Assessment of Bioactive Constituents by GC-MS of *Catharanthus pusillus* (Murr.)G.Don(Apocynaceae)

[1]Yokeswari Nithya, P [2]Mary Jelastin Kala S , [3*]Mohan, V.R

[1] Assistant Professor of Chemistry, [2] Assistant Professor of Chemistry,[3 *]Head & Associate Professor of Botany.

[1]Department of Chemistry,A.P.C.Mahalaxmi College for Women,Tamilnadu.

ABSTRACT

Catharanthus pusillus belonging to family Apocynaceae is known with various names in India and all over the world. It is widely used as various treatments of diseases and traditionally used as herbal medicine.The present investigation deals with the GC-MS determination of methanol extract of the above said plant. Thirty three phytochemical constituents have been identified by comparing the chromatogram, peak value of unknown compound with entries in NIST database. The presence of various bioactive compounds confirms the application of *C. pusillus* for various ailments. However, isolation of individual phytochemical constituent may ensue to find a novel drug. This is the first report of identification of active constituents from whole plant of *C. pusillus.*

Keywords: *Catharanthus pusillus*,Stigmasterol,Camposterol

I.INTRODUCTION

Medicinal plants are the richest bioresources 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). These plants exhibit a wide range of biological and pharmacological activities such as anticancer, antiinflammatory, diuretic, oxytoxic, laxative, antispasmodic, antihypertensive, antidiabetic, and antimicrobial functions. The secondary metabolites of plants provides humans with numerous biological active products which has been used extensively as drugs, foods, additives, flavors, insecticides, colorants, fragrances and chemicals(Koduru *et al* 2006). Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action.

Gas chromatography has gained widespread acceptance in numerous application areas, such as process control in chemical plants, quality control in the food industry, monitoring sample composition in the oil-industry, environmental and biomedical sciences. These are just a few examples in which gas chromatography has been applied. The combination of speed, sensitivity and a high resolving power in gas chromatography provides a very adequate technique for the separation of complex samples. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants.

There are three different stages in GC analysis:
1. The preparation of the sample.
2. The development of the separation and the production of the chromatogram.
3. The processing of the data and the production of the results

Because of its simplicity, sensitivity, and effectiveness in separating components of mixtures, gas chromatography is one of the most important tools in chemistry. The aim of this study is to determine the organic compounds present in the active fraction of *Catharanthus pusillus* whole plant extract with the aid of GC-MS Technique, which may provide an insight in its use in traditional medicine.

II.MATERIALS AND METHODS:

2.1 Collection of plant material

The whole plant of *Catharanthus pusillus* (Murr.) G.Don were collected from Pechiparai, Kanayakumari District, Tamil Nadu.With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology unit, Research department of Botany, V.O.Chidambaram College, Thoothukudi, Tamil Nadu for further references.

2.2 Hot maceration method using Soxhlet apparatus

The freshly collected plant materials were dried in shade and then coarsely powdered in a blender. 100 gram of the coarse powder was extracted with 250 ml of petroleum ethanol in a Soxhlet apparatus for 24 h. The ethanol extract was concentrated in a rotary evaporator. The concentrated ethanol extract was used for GC-MS analysis.

2.3GC-MS Analysis:

GC-MS analysis of ethanol extract of *C.pusillus* was performed using a GC Clarus 500 Perkin-Elmer system comprising a AOC – 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (330 mm × 0.25 mm ID × 1μm df, composed of 100% Dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/minute and an injection volume of 0.5μl was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 40 to 550 Da. Total GC running time was 36 minutes. The relative percentage of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass.

2.4 Identification of Components:

Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62, 000 patterns. The spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

III.RESULTS

The chemical composition of whole plant of *C.pusillus* were analysed by using GC-MS. The chromatogram of ethanol extract of whole plant of *C.pusillus* is given in **Figure 1.** The identification of the phytochemical compounds was confirmed based on the retention time (RT), molecular formula, molecular weight (MW) and peak area in percentage. They are presented in **Table 1 .**The GC-MS analysis of *C.*pusillus whole plant revealed the presence of thirty three compounds. The first compound identified with less retention time (4.66 min) was 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl whereas, Stigmasterol was the last compound which took longest retention time (35.78 min) to identify. The prevailing compounds were, 2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran-4-one, Catechol, 5-Hydroxymethylfurfural, 2-(1,1-dimethylethyl) Phenol, 2, 5-dihydroxy- ethyl benzoate, (3-Nitrophenyl) methanol, isopropyl ether, α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-, 1-(3, 4-dimethoxyphenyl)-Ethanone, , 2, 6, 6-trimethyl- Bicyclo(3.1.1)heptane-2, 3-diol, Ethyl α-d-glucopyranoside,, α-d-Riboside, 1-O-dodecyl-, , 4-C-methyl-Myo-Inositol, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, Phytol, acetate, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Phytol,, 9-Octadecenoic acid, (E)-, Oleic Acid, Octadecanoic acid, 17-methyl,methyl dodecanoate, 17-methyl-, methyl ester, 9, 10-Secocholesta-5, 7, 10(19)-triene-3, 21, 25-triol, (3β, 5Z, 7E)-, Eicosanoic acid, Eicosanebioic acid, dimethyl ester, 11, 14-

Eicosadienoic acid, methyl ester, 4-chloro-17-hydroxy-(17β)- Androst-4-en-3-one, Squalene, 2-butyl-Quinoline,3-ethyl-5-(2-ethylbutyl)- Octadecane, Methylprednisolone Acetate, , 1, 1', 2, 2'-tetrahydro-1, 1'-dimethoxy- Carotene, dl-α-Tocopherol, Campesterol and Stigmasterol. The phytochemicals identified through GC-MS analysis showed many biological activity are listed in the Table 2.The mass spectra of some of the detected compounds from the whole plant of *C.pusillus* are presented in the **Figure 2.**

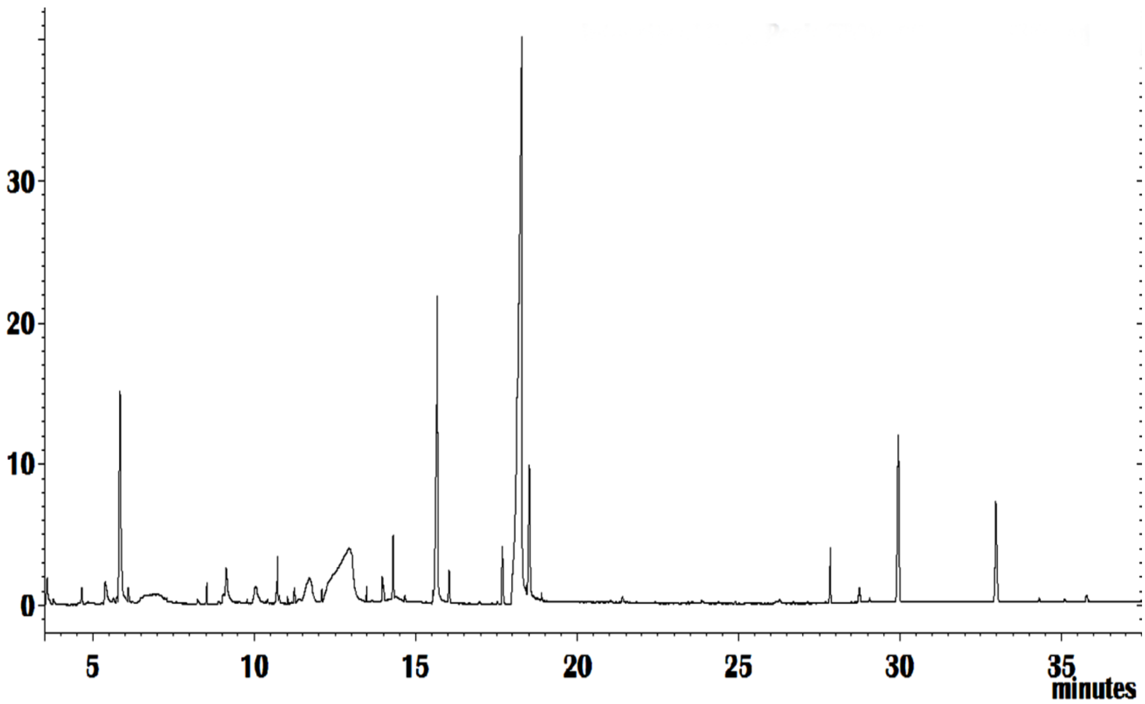


Figure 1: GC-MS Chromatogram of ethanol extract of whole plant of *C.pusillus*

Table 1: Compounds detected in the ethanol extract of whole plant of *C.pusillus*

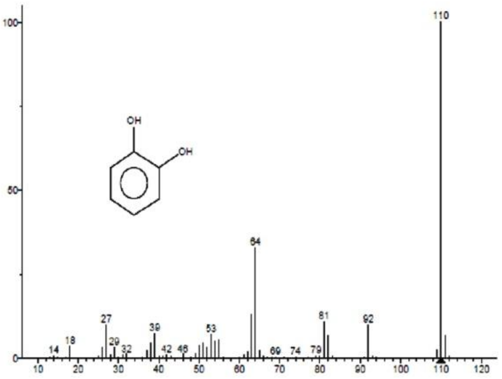
No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	4.66	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	0.21
2.	5.36	Catechol	$C_6H_6O_2$	110	0.25
3.	5.83	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	1.78
4.	7.26	Phenol, 2-(1,1-dimethylethyl)-	$C_{10}H_{14}O$	150	0.34
5.	8.51	Benzoic acid, 2,5-dihydroxy-, ethyl ester	$C_9H_{10}O_4$	182	0.15
6.	9.12	(3-Nitrophenyl) methanol, isopropyl ether	$C_{10}H_{13}NO_3$	195	0.73
7.	10.04	α-D-Glucopyranose, 4-O-β-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	342	0.04
8.	10.72	Ethanone, 1-(3,4-dimethoxyphenyl)-	$C_{10}H_{12}O_3$	180	0.21
9.	11.24	Bicyclo(3.1.1)heptane-2,3-diol, 2,6,6-trimethyl-	$C_{10}H_{18}O_2$	170	0.24
10.	11.72	Ethyl α-d-glucopyranoside	$C_8H_{16}O_6$	208	0.09
11.	12.42	α-d-Riboside, 1-O-dodecyl-	$C_{17}H_{34}O_5$	318	1.25
12.	12.87	Myo-Inositol, 4-C-methyl-	$C_7H_{14}O_6$	194	0.23
13.	14.00	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.99
14.	14.54	Phytol, acetate	$C_{22}H_{42}O_2$	338	0.17
15.	15.67	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.03
16.	16.03	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.71

17.	17.69	Phytol	$C_{20}H_{40}O$	296	0.86
18.	18.27	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282	61.32
19.	18.51	Oleic Acid	$C_{18}H_{34}O_2$	282	7.67
20.	18.88	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}H_{40}O_2$	312	0.68
21.	20.90	9,10-Secocholesta-5,7,10(19)-triene-3,21,25-triol, (3β,5Z,7E)-	$C_{27}H_{44}O_3$	416	0.24
22.	21.43	Eicosanoic acid	$C_{20}H_{40}O_2$	312	0.04
23.	23.55	Eicosanebioic acid, dimethyl ester	$C_{22}H_{42}O_4$	370	0.18
24.	26.30	11,14-Eicosadienoic acid, methyl ester	$C_{21}H_{38}O_2$	322	0.02
25.	26.68	Androst-4-en-3-one, 4-chloro-17-hydroxy-, (17β)-	$C_{19}H_{27}ClO_2$	322	0.21
26.	27.83	Squalene	$C_{30}H_{50}$	410	0.77
27.	28.72	Quinoline, 2-butyl-	$C_{13}H_{15}N$	185	0.63
28.	29.04	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	0.13
29.	29.93	Methylprednisolone Acetate	$C_{24}H_{32}O_6$	416	3.55
30.	30.44	psi.,.psi.-Carotene, 1,1',2,2'-tetrahydro-1,1'-dimethoxy-	$C_{42}H_{64}O_2$	600	0.14
31.	32.94	dl-α-Tocopherol	$C_{29}H_{50}O_2$	430	1.66
32.	35.09	Campesterol	$C_{28}H_{48}O$	400	1.50
33.	35.78	Stigmasterol	$C_{29}H_{48}O$	412	1.42

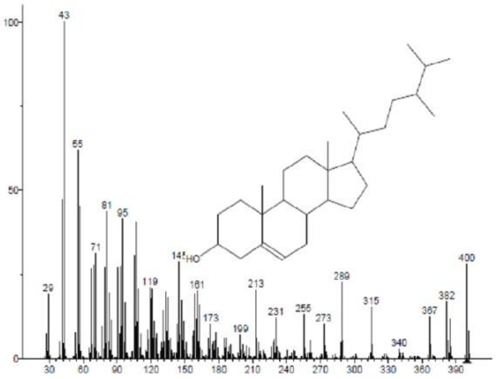
IV. DISCUSSION

The more precise information in qualitative analysis can be obtained by gas-chromatography coupled with mass spectrometry (GC-MS) (Cong *et al*., 2007). The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fraction of the ethanol extract of *C. pusillus*. These compounds were identified through mass spectrometry attached with GC. Thirty three compounds were identified in the whole plant extract of *C. pusillus.* Among the identified compounds, Hexadecanoic acid, methyl ester is a fatty acid ester having antioxidant activity (Jegadeeswari *et al*., 2012).

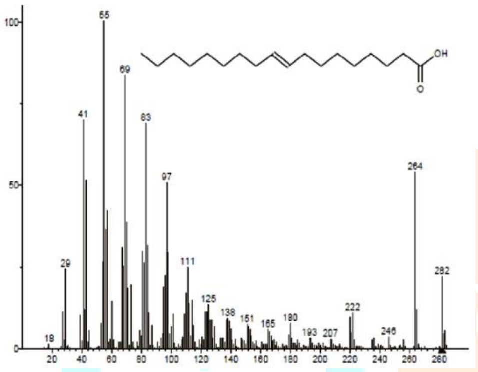
Squalene is used in cosmetics as a natural moisturizer**.**(Devi *et al*., 2009). Recently it has been found that; squalene possesses hemo-preventive activity against the colon carcinogenesis. (Rao *et al*., 1998,*)*. Campesterol and stigmasterol, possess anticancerous activity (Bradford *et al*., *2007*). Phytol is detected in whole plant of *C.pusillus* and is found to be effective at different stages of the arthritis. It gives good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases (Ogunlesi *et al*., 2009). Phytol-diterpene is an antimicrobial, anticancer, antiinflammatory and diuretic agent (Praveenkumar *et al*., 2010). Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al*., 2005). Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies. (Mangunwidjaja *et al*.,2006) reported that stigmasterol is used as a precursor in the manufacture of semi-synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D3 (Sundararaman and Djerassi 1977; Kametani and Furuyama, 1987).

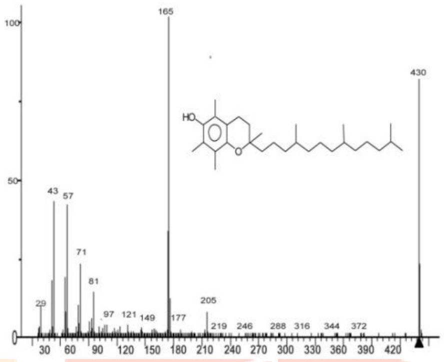


Catechol



Campesterol





9-

Octadecenoicacid(E)-

Vitamin E

Figure 2: Mass spectra of some compounds present in the ethanol extract of *C.pusillus* whole plant

The major phytocompounds detected in the whole plant of *C.pusillus* through GC-MS study and their biological activities are tabulated below.

S.No	Name of the compound	Compound Nature	**Activity
1	2, 3-dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran-4-one	Flavonoid Compound	Antimicrobial Anti-inflammatory
2	Catechol	Phenolic compound	Anticancer, Antitumor Antiviral, Pesticide Antioxidant, Dermatitigenic, Antiseptic, Convulsant Herbicide
3	5-Hydroxymethylfurfural	Aldehyde compound	Antimicrobial Anti-inflammatory
4	2-(1, 1-dimethylethyl)- Phenol	Phenolic compound	Antimicrobial, Antiinflammatory, Antioxidant, Analgesic
5	2, 5-dihydroxy- ethyl benzoate	Phenolic compound	Antimicrobial, Antiinflammatory, Antioxidant, Analgesic

6	(3-Nitrophenyl) methanol, isopropyl ether	Nitrogen compound	Antimicrobial
7	4-O-β-D-galactopyranosyl- α-D-Glucopyranose	Sugar moiety	Preservative
8	Ethyl α-d-glucopyranoside	Sugar moiety	Preservative
9	Myo-Inositol, 4-C-methyl-	Inositol compound	Antidiabetic
10	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial, Anti-inflammatory
11	Phytol, acetate	Diterpene compound	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
12	n-Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic
13	Hexadecanoic acid, ethyl ester	Palmitic acid ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic
14	Phytol	Diterpene	Antimicrobial, Anti-inflammatory, Anticancer, Diuretic
15	9-Octadecenoic acid	Oleic acid compound	Cancer preventive Flavor, Hypocholesterolemic, 165-Alpha reductase, inhibitor, Antiandrogenic Perfumery, Insectifuge Anti-inflammatory, Anemiagenic Dermatitigenic, Choleretic
16	Oleic Acid	Oleic acid compound	Cancer preventive Flavor, Hypocholesterolemic, 5-Alpha reductase inhibitor, Antiandrogenic Perfumery, Insectifuge Antiinflammatory, Anemiagenic,

			Dermatitigenic, Choleretic
17	9, 10-Secocholesta-5, 7, 10(19)-triene-3, 21, 25-triol, (3β, 5Z, 7E)-	Steroids	Antimicrobial, Antiinflammatory, Anticancer, Antiasthma Hepatoprotective, Diuretic
18	11, 14-Eicosadienoic acid, methyl ester	Unsaturated fatty acid compound	Cardio friendly
19	4-chloro-17-hydroxy-, (17β)- Androst-4-en-3-one	Steroids	Antimicrobial, Anti-inflammatory, Anticancer, Antiasthma Hepatoprotective, Diuretic
20	Squalene	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
21	2-butyl- Quinoline	Alkaloid	Antimicrobial, Anti-inflammatory
22	3-ethyl-5-(2-ethylbutyl)- Octadecane	Alkane compound	No activity reported
23	Methylprednisolone Acetate	Glucocorticoid Compound	Anti-inflammatory
24	1, 1', 2, 2'-tetrahydro-1, 1'-dimethoxy-Carotene	Carotene compound	Antimicrobial, Anticancer, Antioxidant
25	dl-α-Tocopherol	Vitamin E compound	Antiageing, Analgesic,Antidiabatic Antiinflammatory, Antioxidant,Antidermatitic, Antileukemic,Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, .Antibronchitic,Anticoronary
26	Campesterol	Steroids	Antimicrobial, Anti-inflammatory,

			Anticancer, Antiasthma Hepatoprotective, Diuretic
27	Stigmasterol	Steroids	Antioxidant, Antiinflammatory, Sedative Antihepatotoxic Caner-preventive, Antiviral, Ovulant, Hypocholesterolemic, Estrogenic Artemicide

V.CONCLUSION

Thus, GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. Further, investigations in the pharmacological importance of whole plant of C.Pusillus, their diversity and detailed phytochemistry may add new knowledge to the information in the traditional medicinal systems.

VI. REFRENCES

1. 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.
2. Bradford, P.G. and Awad A.B. 2007. Phytosterolsas anticancer compounds. Mol Nutr Food Res, 51 (2): 161-70.
3. Cong, Z., Meiling, Q., Qinglong, S., Shan, Z. and Ruonong, F.J. 2007. Pharm. Biomed. Anal. 44: 464.
4. Devi P, Nagarajan M, Christina AJM, Meera R and Merlin NJ: GC-MS analysis of *Euphorbia longan* leaves. *Int. J. of Pharmaceutical Res and Development* 2009; 8: 14.
5. Inoue Y, Hada TA, Shiraishi K, Hirore, H Hamashima and Kobayashi S: Biphasic effects of Geranylgeraniol, Terpenone and Phytol on the growth of *Staphylococcus aureus*. *Antimicrobial agents and Chemother*2005; 49(5): 1770-1774.
6. Jegadeeswari P., Nishanthini A., Muthukumaraswamy S.and Mohan VR, (2012). J. Curr. Chem. Pharm. Sci.; 2: 226-236
7. Kametani, T and Furuyama, H. 1987. Synthesis of vitamin D3 and related compounds. *Med. Res. Rev.* 7: 147–171.
8. Koduru S, Grierson DS, Afolayan A. Antimicrobial activity of *Solanum aculeastrum*. Pharm Biol 2006; 44:283- 286.
9. Mangunwidjaja DS, Kardono SR and Iswantini LBSD, Gas chromatography and Gas Chromatography-Mass Spectrometry analysis of Indonesian *Croton tiglium* seeds. *J. Applied Sci.* 2006;6: 1576-1580.
10. 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
11. Ogunlesi, M., Okiei, W., Ofor, E. and Osibote, A.E. 2009. Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn. (Euphorbiaceae), a potential medication for asthma. *African J. Biotech*. 8: 7042 – 7050
12. Praveen kumar, P. Kumaravel, S. and Lalitha, C. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of Vitex negundo. Afr. J. Biochemistry. Res. 4 : 191-195.

13. Rao, C.V., Newmark, H.L. and Reddy, B.S. 1998. Chemopreventive effect of squalene on colon cancer. Carcinogen. 19: 287-297
14. Sundararaman, P. and Djerassi, C. 1977. A convenient synthesis of progesterone from stigmasterol. *J. Org Chem.* 42: 3633–4