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Research Article

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GC-MS analysis of methanol wild plant and callus extracts from three *Cissus* species, Family Vitaceae

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ABSTRACT

The medicinally important plants Cissus xavierensis, C. quadrangularis var. rotundus and C. vitiginea are analyzed and the phytoconstituents present are identified by GC-MS (Gas Chromatography-Mass Spectrometry) analysis. The analysis was carried out with the methanol extracts of the dried wild plant and callus of the three selected plants. The results concluded that the callus have more phytoconstituents than the wild plant extracts.

Key words :*Cissus* species, wild plant and callus, GC-MS analysis, phytocomponents

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INTRODUCTION

The most traditional medicines are developed from nature. They have not yet fulfilled the scientific requirements so as to be classified as modern medicines. For the purposes of scientific back up, a study is needed to examine their bioactive components. Due to many scientific, economic and ecological advantages of plant tissue culture, it is considered an important strategy for *in vitro* production of bioactive compounds for drug and food industries [1-3]. One of the plants having medicinal activity is *Cissus* species, which belongs to the family Vitaceae. The plants are tendril climbers. The entire plants have high medicinal value. The various phytochemicals present in the plants are thought to be responsible for the medicinal value of the plant [4-6].

The natural vegetation of *Cissus* species is negligible, because of low seed production. It propagates mainly through vegetative mode of reproduction. But its propagation rate is very slow to meet commercial demand of high quality planting material for its commercial cultivation. The plant tissue culture, a tool in biotechnology, is an option that provides a method for their increased biomass production, as well as serving as a tool for the increased production of phytocomponents and conservation [7-8].

One of the studies [9] showed that the tissue grown as callus mass can yield high amount of secondary metabolites. A protocol was developed [10] for the callus induction in *Tridax procumbens* from various explants like leaf, internodes and shoot apical buds and the *in vitro* generated callus was used as a source for the isolation of secondary metabolites from *T. procumbens*.

Due to the medicinal values of these rare plants, it is decided to identify the phytochemicals present in these plants and its callus by GC-MS analysis [11-13].

EXPERIMENTAL SECTION

The plant *C. xavierensis* was collected from Sivanthipetti hills in Tirunelveli district, Tamilnadu; *C. quadrangularis* var. *rotundus* from the medicinal plant garden, Pechiparai in Kanyakumari district of Tamilnadu and *C. vitiginea* from Lower Kodayar hills in Kanyakumari district, Tamilnadu. The duration of plant collection is from September 2009 to December 2009. The collected plants were shade dried at room temperature (25-30°C), for about a month and ground well into powder by using an electric blender. About 15 gms of the powdered plant material of the plant species was taken in a digestion flask fitted to the soxhlet apparatus and extracts were obtained separately with petroleum ether, benzene, chloroform, methanol and distilled water. The extracts were concentrated and kept in brown bottles and then diluted with methanol and used for the GC-MS analysis.

Extract used for the GC-MS analysis

The methanol extract exhibited a better antibacterial activity than others. Hence GC-MS analysis was performed on methanol extract. The extracts used for phytochemical tests were concentrated by evaporation and stored at 4°C and used for GC-MS analysis.

GC-MS analysis

GC-MS analysis was carried out in a GC-MS: HP5890 fitted with a 1.4 µm column RTx-502.20, size-60 m, 0.25 mm. Carrier gas helium with a flow rate of 1 ml/min; column temperature; initial temperature 70°C, injector temperature 250°C and detector temperature 300°C, followed by a linear programmed temperature from 70 to 250°C at a rate of 10°C/min, operating in electron impact mode. The constituents were identified based on the RT values using the NIST 98 library.

RESULTS AND DISCUSSION

The chemical composition of methanol wild plant and callus extracts of *C. xavierensis, C. quadrangularis* var. *rotundus* and *C. vitiginea* were analyzed using GC-MS analysis. The identified compounds, their retention time and area percentage are summarized in Tables 1 to 6.

Table 1 Phyto-Components identified in the methanol extract of *C. xavierensis* (wild)

RT	Area %	Name of the Compounds
6.59	0.44	3-ethyl-2-pentanol
7.54	8.52	2-methyl-pentane
7.97	10.40	4-methyl- 2-heptane
8.45	35.09	*Hexane
10.85	3.54	2, 5-dihydro-3-methyl-1-butene
14.04	0.33	1, 1-dimethyl-heptanoic acid
14.63	0.70	2-decanol, 2, 3-butanediol
14.89	0.66	1, 3-butanediol, 2-hexanol
19.62	0.36	3(5)-[[1,2-dihydroxy-3-propoxy]met, 2(R), 3(S)-1,2,3,4-butanetetrol
23.30	0.36	1-hydroxy-1-phenyl propanone-2, 1,2-ethanediol, ethyl (S)-(+)-mandelate
24.88	1.10	2, 6-dimethyl benzaldehyde, 1*H*-inden-5-ol, 2, 3-dihydro benzaldehyde
26.85	0.20	3,4-methylenedioxyphenol acetone, 1-(4-hydroxymethylphenyl) ethanone
27.11	1.18	4-ethyl-2, 6-dimethyl-1,3-benzodioxole
28.74	0.35	Nonadecanoic acid, 10-methyl-octadecanoic acid
29.24	1.83	2-(5*H*)-furanone, Phosphinic acid
30.23	9.55	benzoic acid, anisyl propionate

**May be the solvent in the GC column*

Table 2 Phyto-Components identified in the methanol extract of *C. xavierensis* (callus)

RT	Area %	Name of the compounds
11.76	2.80	2,3-butanediol, 1,3-butanediol
12.12	0.29	Ethylamine, 2-propanol
16.36	0.86	2,5-hexanedione, 3, 4-dihydroxy-3,4,*N*-hydromethyl acetamide
16.50	0.87	Oxirane-2-carboxylic acid, ethyl ester
16.91	0.55	1-heptadecanamine, azetidine, 4-aminobutanoic acid
16.99	0.44	1- heptadecanamine, *N*,*N*-di-t-butylethylenediamine
17.44	1.45	1-propanol, propanoic acid, 2-methyl-octyl ester
18.03	13.32	Propanoic acid, propane, 2,2,2–[methyldyne]tris propanoic acid
18.14	2.26	Propanoic acid, 2-methyl-pentyl butanal
18.81	0.23	Cyclopentanol, 2-aminomethyl-t-spirohexan-4-one
19.07	0.43	2-piperidinone, 2-pyrrolidinone
19.17	0.37	Cyclohexene-4
19.44	0.68	11, 15-dimethylheptatriacontane
19.56	0.27	Cyclohexene-4, 5-dicarboxylic acid
19.94	1.92	1, 4-benzenedicarboxaldehyde
20.03	0.50	Pentanol
20.89	1.86	Undecanoic acid
21.28	3.29	Cyclobutanone, oxime azetidine
21.62	3.67	Dotriacontane
21.97	2.54	Phosphinic acid, diisopropyl-, 2(5h)-furanone
22.03	1.64	Bis(1,1-dimethylethyl)Phenol, 2,5-bis(1,1-dimethylethyl)Phenol, 2,4-bis (1,1-dimethylethyl)phenol
22.33	5.65	Benzoic acid, 4-(3-Hydroxyphenyl)-4-oxobutyric acid
22.48	1.07	Diethyl-3-chloro-2-hydroxypropylamine
22.79	1.95	diethyl 4-methyl-3,5-heptan
22.90	4.65	Eicosane, 9-octyl- hexadecane, 8-hexyl-8-pentyl-octadecane
23.22	4.70	9-ethyl-9-heptyloctadecane, Dotriacontane
23.34	1.93	3-ethyl-5-(2-ethylbutyl)octadecane, docosane
23.59	2.04	1,2-hydrazinedicarboxylic acid
24.16	1.00	Benzamide, hydrazinecarboxylic acid ethyl ester
24.28	0.79	Diethyl 3-chloro-2-hydroxypropylamine
24.62	3.65	Octadecane, eicosane
24.99	7.33	decahydro-1,1,4a-trim-8a(2*H*)-phenanthrenol
25.15	13.11	1,2-dihydro-9-methyl-6-phenoxycarbacetamide
26.36	0.87	Benzenebutanamine

Table 3 Phyto-Components identified in the methanol extract of *C. quadrangularis* var. *rotundus* (wild)

RT	Area %	Name of the Compounds
8.50	12.39	Acetic acid
10.41	2.75	Mercaptamine, 4-amino-1-butanol
18.07	1.38	Cyclopropane, Furazan
18.37	1.03	Ethenamine
19.09	7.65	3,5-dithiahexanol 5,5-dioxide
19.54	0.72	1-octanamine, urea
20.83	4.79	2-pyrrolidinethione, butanedioic acid, monomethyl ester
21.98	1.75	hydroxyldimethylbutanedioic acid, dimethyl-2-propoxy-succinic acid
22.44	0.97	Propane, 1-undecanamine
22.79	1.47	4*H*-pyran-4-one
23.66	3.82	*N*-methyl-piperazine adipate
24.16	2.85	2-hexynoic acid
24.33	2.54	Di(1-methylcyclobutyl) ether, 2-butenoic acid
25.26	2.97	N-acetyl-7-[beta-D-ribofuranosyl] imidazole
25.70	9.70	2-cyclohexen-1-one
25.94	2.04	Benzene, methanol, 2*H*-pyran-2-one
26.36	14.29	1,3,2-oxazaborolane
26.72	1.61	Tricyclo[4,3,1,13,8]undecane-1-carbaldehyde
27.27	11.20	L-proline, methyl ester
28.69	4.10	2-acetamido-d-mannitol
29.36	3.40	Benzoic acid, 4-ethoxyethyl ester
29.87	1.11	Tris(dimethylamino)methane
30.09	5.45	2,6-dimethyl- 3-methyl-isoxazol-5(4*H*)-one, 3-methyl-5-hydroxy-isoxazole

Table 4 Phyto-Components identified in the methanol extract of *C. quadrangularis* var. *rotundus* (callus)

RT	Area %	Name of the compounds
10.42	0.51	1*H*-pyrrole-2,5-dione
10.85	0.98	Cyclopropane, Muscimol, 5-(Aminomethyl) isoxazoe
11.68	1.45	2,4-dimethyl-heptane
12.74	0.32	7-aazabicyclo[4,1.0]heptanes
13.08	0.43	1-penten-3-one, propanenitrile
15.56	0.11	Dibutyl squarate
16.35	0.87	4-methyl-decane, octane
16.88	4.30	1-iodo-2-methylonane
16.88	4.30	Heptadecane, undecane
17.30	0.36	1,2,5-oxadiazole, 1h-azonine, octahydro-,
17.49	4.08	Undecane
18.64	0.28	Octadecane, dodecane
18.76	0.28	Methyl-oxirane-2-carboxylic acid, tetradecanoic acid
19.24	1.75	5-methyl-tetradecane, undecane
19.42	6.82	Pentadecane, dodecane
19.85	5.00	Octane, dodecane, tetradecane
20.48	0.38	1-imidazole-1-yl-2,2-dimethylpropane, 1-hexanol
20.64	0.28	2H-pyrane, 1*H*-Imidazole
20.98	1.77	Cyclobutanone oxime
21.26	6.99	Heptacosane, docosane
21.59	8.07	Heptacosane
22.02	1.64	Hexadecane, eicosane
22.16	0.67	4-trifluoroacetoxytetradecane,1-tetradecanol
22.31	5.08	4-ethoxybenzoic acid
22.61	1.37	Octadecane, decane
22.88	7.98	Eicosane, triacontane
23.20	8.01	Heptadecane
23.51	2.35	Octadecane
23.67	1.68	Cadina-1-(10),6,8-triene, 3,3,4,5,7-pentamethyl-1-indanone
23.89	1.31	Octadecane
24.32	2.40	Nonadecane, octadecane
24.58	4.00	Heptadecane, pentacosane
24.95	4.75	Eicosane
25.25	3.50	1,2-benzenedicarboxylic acid
26.06	0.46	Diethyl-3-chloro-2-hydroxypropylamine
26.30	0.87	Dibutylphthalate
26.71	2.00	Octadecane, eicosane
27.04	0.83	Octadecane, 2,3-dimethylnonadecane
27.21	1.76	Eicosane, octadecane
27.58	0.97	2-methyl-icosane
31.56	0.06	Acetamide

It was identified that sixteen compounds were present in wild and forty five compounds in callus methanol extract of *C. xavierensis*. The major compounds of the wild extract were found to be 4-methyl-2-heptane, Benzoic acid, anisyl propionate, 2-methyl- pentane and 2, 5-dihydro-3-methyl-1-butene.

In callus extract the major compounds identified were propanoic acid, propane, 2,2,2–[methylidyne]tris propanoic acid, 1,2-dihydro-9-methyl-6-phenoxycarbacetamide, decahydro-1,1,4a-trim-8a(2*H*)-phenanthrenol, benzoic acid, 4-(3-hydroxyphenyl)-4-oxobutyric acid, octadecane, 9-ethyl-9-heptyldotriacontane, eicosane, 9-octyl- hexadecane, 8-hexyl-8-pentyloctadecane, dotriacontane 1 32-dibromo-octadecane and cyclobutanone, oxime azetidine. The rest of the compounds were found less than 3%.

In the GC-MS analysis, 23 compounds were identified in the wild methanol extract of *C. quadrangularis* var. *rotundus*, but in callus extract 46 compounds were detected. The major compounds in the wild plant extract were 1,3,2-oxazaborolane, acetic acid, L-proline, methyl ester, 2-cyclohexen-1-one, 3,5-dithiahexanol 5,5-dioxide, 2,6-dimethyl-3-methyl-isoxazol-5(4*H*)-one, 3-methyl-5-hydroxy-isoxazole, 2-pyrrolidinethione butanedioic acid, monomethyl ester, 2-acetamido-d-mannitol, *N*-methylpiperazine adipate and 4-ethoxybenzoic acid ethyl ester.

In callus extract, the major compounds identified were heptacosane, heptadecane, eicosanetriacontane, heptacosane, docosane, pentadecane, dodecane, 4-ethoxybenzoic acid, eicosane, 1-iodo-2-methylonane, heptadecane, undecane, pentacosane and 1,2-benzenedicarboxylic acid. The rest of the compounds were found less than 3%.

Table 5 Phyto-Components identified in the methanol extract of *C. vitiginea* (wild)

RT	Area %	Name of the Compounds
18.80	1.75	1-octanol, 3-butyn-1-ol
20.63	2.35	1-octadecanamine
21.09	0.84	1-nonanol
21.73	1.75	3-butenoic acid ethyl ester
21.90	1.20	Bicycle[3,1,1]hept-3-ene-2-spiro-4-oleylamine
22.38	2.88	2-bromo-18-nonadecen-1-amine
22.56	1.11	1-dodecanamine, propane
22.85	1.60	1-octadecanamine, 1-tetradecanamine
23.17	1.18	Pentadecylamine
23.30	0.83	Phenol
24.02	1.73	*N,N*-di-n-butylurea
24.51	4.50	18-nonadecen-1-amine, Carbamic acid
25.29	12.55	1-tetradecanamine
25.57	2.99	Phenylethanolamine
25.92	16.18	Dibutyl phthalate, 1,2-benzenedicarboxylic acid
26.30	3.61	Hex-5-enylamine cyclobutane
26.88	5.78	Ethanol, 2-bromo-acetamide
27.17	3.81	1-nonanamine
27.50	5.66	2,3-dimethyl-2-nitrobutane
28.47	4.43	1-propanol
28.73	3.31	Ethanol, 2-bromo- 9-octadecene
29.16	8.43	Hexadecanoic acid methyl ester
30.35	2.45	2-bromopropiolic acid

Table 6 Phyto-Components identified in the methanol extract of *C. vitiginea* (callus)

RT	Area %	Name of the Compounds
18.15	2.39	2-(dimethylamino)-*N,N*-diethyl-p-nitroaniline
23.02	1.34	Carbamic acid, Cyclopentane, undecanoic acid
23.26	1.05	*N,N*-di-n-butylurea
24.67	1.66	Bicyclo[3,1,1]hept-3-ene-2-spiro-4
24.90	0.94	4-dodecene-6,8,10-triyn-3-ol
25.24	0.55	2-acetamido-2-deoxy-D-mannolactone, 1-dodecanamine
25.96	0.17	Benzene, benzeneethanamine
26.40	0.36	5-bromo-8-(4-hydroxybenzylidene)
26.99	0.28	Demeton-S-sulfone, diethyl-3-chloro-2-hydroxypropylamine
27.75	2.59	2*H*-1-benzopyran
29.03	0.47	Corydaldine , 2-ethylacridine
29.46	7.69	Z,Z-8,10-hexadecadien-1-ol acetate
29.70	6.32	14-methyl-pentadecanoic acid, hexadecanoic acid methyl ester
30.08	11.74	2-(E-4, 4-dicyano)-3-1,2,3-triazole, 3,4-dimethoxytoluene
30.42	11.61	(Z,Z) 9,12-octadecadienoic acid
30.89	6.28	5-methyl-2-phenylindolizine
31.67	7.09	2-ethylacridine
32.02	4.95	Silicic acid, vanadium
32.20	4.54	Anthracene, 5-methyl-2-phenylindolizine
32.80	3.98	diethyl bis(trimethyl)silicic acid, 2-ethyl acridine
33.27	6.16	9, 10-diethyl-anthracene, 9,10-dihybrallobarbital
33.66	1.32	3-trifluoromethyl-7-phenothiazone
34.58	10.83	2-ethylacridine-1*H*-indole
34.87	4.38	Cyclobarbital, anthracene
35.15	0.45	3-trifluoromethyl-7-phenothiazone, triethyl-(2-phenylethoxy)-silane,
35.39	0.57	*N*-methyl-1-adamantaneacetamide

The results revealed the presence of 23 compounds in the wild plant extract and 27 in the callus extract of *C. vitiginea*. The major compounds in the wild plant extract were dibutyl phthalate 1,2-benzenedicarboxylic acid, 1-tetradecanamine, hexadecanoic acid methyl ester, ethanol, 2-bromoacetamide, 2,3-dimethyl-2-nitrobutane, 18-

nonadecen-1-amine carbamic acid, 1-propanol, 1-nonanamine, hex-5-enylamine cyclobutane and ethanol and 2-bromo- 9-octadecene.

In callus extract the prevailing compounds were 2- (E-4,4-dicyano)-3-1,2,3-triazole, 3,4-dimethoxytoluene, (Z,Z)-9,12-octadecadienoic acid, 2-ethylacridine-1*H*-indole, (Z,Z)-8,10-hexadecadien-1-ol acetate, 2-ethylacridine, 14-methyl-pentadecanoic acid, hexadecanoic acid, methyl ester, 5-methyl-2-phenylindolizine, 9,10-diethyl-anthracene, 9,10-dihybrallobarbital, Silicic acid, vanadium, Anthracene, 5-methyl-2-phenylindolizine, cyclobarbital, anthracene, diethyl bis(trimethyl)silicic acid and 2- ethyl acridine. The rest of the compounds were found less than 3%.

In this study, methanol wild and callus extracts of *C. xavierensis, C. quadrangularis* var. *rotundus* and *C. vitiginea* were analyzed. Among the five different solvent extracts screened for their antibacterial activity, methanol extract showed the highest antibacterial activity, while all other extracts such as petroleum ether, benzene, chloroform and aqueous extract showed a weak antibacterial activity. Hence, the methanol wild plant and callus extracts of *C. xavierensis, C. quadrangularis* var. *rotundus* and *C. vitiginea* were selected for GC-MS analysis.

The callus extract contains certain compounds characteristic of the parent plants such as 2,3-butanediol, 1,3-butanediol, 2-(5*H*)-furanone, phosphinic acid and benzoic acid in *C. xavierensis*; cyclopropane, 2*H*-pyran, undecane and benzoic acid in *C. quadrangularis* var. *rotundus* and 1-dodecanamine, *N,N*-di-n-butylurea, carbamic acid and hexadecanoic acid in *C. vitignea*. Similar results were reported earlier in *Mentha longifolia* and *Pogostemon cablin* [14-15].

The accumulation of compounds in the callus that are not observed in the parent plant is also known [10]. However, the number of compounds detected from *in vitro* biomass was higher than those obtained from wild extracts of the selected plants. These results are comparable with those reported in the literature [15-18].

Among the identified compounds benzoic acid, phenol, 4*H*-pyran-4-one, 2,3, hexadecanoic acid, tetradecanoic acid, dibutylphthalate, 1,2-benzene dicarboxylic acid, octadecanoic acid and eicosane have the antibacterial activity as reported by earlier workers [19-20]. Antimicrobial activity of hexadecanoic acid was discussed by [21-24].

The inflammatory activity and antiarthritic activity of (Z,Z)-9,12-octadecadienoic acid were reported [25]. The anticancer activity of benzaldehyde, phenol, succinic acid, tetradecanoic acid and (Z,Z)-9,12-octadecadienoic acid were reported [26-28]. Based on the literature survey the above said compounds could effectively contribute to the antibacterial activities of selected plants.

The phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms. However, the antimicrobial activity of plant extracts depends not only on phenolic compounds but also by the presence of different secondary metabolite [29] like hydroxyl groups on the active constituents, because of the ability of these substances to bind to bacterial adhesions and disturb the availability of receptors on the surface. The phenols observed in this study are 3,4-methylenedioxyphenol acetone in *C. xavierensis* wild extract, 2,5-bis(1,1-dimethylethyl)phenol and 2,4-bis (1,1-dimethylethyl) phenol in *C. xavierensis* callus extract and phenol, 3-(2-aminoethyl)phenol, in *C. vitiginea* wild extract.

When compared with previously reported data of the *Cissus quadrangularis* species, it was found that (Z,Z) 9,12-octadecadienoic acid, 1,2-benzenedicarboxylic acid, octadecanoic acid, hexadecanoic acid, undecanoic acid, tetradecanoic acid and pentadecanoic acid [30] are found in the methanol extract.

REFERENCES

[1] F Bougard, A Gravot, S Milesi and E Gontier. *Plant Sci*. **2001**, 161, 839-851.
[2] VCY Mulabagal, SF Lee, SM Ho, CL Nalawade and HS Tsay. *Bot. Bull. Acad. Sin*. **2004**, 45, 1-22.
[3] S Srivastava and A Srivastava. *Crit. Rev. Biotechnol*. **2007,** 27, 29-43.
[4] Vandana H Barve, SN Hiremath, Shashikant R Pattan and SC Pal. *J. Chem. Pharm. Res.* **2010**, 2(1), 300-309
[5] Garima Zibbu and Amla Batra. *J. Chem. Pharm. Res.* **2010**, 2(6), 351-358.
[6] P Rane Zab Anish Kumar and Anusha Bhaskar. *J. of Chem. and Pharm. Res.* **2012**, 4(6), 2869-2873.
[7] EA Uyoh, AE Nkang and EE Eneobong. *Afr. J. Biotech*. **2003**, 2(12), 704-709.

[8] JT Opabode, OC Adeboye. *Afr. J. Biotech*. **2005,** (3), 138-142.
[9] P Maheshwari, B Songara, S Kumar, P Jain, K Srivastava and A Kumar. *Biotech. J.* **2007,** 2, 1026-1032.
[10] Minal Wani, Snehal Pande and Nitin. *Int. J Biotech. Appl*. **2010,** 2 (1), 11-14.
[11] N Raja Rajeswari, S Rama Lakshmi and K Muthuchelian. *J. Chem. Pharm. Res.* **2011**, 3(3), 792-798.
[12] S Gopalakrishnan, K Saroja and J Dulcy Elizabeth. *J. Chem. Pharm. Res.* **2011**, 3(3), 477-480.
[13] Anusha Bhaskar, V Nithya and VG Vidhya. *J. Chem. Pharm. Res.* 20**1**1, 3(5), 689-696.
[14] Supawan Bunrathep, George Brian Lockwood, Thanapat Songsak and Nijsiri Ruangrungsia. *Sci. Asia*. **2006,** 32, 293-296.
[15] Alessandra Bertoli, Michele Leonardi1, Justine Krzyzanowska, Wieslaw Oleszek and Luisa Pistelli. *Acta biochimica Polonica***. 2011**, 58, 4581-4587.
[16] DY Banthorpe and GD Brown. *Phytochem***. 1989**, 28(11), 3003-3007.
[17] Hiroki Hamada, Takuji Hoshino and Katsuaki Ohsato. *Plant tissue cult. lett*. **1991**, 8 (3), 190-192.
[18] Marissa G. Noel and Fabian M. Dayrit. *Philip. J. Sci*. **2005**, 134 (1), 5-19.
[19] ME Ramos-Nino, CA Ramirez-Rodriguez and MN Clifford. *J. Appl. Microbiol*. **1998,** 84(2), 207-212.
[20] Manas Mathur and Raka Kamal. *Brazil. J. Pharm*. **2011**.
[21] MK Woolford. *J. Sci. Food and Agri*. **1975**. 26, 219-228.
[22] G Bergsson, J Arnfinnsson, O Steingrímsson and H Thormar. *Int. J. Antimicrobial Agents***. 2002**, 20, 258-262.
[23] PL Dawson, GD Carl, JC Acton and IY Han. *Poultry Science*. **2002**, 81, 721-726.
[24] JY Lee, YS Kim and DH Shin. *J. Agri. and Food Chem*. **2002**, 50, 2193-2199.
[25] PJ Jones. *CMAJ*. **2002**, 166, 1555-1563.
[26] Frank D. Popp. *J. Med. Chem*. **1962**, 5 (3), 627-629.
[27] RW Owen, A Giacosa, WE Hull, R Haubner, B Spiegelhalder and H Bartsch. *Eur. J. Cancer*. **2000**, 36(10), 1235-47.
[28] A Maruthupandian and VR Mohan. *Int. J. Chemtech. Res*. **2011**, 3 (3), 1652-1657.
[29] SC Gordana, MC Jasna and MD Sonja. *Int J Mol Sci*. **2007**, 8, 1013-1027.
[30] G Sathyaprabha, S Kumaravel, D Ruffina and P Praveenkumar. *J. Pharm. Res*. **2010,** 3(12), 2970-2973.