal action of cinnamaldehyde against *Listeria monocytogenes* and of eugenol against *L. monocytogenes* and *Lactobacillus sakei*. Applied and environmental microbiology. 2004; 70(10):5750-5755.
37. Ali SM, Khan AA, Ahmed I, Musaddiq M, Ahmed KS, Polasa H *et al*. Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori. Annals of clinical microbiology and antimicrobials. 2005; 4(1):20.