Research Article

Antioxidant, cytotoxic and nutritive properties of *Ipomoea staphylina* Roem & Schult. plant extracts with preliminary phytochemical and GCMS analysis

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Received: 1 May 2018 Revised: 25 May 2018 Accepted: 30 May 2018

Abstract

Objective: *Ipomoea staphylina* Roem & Schult. plant ethanolic extract were subjected to Antioxidant, cytotoxic and nutritive experiment by using standard method. **Materials and Methods:** Ethanolic extract of *I. staphylina* was investigated for antioxidant experiment by using DPPH, ABTS, superoxide radical scavenging, Hydroxy radical scavenging, Metal chelating assays. Cytotoxic experiment is done by trypan blue exclusion test using DLA and EAC cancer cells. Nutritive value is performed by double acid digestion followed by Atomic absorption spectroscopy. **Results:** Antioxidant experiment revealed that *I. staphylina* plant ethanolic extract has excellent antioxidant activity in all tested experiments but comparably less with the standards used. *I. staphylina* plant ethanolic extract has negligible toxicity compared to the standard curcumin used. From nutritive value experiment it is revealed that *I. staphylina* plant has high iron content with rich macro and micronutrients. **Conclusion:** *I. staphylina* plant could be exploited as a valuable source of antioxidant agent enriching with nutrients.

***Keywords*:** *Ipomoea staphylina* Roem & Schult., antioxidant activity, cytotoxic activity, nutritive properties

Introduction

Plants have been a valuable source of natural products for maintaining human health, especially, in the last decade with more intensive studies for natural therapies (Nascimento et al., 2000). Medicinal herbs are widely used with a greater number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. Recently, considerable attention has been paid to utilize eco-friendly and bio-friendly plant-based products for the prevention and cure of different human diseases. It has been recorded that 80% of the world's population has fidelity in traditional medicine, particularly plant-based drugs for their primary health care (Valdiani et al., 2011).

India is also one of the mega biodiversity countries in the world. The total number of plant species of groups recorded from India is 45000. Of these seed-bearing account for nearly 15,000-18,000. In India, more than 1000 species were used in several countries in the traditional system of medicine viz. Ayurveda, Siddha, and Unani which has survived through 3000 years mainly using plant-based drugs. The ancient texts like Rigveda (4500 – 1600 B.C) and Athrvana Veda mention the use of several plants as medicine. The books on Ayurvedic medicine such as Charaka Samhita and Sushruta Samhita referred to the use of more than 700 herbs (Aggarwal et al., 2007).

Ipomoea staphylina Roem. & Schult plant description

Ipomoea staphylina Roem. & Schult is commonly known as Clustered Morning Glory, Kannada: Ugina kodi, Unang kodi, Sunang kodi, Tamil: Onaankodi, Onan Kodi.

Ipomoea staphylina Roem. & Schult is a climber plant grows

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DOI: https://doi.org/10.31024/ajpp.2018.4.4.16

near water resources and distributed throughout India, China, and Sri Lanka Deciduous forests. Leaves were stout stragglers. 15 x 10 cm dimension, broadly ovate, base cordate, apex acute, membranous, nerves oblique; petiole 6.5 cm. Flower is Panicle of cymes axillary, to 15 cm; pedicels 0.5-1 cm; bracts minute; outer sepals 5 x 4 mm, oblong, obtuse, inner obovate with hyaline margins, 6 x 5 mm; corolla 2 cm long, shallowly 5-lobed, funnel-shaped, pink; stamens 5, included, base dilated, hairy, filaments 7-8 mm; anthers 3 mm; ovary 2 mm; style 1.5 cm, stigma 2, globose. In axillary or subterminal panicles; white with a purple throat. Flowering from December-March.Fruit is subglobose capsule; seeds oblong, subtrigonous, hairy at top. Fruiting is from January-April. (Gamble*: Ipomoea staphylina* Vol- II, 1883*)*.

The plant *Ipomoea staphylina* has been used in different systems of traditional medication for the treatment of diseases and ailments of human beings. It has been reported for its analgesic (Nagariya et al., 2010), anti-inflammatory (Sarvalingam *etal*., 2011; Kirtikar et al., 1995), anti-diarrheal, gastroprotective properties (Suresh et al., 2011).

Ipomoea staphylina is used as purgative, dyspepsia, anthelmintic, bronchitis (Savitramma et al., 2015) and the *Ipomoea staphylina* is used for respiratory disorders (Reddy et al., 2013). A literature review reveals anti-inflammatory activity (Firdous [a,b] et al., 2012), 5-lipoxygenase, α-glucosidase and α-amylase inhibitory activity of *Ipomoea staphylina*. Bioactive chemical constituents reported from the leaves of *Ipomoea staphylina* include Sitosteryl-3-O-β-D-glucoside and chiro deoxy inositol (Reddy et al., 2013).

Analgesic activity of water and the methanolic extract was also reported against acetic acid-induced writhing test (Kumar et al., 2013). Some report proved that *Ipomoea staphylina* has anti-ulcer properties (Banerjee et al., 2015), Antidiabetic properties (Firdous et al., 2016), leaves of *Ipomoea staphylina* has Hepatoprotective and nephroprotective activity (Bag et al., 2013).

In Tamil Nadu Kanikkars tribal people of Tirunelveli District, used *Ipomoea staphylina* leaf latex to cure foot crack (Muralidharan et al., 2012), in Coimbatore, Tamil Nadu Irulas and Palliyars tribes were eaten the plant leaves in raw and roots were used as a anti dote for snake-bite (Sarvalingam et al., 2014; Balasubramanian et al., 1997; Arinathan et al., 2007), root tubers were rich with starch were eaten in raw (Mohan et al., 2010), Valaiyans tribes of Karandamalai, Dindigul District, Tamil Nadu Ipomea plant leaves were boiled and made decoction used in the treatment of stomach disorders (Kottaimuthu, 2008), In Andhra Pradesh, the tribes called Chenchus used the plant leaves in the treatment of piles (Kumar et al., 1999).

It has been reported as a analgesic (Nagariya et al., 2010; Kumar et al., 2013), anti-inflammatory (Sarvalingam et al., 2011; Kirtikar et al., 1995); Firdous [a,b] et al., 2012), anti-diarrheal, gastroprotective effect (Suresh et al., 2011), anti-ulcer properties (Banerjee et al., 2015), Antidiabetic properties (Firdous et al., 2016), α-amylase inhibitory activity (Kumar et al., 2013), antioxidant (Firdous et al., 2014), Hepatoprotective and nephroprotective activity (Bag et al., 2013). Bioactive chemical constituents like Sitosteryl-3-O-β-D-glucoside and chiro deoxy inositol were reported from the leaves of Ipomoea staphylina (Reddy et al., 2013).

The aim of the research topic is to evaluate the antioxidant, cytotoxic and nutritive properties *Ipomoea staphylina* Roem. & Schultin and compare it with the traditional use and old data.

Materials and methods

Plant collection and authentication

The plant materials were collected near Tomlinson church, Tumkur District, Karnataka in January 2018. (13.367190° N, 77.101176° E) The plant was identified by Dr.Y.N.Seetharam, Tumkur University and voucher specimen was conserved under the reference number TU/BD/PMS/001 (Figure 1 and 2).

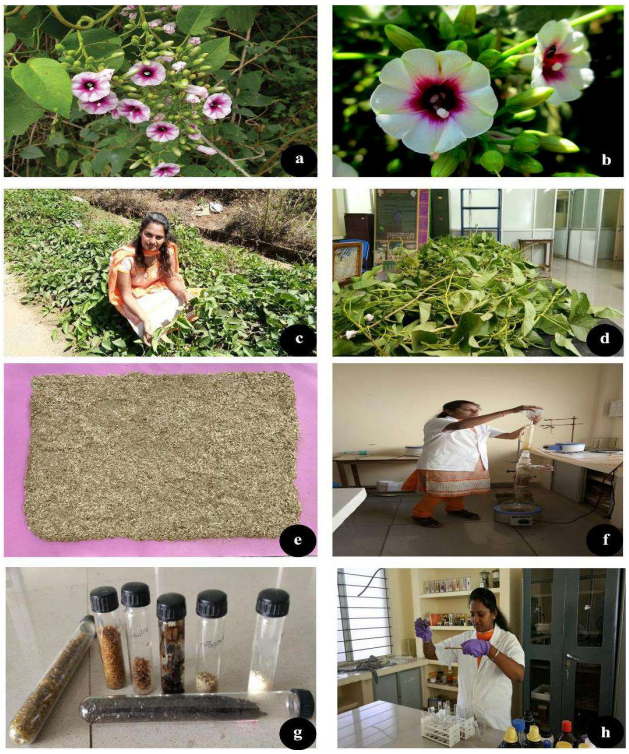

Figure 1. *Ipomoea staphylina* Roem. & Schult. a - plant habit, b - plant flower c - collection of plant material, d- shade drying of the plant sample, e - plant sample grinded. f - soxhlet extraction with different solvents, g - extracts collected in glass container. h- priliminary phytochemical analysis of plant extracts.

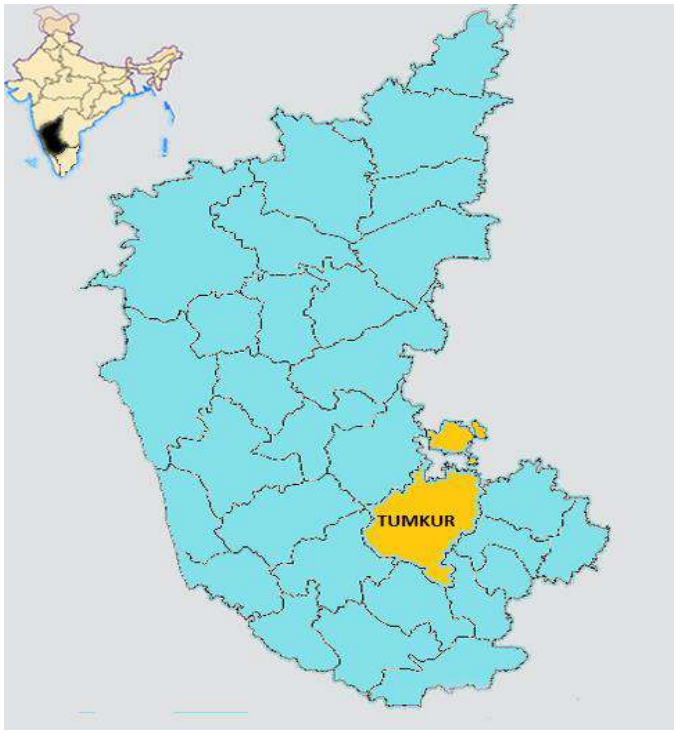

Figure 2. location where experiment plant was collected

Plant preparation and extraction

The plant samples were dried in shade for 20 to 25 days, mechanically powdered and subjected to Soxhlet extraction using petroleum ether, chloroform and ethanol (De-Castro and Ayuso. 1998). The crude extracts were collected in air-tight plastic containers and stored in cool condition.

Chemicals required

Mayer's reagent, hydrochloric acid, Wagner's reagent, ferric chloride, magnesium, sulphuric acid, acetic anhydride, bromine water, gelatin solution,Peptone, beef extract, agar, sodium chloride, sabouraud Dextrose Agar (SDA), Preliminary phytochemical reagents, 2,2-Diphenyl-2-picrylhydrazyl(DPPH), 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), ascorbic acid, butylated hydroxyl anisole (BHA), Ferrozine, gallic acid, ferrous chloride, Folin–Ciocalteu reagent, nitro blue tetrazolium sodium salt (NBT), Nicotinamide adenine dinucleotide phosphate reduced (NADH), phosphate buffered saline (PBS), trichloroacetic acid (TCA) and Trypan blue all other chemicals and solvents used were of analytical grade.

Preliminary phytochemical screening

Soxhlet extracted solvent crude extracts were screened for the presence of tannins, alkaloids, saponin, glycosides, flavonoids, steroids/sterols and phenols using standard methods (Ajaiyeoba. 2000; Harborne, 1998).

Each extract was subjected to phytochemical investigation, to study the presence of the following constituents *viz*., Alkaloid, Flavonoids, Glycosides, Saponin, Steroids, Tannins and Phenols.

GC-MS analysis

Plant extracts were subjected to Gas Chromatography and mass spectroscopy (GC-MS) obtained spectra was analyzed. GC Model: Thermo Trace GC Ultra, MS Model: Thermo DSQ II, Ionization: Electron Impact Ionisation (EI), Chemical Ionisation (CI), Mass Range: 1 - 1074 m/z. The column used is HP-5MS UI (cross-linked 5 % methyl phenyl Silox) capillary column (30 m x 0.25 mm) and the film thickness is 0.25 µm. The oven temperature was increased from 50-200 °C at a rate of 10ºC/min. Then, continued 200-300°C at rate 30 °C/min. Then post run for 10 minutes in 300 °C. Pure helium gas was used as the carrier gas with flow rate of 1 mL/min. Injector and detector temperatures were 250 °C. GC-MS was done by injecting 1 µL of sample (0.1 % in absolute methanol).

***In vitro* Antioxidant activity**

Scavenging of superoxide radicals

Superoxide radical scavenging activity was determined by the NBT reduction method (McCord & Fridovich, 1969). The reaction mixture contained 6 µM EDTA, 0.0015% NaCN, 2 µM riboflavin, 50 µM NBT, various concentrations of extract, and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured at 560 nm before and after illumination. The percentage inhibition of superoxide radical generation was evaluated by comparing the absorbance values of control and experimental tubes.

Scavenging of hydroxyl radical

Hydroxyl radicals generated from Fe^{2+}/ ascorbate/ H system degrades deoxyribose producing thiobarbituric acid reacting substance (TBARS) (Kunchandy & Rao, 1990). The efficacy of the extracts to inhibit TBARS formation was assessed. The reaction mixture contained 2.8 mM deoxyribose, 0.1 mM $FeCl_3$, 0.1 mM EDTA, 1 mM H_2O_2, 0.1 mM ascorbic acid, 20 mM KH_2PO_4–KOH (pH 7.4), and various concentrations of extracts in a final volume of 1 mL. The reaction mixture was incubated for 1 h at 37°C. The TBARS formed was measured by the method of Ohkawa et al. (1979) and the percentage inhibition was calculated from the optical measurements of control and experimental tubes.

Scavenging of DPPH radicals

Stable radical, 2, 2-diphenyl-1-picryl hydrazyl (DPPH) in methanol was used as a substrate to evaluate antioxidant

activity. The method is based on the reduction of DPPH radical in the presence of a hydrogen donating antioxidants leading to the formation of a non-radical form DPPH-H by the reaction. DPPH in its radical form has an absorption peak at 515 nm which disappeared upon the reduction of antioxidant compounds. Absorbance was measured 20 min after the reaction was started.

Radical scavenging activity was calculated using the following formula:

$$Percentage\ inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

IC_{50} value was calculated using the following formula:

$$IC50 = \frac{Sum\ of\ extract\ concentration}{Sum\ of\ percentage\ of\ inhibition\ at\ diff.conc.} \times 50$$

Scavenging of ABTS radicals

ABTS (2, 2-azobis-3-ethylbenthiozoline-6-sulfonic acid) radical scavenging activity of the extract was determined by the method described by Alzoreky and Nakahara (2001). The principle involves the oxidation of ABTS to its cation radicals by ferryl myoglobin formed in the reaction of H_2O_2 and metmyoglobin. Briefly, the stock solutions of 500 µM ABTS diammonium salt, 400 µM myoglobin (MbIII), 740 µM potassium ferricyanide, and 450 µM H_2O_2 were prepared in PBS (pH 7.4). Metmyoglobin was prepared by mixing equal volumes of myoglobin and potassium ferricyanide solutions. The reaction mixture (2 mL) contained ABTS (150 µM), MbIII (2.25 µM), and varying concentrations of extracts in PBS. The reaction was initiated by adding 75 µM H_2O_2 and oxidation reaction was monitored at 734 nm.

Metal chelating activity

The chelation of ferrous ions was determined according to the method of Dinis et al., 1994. About 3 ml of extracts at different concentrations were taken in different test tubes followed by the addition of 50 µl of ferrous chloride (2 mM). The reaction was initiated by the addition of 20 µL ferrozine (5 mM), and then the mixture was shaken vigorously and allowed to stand for 10 min at room temperature. After equilibrium, the absorbance of the solution was measured at 562 nm against the blank. EDTA was used as a standard for comparison. Percentage of inhibition and the IC_{50} value was calculated using Equation (1) and Equation (2).

***In vitro* cytotoxicity assay**

Cell lines

EAC (Ehrlich's Ascites Carcinoma): Paul Ehrlich found the initial tumor for the Ehrlich's Ascites carcinoma in 1905. The ascites variant was obtained in 1932 by intraperitoneal transplantation of Ehrlich's solid adenocarcinoma.

DLA (Dalton's Lymphoma Ascites): The initial tumor for the DLA arose as a Spontaneous Carcinoma in the thymus of mice in 1947.

The cell lines were obtained from Amala Cancer Research Centre, Thrissur.

Trypan blue dye exclusion technique

Any compound, which is cytotoxic to cells, inhibits the cell proliferation and kills the cells. Trypan blue (Moldeus et al., 1978) has the ability to penetrate into the dead cells and give it a blue color. This method gives an exact number of dead and viable cells (Kuttan et al., 1985).Cells were aspirated from the peritoneal cavity of tumor-bearing mice and it was washed three times using PBS. The viability of cells was checked using trypan blue (cell viability should be above 98%).

The cell suspension was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered saline (PBS). Control tubes containing only cell suspension. These assay mixtures were incubated for 3h at 37ºC and then 1ml of trypan blue was added after incubation and the number of the dead cells was counted using a hemocytometer (Shrivastava and Ganesh, 2010). The percentage cytotoxicity was calculated using the following equation:

$$\%\ Cytotoxicity = \frac{No.of\ dead\ cells}{No.of\ viable\ cell + No.of\ dead\ cells} * 100$$

Elemental composition of *I. staphylina* aerial parts

The microelements, calcium, magnesium, zinc, copper, manganese, lead, and cadmium were analyzed by atomic absorption spectra GBC 932 AA/AAS. plant samples were predigested with nitric acid (HNO_3) and HCl in the ratio of 1:3 for 1-4 hour depending upon the plant sample. Then, the sample is kept over hot water bath (95º C) for 4-5 hours till the sample completely dissolved (Ang et al., 2005; Uddin et al., 2016).

Statistical analysis

For statistical analysis we used Prism 04 software and all the experiment were triplicated and the values were expressed in mean ± standard error of mean (SEM).

Results

Extracts yield of *Ipomoea staphylina* Roem. & Schult. leaf

Soxhlet extraction of *Ipomoea staphylina* Roem. & Schult. leaves (700 grams) with solvents like petroleum ether gives 1.30 grams (3.95%), with chloroform 2.88 grams (8.76%) , and with ethanol 28.69 grams (87.28%). (Table 1; Figure 3). The result shows that the highest yield is obtained in ethanol followed by chloroform and least is petroleum ether.

Table 1. The percentage yield of crude extracts

Organic Solvent used	Yield of extracts in grams	% of Yield
Petroleum ether	1.305	3.95
Chloroform	2.88	8.76
Ethanol	28.693	87.28

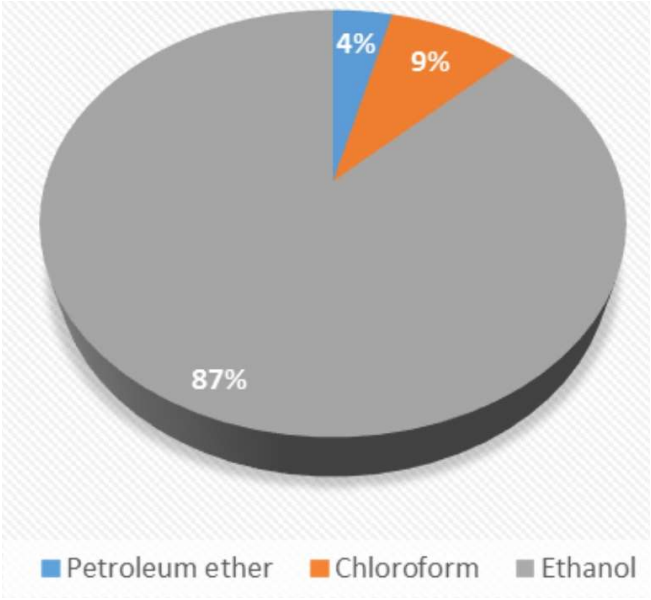

Figure 3. Yield of crude extracts in percentage

Preliminary qualitative phytochemical analysis of *Ipomoea staphylina* Roem. & Schult. Leaf extracts

The preliminary phytochemical analysis of *Ipomoea staphylina* Roem. & Schult. leaf extracts were analysed results showed that, the petroleum ether extract reveals the presence of soponins, steroids, glycosides and sterols. The chloroform extracts shows the presence of saponins, steroids, sterols. The ethanolic extract give positive result for alkaloids, saponins, flavonoids, steroids, glycosides, phenols and sterols (Table 2).

Quantitative GC-MS analysis of *Ipomoea staphylina* Roem. & Schult. leaf extracts

Due to the less extract yield and less secondary metabolites we took only ethanolic extract of *Ipomoea staphylina* Roem. & Schult. leaf for Gas chromatography mass spectroscopy (GC-MS) analysis.

(GC-MS) analysis of *Ipomoea staphylina* Roem. & Schult. ethanolic leaf extract confirms the presence of 79 compounds, out of these 24 compounds were unknown and 55 compounds were known for its medicinal properties, most of them were antimicrobial agents 18 in numbers, followed by 16 food additive and flavoring agents, 15 compounds were antioxidant, 14 compounds have anticancer properties, 14 compounds were Anti-hypercholesterolemic, 12 compounds were anti-inflammatory agents, 6 compounds were cytotoxic,

Table 2. Preliminary qualitative phytochemical analysis of *Ipomoea staphylina* Roem. & Schult. leaf extracts

Secondary Metabolites	Name of the Test	Petroleum ether Extract	Chloroform Extract	Ethanolic Extract
Alkaloids	Mayer's test	-	-	+
	Wagner's test	-	-	+
Saponins	Foam test	+	+	+
Tannins	Ferric chloride test	-	-	-
	Ferric chloride test	-	-	+
	Shenoda test	-	-	+
Flavonoids	Zinc HCl reduction test	-	-	-
	Alkaline reagent test	-	-	+
	Lead acetate test	-	-	+
Steroids	Salkowski test	+	+	+
	Keller-Kiliani test	+	+	+
Glycosides	Legal's test	+	-	-
	Ferric chloride test	-	-	+
Phenols	Ellagic acid test	-	-	-
Sterols	Liebermann Burchard test	+	+	+

6 compounds were used in cosmetics and perfumeries, 6 compounds have hepatoprotective properties, 5 compounds have antiviral properties, 5 compounds were analgesic, 4 compounds were insect pheromones, rest of them were allergenic, anesthetic, antimutagenic, antispasmodic, choleretic, dermatitigenic, fungicide, herbicide, laxative, pesticide, lipoxygenase-inhibitor, pesticide, tyrosinase inhibitor, vermifuge etc. major compound is Dodecanoic acid, 3-hydroxy- (10.41%), followed by 9-Hexadecen-1-ol (9.52%), 9-Octadecen-1-ol (8.56%), Hydroperoxide, 1-ethylbutyl (5.88%) etc. and the least percentage is 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)] undecan-1-ol (0.06%) (Figure 4, 5; Table 3).

In vitro Antioxidant properties of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Ipomoea staphylina Roem. & Schult. leaf ethanolic extract subjected to different antioxidant experiements like DPPH radical scavenging activity, ABTS radical scavenging activity, NBT superoxide radical scavenging activity, Hydroxy radical scavenging and Metal chelating activities. The experiments were triplicated and values were expressed in terms of mean±standard error of mean (SEM).

Sample Information

Analyzed by	: Admin
Analyzed	: 2/26/2018 3:00:09 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: ISE-1
Sample ID	: 3973
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 3
Injection Volume	: 1
Data File	: D:\GCMS-QP2010+\Data\2018\FEB\26.2.18\3973.qgd
Org Data File	: D:\GCMS-QP2010+\Data\2018\FEB\26.2.18\3973.qgd
Method File	: D:\GCMS-QP2010+\Data\2018\FEB\26.2.18\ARML SCAN.qgm
Org Method File	: D:\GCMS-QP2010+\Data\2018\FEB\26.2.18\ARML SCAN.qgm
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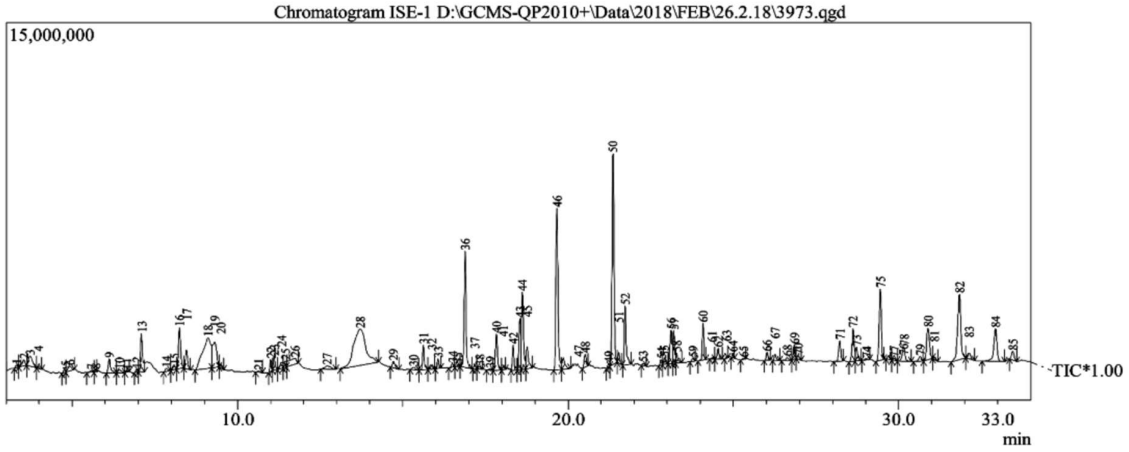

Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	3.308	3.242	3.375	412606	0.11	2-Furanmethanol
2	3.535	3.375	3.617	1510768	0.40	Propane, 1-(1-methylethoxy)-
3	3.724	3.617	3.925	3686382	0.98	2-Propanone, 1,3-dihydroxy-
4	3.973	3.925	4.075	226739	0.06	Butyrolactone
5	4.784	4.683	4.808	848334	0.23	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
6	4.924	4.808	5.058	2014770	0.54	Diglycerol
7	5.568	5.442	5.650	358597	0.10	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-
8	5.702	5.650	5.750	215591	0.06	Pentanoic acid, 4-oxo-
9	6.118	6.025	6.333	3215844	0.86	Cyclopentane, 1-acetyl-1,2-epoxy-
10	6.424	6.333	6.592	857595	0.23	Tetrahydro-4H-pyran-4-ol
11	6.661	6.600	6.725	318315	0.08	Uracil, 1-methyl-
12	6.941	6.875	7.000	385607	0.10	Alpha-amino-gamma-butyrolactone
13	7.093	7.000	7.183	7196144	1.92	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
14	7.847	7.758	7.967	782624	0.21	1,2-Benzenediol
15	8.080	7.983	8.158	770867	0.21	2-[2-(5-Norbornenyl)oxy]-tetrahydropyran
16	8.241	8.158	8.375	7585855	2.02	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
17	8.459	8.375	8.583	3648716	0.97	1,2,3-Propanetriol, monoacetate
18	9.107	8.717	9.217	22087687	5.88	Hydroperoxide, 1-ethylbutyl
19	9.311	9.217	9.442	10046176	2.68	1-Deoxy-d-arabitol
20	9.495	9.442	9.583	675750	0.18	2-Methoxy-4-vinylphenol
21	10.639	10.550	10.750	294837	0.08	3,4-Altrosan
22	11.000	10.933	11.050	1517681	0.40	Caryophyllene
23	11.109	11.050	11.217	2182493	0.58	Benzaldehyde, 2-hydroxy-6-methyl-
24	11.331	11.217	11.383	1424434	0.38	Sucrose
25	11.451	11.383	11.483	1368261	0.36	.alpha.-Caryophyllene
26	11.735	11.483	11.833	4339344	1.16	Sucrose

Figure 4. GC-MS analysis of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
27	12.714	12.525	12.975	1773049	0.47	DL-Arabinitol
28	13.703	13.108	14.267	39104314	10.41	Dodecanoic acid, 3-hydroxy-
29	14.717	14.625	14.875	1371293	0.37	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
30	15.327	15.217	15.467	642227	0.17	Cyclohexanol, 1R-4-trans-acetamido-2,3-trans-epoxy-
31	15.625	15.483	15.767	3747483	1.00	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
32	15.881	15.767	15.967	738254	0.20	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
33	16.067	15.983	16.150	1407786	0.37	Oxirane, hexadecyl-
34	16.503	16.392	16.567	574932	0.15	Hexadecanoic acid, 15-methyl-, methyl ester
35	16.668	16.575	16.733	274324	0.07	Oleic Acid
36	16.883	16.733	17.108	20753744	5.53	Pentadecanoic acid
37	17.172	17.117	17.233	573532	0.15	Hexadecanoic acid, ethyl ester
38	17.320	17.233	17.417	434186	0.12	d-Mannose
39	17.646	17.533	17.733	360970	0.10	Oleic Acid
40	17.839	17.733	17.983	7408321	1.97	Oleyl Alcohol
41	18.045	17.983	18.108	464853	0.12	1-Heptadecanol
42	18.342	18.275	18.458	3455623	0.92	Phytol
43	18.546	18.458	18.575	8644771	2.30	9,12-Octadecadienoic acid (Z,Z)-
44	18.616	18.575	18.708	13297413	3.54	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-
45	18.764	18.708	18.917	4553929	1.21	Octadecanoic acid
46	19.667	19.567	19.800	32151116	8.56	9-Octadecen-1-ol, (E)-
47	19.857	19.800	20.067	2719470	0.72	1-Nonadecanol
48	20.519	20.433	20.608	2549927	0.68	12-Chlorobicyclo[8.2.0]dodecan-11-one
49	21.227	21.167	21.267	559170	0.15	Z,E-2,13-Octadecadien-1-ol
50	21.355	21.267	21.483	35735284	9.52	9-Hexadecen-1-ol, (Z)-
51	21.537	21.483	21.642	1651137	0.44	1-Tetracosanol
52	21.726	21.650	21.908	9717094	2.59	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
53	22.265	22.208	22.375	525810	0.14	Ethanol, 2-(9-octadecenyloxy)-, (Z)-
54	22.818	22.742	22.867	936686	0.25	cis-9-Hexadecenal
55	22.918	22.867	23.025	1108080	0.30	Oleyl Alcohol
56	23.117	23.025	23.158	6124517	1.63	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
57	23.192	23.158	23.250	4810083	1.28	Methyl (Z)-5,11,14,17-eicosatetraenoate
58	23.281	23.250	23.442	1863948	0.50	Octadecanoic acid, 2,3-dihydroxypropyl ester
59	23.775	23.700	23.850	553091	0.15	Octacosanoic acid, 2,4,6,8-tetramethyl-, methyl ester, [2R-(2R*,4R*,6R*,8R*)]-
60	24.079	23.933	24.175	5246157	1.40	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-
61	24.356	24.283	24.442	235563	0.06	1,5,9,9-Tetramethyl-2-oxatricyclo[6.4.0.0(4,8)]dodecane
62	24.539	24.442	24.658	1875645	0.50	1-Hexacosanol
63	24.788	24.733	24.833	210154	0.06	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
64	24.983	24.933	25.042	611960	0.16	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (
65	25.311	25.225	25.367	211250	0.06	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol
66	26.020	25.942	26.125	1290445	0.34	.gamma.-Tocopherol
67	26.261	26.192	26.408	1057299	0.28	1-Triacontanol
68	26.628	26.467	26.758	745543	0.20	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
69	26.845	26.767	26.908	2117147	0.56	Vitamin E
70	26.959	26.908	27.050	550224	0.15	beta.-Sitosterol
71	28.224	28.033	28.325	3878875	1.03	Campesterol
72	28.623	28.500	28.683	6655430	1.77	Stigmasterol
73	28.724	28.683	28.875	2572782	0.69	4-Acetoxycinnamic acid
74	29.020	28.883	29.125	358118	0.10	Squalene
75	29.444	29.308	29.558	14170282	3.77	.gamma.-Sitosterol
76	29.687	29.617	29.783	609515	0.16	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-
77	29.861	29.792	29.958	413622	0.11	Cyclohexanecarboxylic acid, 4-butyl-, 4-pentylphenyl ester
78	30.155	29.967	30.383	3568533	0.95	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-oc
79	30.647	30.458	30.742	1049291	0.28	9,19-Cyclolanost-24-en-3-ol, acetate
80	30.891	30.758	31.025	8221963	2.19	Lupeol
81	31.077	31.025	31.183	377198	0.10	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-
82	31.842	31.592	32.033	18408044	4.90	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-, (1.alpha.,2.beta.,3.beta.,5.alpha.)-
83	32.136	32.033	32.308	1249104	0.33	2-Propenoic acid, 3-(4-hydroxyphenyl)-
84	32.932	32.525	33.200	9222304	2.46	D:A-Friedooleanan-3-ol, (3.alpha.)-
85	33.451	33.350	33.575	2003937	0.53	Friedelan-3-one
				375468819	100.00	

Figure 5 (Continue..). GC-MS analysis of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Table 3. GC-MS analysis of *Ipomoea staphylina* Roem. & Schult. Ethanolic extract

S. No	% in crude extract	Chemical name	Properties
1	0.11	2-Furanmethanol	Moderately toxic, Flavoring Agents, important constituent of urine, present in the aroma of coffee, tea, wheat bread, crispbread, soybean, cocoa, rice, potato chips, Adhesives and Sealants, anti-oxidative activity (Fuster et al., 2000; Yanagimoto et al., 2002)
2	0.4	Propane, 1-(1-methylethoxy)-	Inhibitors of Hepatitis C Virus, used in the treatment of mental disorders (Pinard et al., 2010)
3	0.98	2-Propanone, 1,3-dihydroxy-	Used in the treatment of vitiligo, in cosmetics, antifungal agent in creams, Flavoring Agents, intermediate of bacterial metabolism, less toxic commonly derived from sugar beets and sugar cane (Kenar, 2007)
4	0.06	Butyrolactone	cdc2 and cdk2 kinases inhibitor, anti-cancer activity, antimicrobial, antidepressant, Flavoring Agents (Giarman et al., 1963; Nishio et al., 1996; Kitagawa et al., 1994)
5	0.23	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	Food-grade flavor ingredients (Hameed et al., 2015)
6	0.54	Diglycerol	skin moisturizers, Hand cleaners, Insect repellent lotions and sprays, Deodorants, Chewing gums, combinational drugs used in the treatment of respiratory and urinary track disorders (Nelson et al., 1989)
7	0.1	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-	Unknown
8	0.06	Pentanoic acid, 4-oxo-	Hepatoprotective, Flavoring Agents (Ueno et al., 2007)
9	0.86	Cyclopentane, 1-acetyl-1,2-epoxy-	Anti-inflammatory, antiviral and bronchodilatory properties (Awakan et al., 2018)
10	0.23	Tetrahydro-4H-pyran-4-ol	Hepatitis C virus inhibitors, treatment of respiratory and urinary track disorders (Bianchi et al., 2017)
11	0.08	Uracil, 1-methyl-	Antiviral Compounds (Krzysztof et al. ,1987)
12	0.1	Alpha-amino-gamma-butyrolactone	Unknown
13	1.92	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Mutagen, Antimicrobial, anti-inflammatory and antioxidant capacity (Hiramoto et al., 1997; Kumar et al., 2010; Yu et al., 2013)

Table 3. Continue...

S. No	% in crude extract	Chemical name	Properties
14	0.21	1,2-Benzenediol	Antibacterial, Flavoring Agents, Antioxidants, moderately toxic, treatment of respiratory and urinary track disorders (Xu et al., 2003)
15	0.21	2-[2-(5-Norbornenyl)oxy]-tetrahydropyran	Unknown
16	2.02	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	Antimicrobial, Preservative (Gopalakrishnan et al., 2011)
17	0.97	1,2,3-Propanetriol, monoacetate	Food Additives (Marielle et al., 2000)
18	5.88	Hydroperoxide, 1-ethylbutyl	Unknown
19	2.68	1-Deoxy-d-arabitol	Unknown
20	0.18	2-Methoxy-4-vinylphenol	Can induce cell cycle arrest, antibacterial, Anti-inflammatory, antioxidant, flavoring agent, also acts as insect pheromones (Jeong et al., 2010; Silici et al., 2005; Jeong et al., 2011; Fukai et al., 2009)
21	0.08	3,4-Altrosan	Bacteriostat, Fungicide (Jadhav et al., 2014)
22	0.4	Caryophyllene	Local anaesthetic, Non-Steroidal Anti-inflammatory, Anticancer, Analgesic, Gastric cytoprotective, Antimicrobial, induces apoptosis, moderate cytotoxic, antioxidant, anticancer antipyretic, platelet-inhibitory and Inhibition of prostaglandin synthesis, sedative, fungicide (Ghelardini et al., 2001; Tambe et al., 1996; Sabulala et al., 2006; Yang et al., 2000; Huang et al., 2012; Park et al., 2011; Calleja et al., 2013; Dahham et al., 2015; Kumar et al., 2010)
23	0.58	Benzaldehyde, 2-hydroxy-6-methyl-	Pheromone of the Acarid Mite Tyrophagus perniciosus, Collohmannia gigantea, Dermatophagoides farinae, Acarus siro, Tyrophagus neiswanderi. Used in the treatment Cancer, sexual or genital disorder, antipyretic, anti-inflammatory, analgesic, treatment in immunological or allergic disorder (Leal et al., 1988)
24	1.54	Sucrose	As a sweetener in foods and soft drinks, in the manufacture of syrups, in invert sugar, confectionery, preserves and jams, demulcent, beverages, medications, pharmaceutical products, and caramel (Karen, 2004)
25	0.36	alpha.-Caryophyllene	Essential oil present in Humulus lupulus, anti-inflammatory, Antitumor activity, analgesic, anti-inflammatory,antiseptic, immunostimulant, perfumes (Fernandes et al., 2007; Legault et al., 2003; Legault et al., 2007)
26	0.47	DL-Arabinitol	Indicator of liver cirrhosis, gastrointestinal candidiasis etc in serum and urine (Wong et al., 1990)
27	10.41	Dodecanoic acid, 3-hydroxy-	In the treatment of Fatty Acid Oxidation disorder, intermediate of liver fatty acid metabolism (Jones et al., 2000)
28	0.37	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Antimicrobial, Antioxidant, Antiinflammatory, Analgesic (Gopalakrishnan et al., 2011)
29	0.17	Cyclohexanol, 1R-4-trans-acetamido-2,3-trans-epoxy-	Camphor like odor and are used in making soaps, insecticides, germicides, dry cleaning, and plasticizers (Yasuko et al., 2000)
30	1.2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Treatment in asthma, antimicrobial, cancer preventive, Anti-inflammatory (Ogunlesi et al., 2009; Yu et al., 2008; Ponnamma et al., 2012; Srinivasan et al., 2014;)
31	0.37	Oxirane, hexadecyl-	Unknown
32	0.15	Hexadecanoic acid, 15-methyl-, methyl ester	Unknown
33	0.17	Oleic Acid	Antimicrobial, edible oils, Fish Oil Supplementation, Colorectal Cancer Prevention, Flavoring Agents, Insecticide, Acaricide, Herbicide, Plant growth regulator, Surfactants Lubricants, Paint additives (Dilika et al., 2000)
34	5.53	Pentadecanoic acid	Adhesives and sealant chemicals, Agricultural chemicals (non-pesticidal), Finishing agents, Lubricants and lubricant additives, Surface active agents, serum as a marker for intake of milk fat (Smedman et al., 1999)
35	0.15	Hexadecanoic acid, ethyl ester	Antioxidant, lubricant, hypocholesterolemic nematicide, pesticide, anti-androgenic, flavoring agent, hemolytic, 5-Alpha reductase inhibitor (Kumar et al., 2010; Maruthupandian et al., 2011)
36	0.12	d-Mannose	protein quality control in human body (Lee et al., 1988)
37	2	Oleyl Alcohol	Savory, emulsion stabilizers, surfactant - emulsifying agents, antifoaming agents, and skin conditioning agents, cosmetics, protect the outer surface of plants and animals from water loss, chemical intermediate, automotive lubricant, defoamer, cosolvent and plasticizer for printing ink, Oleyl alcohol is a natural product in fish oils (Billich et al., 2004)
38	0.12	1-Heptadecanol	Flavoring Agents, Insect sex pheromone, antiacne agents, antibiotic (Butler et al., 391981; Kubo et al., 1994)
39	0.92	Phytol	Antimicrobial, Anti-inflammatory (Srinivasan et al., 2014)
40	2.3	9,12-Octadecadienoic acid (Z,Z)-	Anticoronary, Antialopecic, Antiarteriosclerotic, Antiarthritic, antianaphylactic, Antieczemic, Cancer preventive, antiprostatic, hepatoprotective, Hypocholesterolemic, Metastatic, Nematicide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, (Ponnamma et al., 2012; Maruthupandian et al., 2011; Kalaivani et al., 2012)
41	3.54	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Anti-inflammatory, Hypocholesterolemic, Antihistaminic (Srinivasan et al., 2014)
42	1.21	Octadecanoic acid	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propecic, Suppository (Ponnamma et al., 2012)
43	8.56	9-Octadecen-1-ol, (E)-	pheromonal component from the sting of the honey bee, (Pickett et al., 1982)
44	0.72	1-Nonadecanol	Unknown
45	0.68	12-Chlorobicyclo[8.2.0]dodecan-11-one	Unknown

Table 3. Continue...

S. No	% in crude extract	Chemical name	Properties
46	0.15	Z,E-2,13-Octadecadien-1-ol	Insect sex pheromone component (Schwarz et al., 1983)
47	9.52	9-Hexadecen-1-ol, (Z)-	Cosmetics, anti-hair fall agent, present in sex pheromones of *Heliothis subflexa* (Teal et al., 1981; Choi, 2013)
48	0.44	1-Tetracosanol	Unknown
49	2.85	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Unknown
50	0.14	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Unknown
51	0.25	cis-9-Hexadecenal	Insect pheromone (Berg et al., 2005)
52	1.63	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	hypocholesterolemic, antieczemic, Nematicide, hepatoprotective (Gnanavel et al., 2013)
53	1.28	Methyl (Z)-5,11,14,17-eicosatetraenoate	Unknown
54	0.5	Octadecanoic acid, 2,3-dihydroxypropyl ester	Food additive in Dairy, Surfactants, Antiviral (Jaafar et al. ,2007)
55	0.15	Octacosanoic acid, 2,4,6,8-tetramethyl-, methyl ester, [2R-(2R*,4R*,6R*,8R*)]-	Unknown
56	1.4	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	Antibacterial, Antioxidant, Cancer-preventive,Antitumor, Immunostimulant, perfumery, Pesticide, Sunscreen (Ponnamma et al., 2012)
57	0.06	1,5,9,9-Tetramethyl-2-oxatricyclo[6.4.0.0(4,8)]dodecane	Unknown
58	0.5	1-Hexacosanol	Naturally in the epicuticular wax and plant cuticle of many plant species (Asperger et al., 1999)
59	0.16	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-,	Unknown
60	0.06	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	Fungistatic, Ginsenol is found in tea. Ginsenol is isolated from ginseng plant rootlets (Aleu et al., 2001)
61	0.34	gamma.-Tocopherol	Anticancer, Antioxidant, Antitumor, Antiinflammatory, Hypocholesterolemic, Cardioprotective (Ponnamma et al., 2012)
62	0.28	1-Triacontanol	plant growth stimulator, (Jones et al., 1979; Khandaker et al., 2013)
63	0.56	Vitamin E	Antiaging, Antialzheimeran, Antidermatitic, Antidiabetic, Antioxidant, Antitumor, Cancer-preventive, Hypocholesterolemic, Immunostimulant, analgesic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, hepatoprotective, ypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary (Ponnamma et al., 2012; Srinivasan et al., 2014; Kumar et al., 2010)
64	0.15	beta.-Sitosterol	Anti-hypercholesterolemia, Reduces blood levels of cholesterol, antioxidant activity, anticancer (Kalaivani et al., 2012)
65	1.03	Campesterol	Antioxidant, Hypocholesterolemic (Ponnamma et al., 2012)
66	1.77	Stigmasterol	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemic (Ponnamma et al., 2012)
67	0.69	4-Acetoxycinnamic acid	Plant growth inhibitor (Hiradate et al., 1999)
68	0.1	Squalene	Antibacterial, Antioxidant, Immunostimulant (Srinivasan et al., 2014)
69	3.77	gamma.-Sitosterol	used to treat Hyperlipidemias, Antioxidant, antibacterial and prophylactic activities (Venkata et al., 2012; Akpuaka et al., 2013)
69	3.77	gamma.-Sitosterol	used to treat Hyperlipidemias, Antioxidant, antibacterial and prophylactic activities (Venkata et al., 2012; Akpuaka et al., 2013)
70	0.16	Cholest-5-en-3-ol, 24-propylidene-, (3.beta.)-	Unknown
71	0.11	Cyclohexanecarboxylic acid, 4-butyl-, 4-pentylphenyl ester	Unknown
72	0.95	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-oc	Unknown
73	0.28	9,19-Cyclolanost-24-en-3-ol, acetate	Unknown
74	2.19	Lupeol	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase inhibitor, Pesticide (Maruthupandian et al., 2011)
75	0.1	D:B-Friedo-B':A'-neogammacer-5-en-3-ol, (3.beta.)-	Unknown
76	4.9	Cyclohexane, 1,2-dimethyl-3,5-bis(1-methylethenyl)-, (1.alpha.,2.beta.,3.beta.,5.alpha.)-	Unknown
77	0.33	2-Propenoic acid, 3-(4-hydroxyphenyl)-	Antibacterial, flavor, Aldose-Reductase-Inhibitor, Allergenic, Anesthetic, Antiinflammatory, Antimutagenic, Antispasmodic, Cancer Preventive; Choleretic; Dermatitigenic,Fungicide, Herbicide, Laxative, Pesticide, Lipoxygenase-Inhibitor, Pesticide, Tyrosinase Inhibitor, Vermifuge (Ponnamma et al., 2012; Kumar et al., 2010)
78	2.46	D:A-Friedooleanan-3-ol, (3.alpha.)-	Unknown
79	0.53	Friedelan-3-one	Unknown

a. DPPH radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Ipomoea staphylina Roem. & Schult. leaf ethanolic extract showed dose dependant radical scavenging activity in all tested concentrations. IC_{50} value of the ethanolic crude extract (45.07±1.72) is almost nearer to the to the value of standard Ascorbic acid (39.48± 0.02) used (Table 4; Figure 6).

b. ABTS radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

In ABTS radical scavenging activity *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract showed dose dependant antioxidant activity in all tested concentrations. IC_{50} value of the ethanolic crude extract (84.37±2.68) is comparable with the value of standard Butylated Hydroxyl Anisole (66.92±0.36) used (Table 5; Figure 6).

Table 4. DPPH radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Concentration in µg/mL	Scavenging activity	IC_{50} value	Standard µg/mL (Ascorbic acid)	IC_{50} value of Standard Ascorbic acid
25	40±0.57	45.07±1.72	76.23±0.23	39.48± 0.02
50	77.33±1.85		82.32±0.43	
75	103.66±3.28		113.11±0.09	
100	119±1.15		134.54 ± 0.91	
125	141.33±1.85		156.43± 0.02	
150	160.66±0.88		176.65 ± 0.34	
175	167.66±2.66		189.41 ± 0.54	
200	188.66±1.45		210.87±0.32	

Table 5. ABTS radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Concentration in µg/mL	scavenging activity	IC_{50} value	Standard µg/mL (Butylated Hydroxyl Anisole)	IC_{50} value of Standard Butylated Hydroxyl Anisole
50	37.33±0.88	84.37±2.68	47.34±0.32	66.92±0.36
100	74.33±1.2		84.65±0.05	
150	92.66±2.72		120.43±0.36	
200	117±2.08		149.68±0.1	
250	141±3.6		185.65±0.3	
300	183±3.05		214.76±0.62	
350	194.6±2.72		254.36±0.06	
400	226.66±5.17		287.98±0.6	

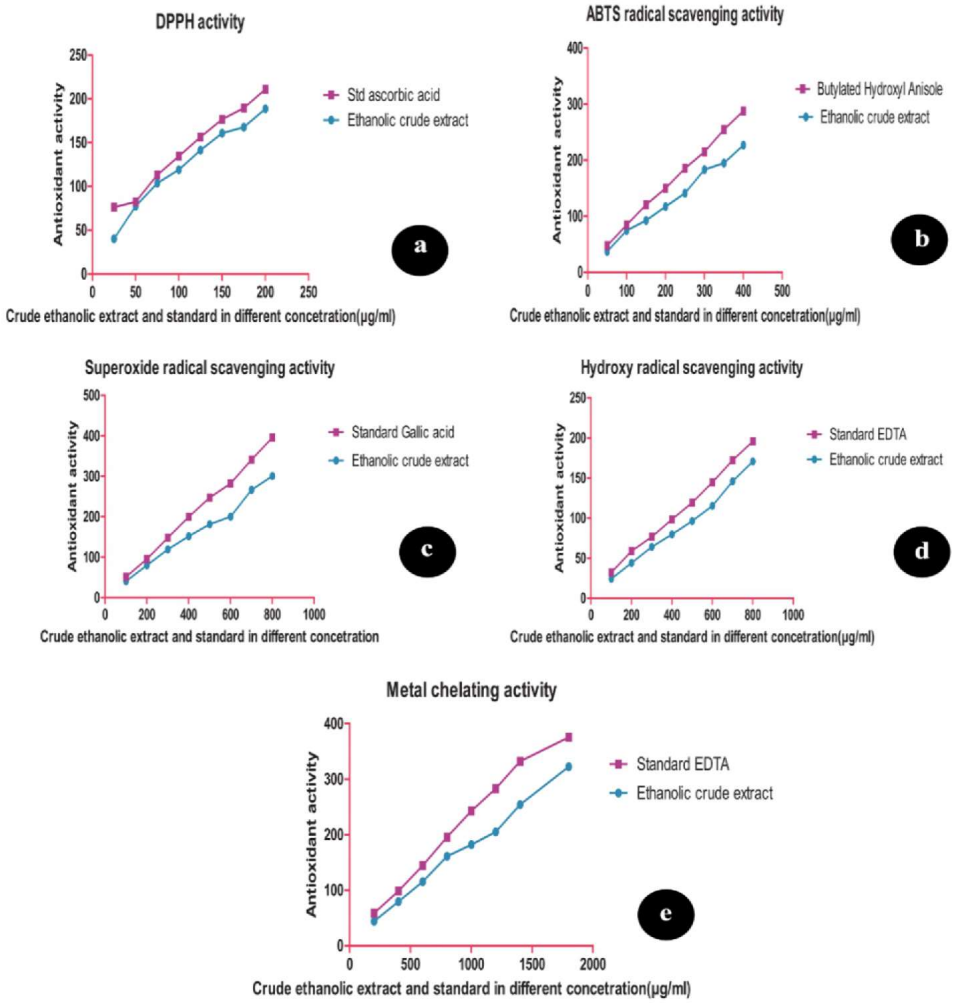

Figure 6. *Ipomoea staphylina* Roem. & Schult. antioxidant activity a - DPPH radical scavenging activity, b - ABTS radical scavenging activity c - Superoxide radical scavenging activity, d- Hydroxy radical scavenging activity, e - Metal chelating activity

c. Metal chelating activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

In Metal chelating activity *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract showed dose dependant antioxidant activity in all tested concentrations. IC_{50} value of the ethanolic crude extract (271.261±1.45) is comparable with the value of standard EDTA (213.69±2.13) (Table 6; Figure 6).

Table 6. Metal chelating activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Concentration in µg/mL	scavenging activity	IC_{50} value	Standard µg/mL (EDTA)	IC_{50} value of Standard EDTA
200	44±1.52	271.261±1.45	58.76±0.32	213.69±2.13
400	79.66±0.66		98.34±0.03	
600	115.33±1.45		144.65±0.32	
800	161±1.15		195.76±0.45	
1000	182±0.57		242.87±0.14	
1200	205.33±0.88		283.24±0.36	
1400	254.33±2.6		332.31±0.05	
1800	322.33±0.88		375.52±0.82	

d. Superoxide NBT radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

In Superoxide NBT radical scavenging activity *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract showed dose dependant antioxidant activity in all tested concentrations. IC_{50} value of the ethanolic crude extract (134.19±1.45) is comparable with the value of standard Gallic acid (102.17±0.49) used (Table 7; Figure 6).

Table 7. Superoxide NBT radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Concentration in µg/mL	scavenging activity	IC_{50} value	Standard µg/mL (Gallic acid)	IC_{50} value of Standard Gallic acid
100	40.66±0.88	134.19±1.45	51.66±0.11	102.17±0.49
200	80.33±0.88		95.67±0.54	
300	119.33±1.2		148.12±0.42	
400	152±2.08		199.77±1.34	
500	181.33±2.33		247.32±0.49	
600	200.33±0.88		282.22±0.19	
700	266.66±2.18		341.21±0.24	
800	300.66±1.2		395.74±0.63	

e. Hydroxy radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

In Hydroxy radical scavenging activity *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract showed dose dependant antioxidant activity in all tested concentrations. IC_{50} value of the ethanolic crude extract (243.133±1.45) is comparable with the value of standard EDTA (200.51±2.45) used (Table 8; Figure 6).

Table 8. Hydroxy radical scavenging activity of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Concentration in µg/mL	scavenging activity	IC_{50} value	Standard µg/mL (EDTA)	IC_{50} value of Standard EDTA
100	24.33±0.88	243.133±1.45	32.14±0.63	200.51±2.45
200	44±1.52		58.76±0.32	
300	64±1.15		76.58±0.98	
400	79.66±0.66		98.34±0.03	
500	96.33±1.33		119.24±0.19	
600	115.33±1.45		144.65±0.31	
700	146±2.51		172.23±0.48	
800	170.66±0.88		195.76±0.45	

In vitro Cytotoxic properties of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Ipomoea staphylina Roem. & Schult. leaf ethanolic extract was subjected to *in vitro* Cytotoxic properties using DLA (Dalton's Lymphoma Ascites) and EAC (Ehrlich's Ascites Carcinoma) cancer cells by Trypan blue dye exclusion technique. The cell lines were maintained at Amala Cancer Research Centre, Amala Nagar, Thrissur, India. The experiments were triplicated and values were expressed in terms of mean±standard error of mean (SEM).

Ipomoea staphylina Roem. & Schult. leaf ethanolic extract was tested for *in vitro* cytotoxic experiment against DLA and EAC cancer cells showed moderate toxicity in all tested concentrations. Ethanolic extract is more toxic to EAC cells (CTC_{50}:155.73±3.14) than DLA cells (CTC_{50}:192.58±6.8) but not up to the mark compared to standard Curcumin (CTC_{50}:54.31±1.5) used (Table 9; Figure 7).

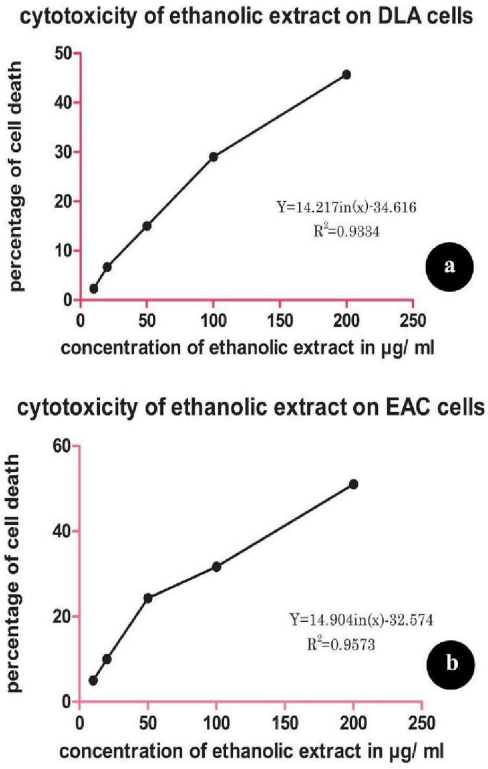

Figure 7. *Ipomoea staphylina* Roem. & Schult. invitro cytotoxic activity a - percentage of cell death of DLA cancer cell, b - percentage of cell death of EAC cell.

Table 9. *In vitro* Cytotoxic properties of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract

Conc (µg/mL)	DLA cells		EAC cells		Standard Curcumin	Standard CTC_{50}
	Percentage cytotoxicity	CTC_{50}	Percentage cytotoxicity	CTC_{50}		
10	2.33±0.33	192.58	5±0.57	155.73	15.4±3.3	54.31±1.5
20	6.66±0.33	±6.8	10±0.57	±3.14	34.4±3.3	
50	15±0.57		24.33±0.33		100±0.5	
100	29±0.57		31.66±0.33		100±0.5	
200	45.66±0.88		51±0.57		100±0.5	

Elemental composition of *Ipomoea staphylina* Roem. & Schult. leaf sample

Ipomoea staphylina Roem. & Schult. leaf sample subjected for nutrient analysis through atomic absorption spectroscopy. The results was found to be saticifying with sufficient quantity of macronutrients like nitrogen (4.27±0.54), phosphorus (0.21±0.04), potassium (1.58±0.02), calcium (0.59±0.13), and magnesium (0.030±0.05) in percentage (Table 10; Figure 8).

Micronutrients like Iron (444.60 ±0.54), manganese (84.50±0.02), zinc (22.60±0.02) and copper (17.25±0.13) in ppm (Table 11; Figure 9).

Table 10. Macronutrient of *Ipomoea staphylina* Roem. & Schult. leaf sample

Samples	Macronutrients in percentage (mean ± st.dev)				
	N	P	K	Ca	Mg
Leaf	4.27±0.54	0.21±0.04	1.58±0.02	0.59±0.13	0.030±0.05
Occurance	Medium	Medium	Medium	Medium	Medium

Table 11. Micronutrients of *Ipomoea staphylina* Roem. & Schult. leaf sample

Samples	Micronutrients in ppm (mean ± st.dev)			
	Fe	Mn	Zn	Cu
Leaf	444.60 ±0.54	84.50±0.02	22.60±0.02	17.25±0.13
Occurance	High	Medium	Medium	Medium

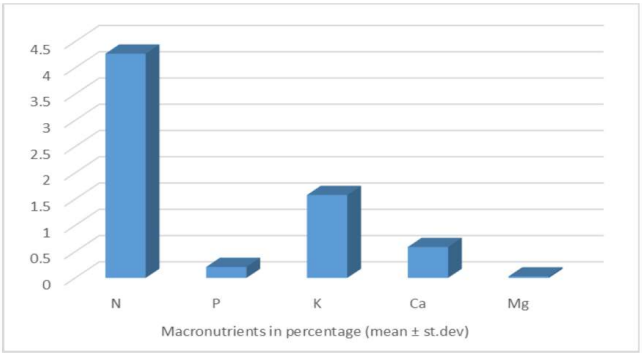

Figure 8. Macronutrients of *Ipomoea staphylina* Roem. & Schult. Leaf samples in percentage

In all the nutrient component of *Ipomoea staphylina* Roem. & Schult. leaf sample iron (444.60 ±0.54) was found to be highest, which is essential micro nutrient mainly help in the treatment of anemic patients having the deficiency of iron in the form of ferrous ion. In developing countries iron deficiency is common factor affects the growth of devolping childerns. Ferrous ions also helps in the heamoglobin formation which essential for human beings.

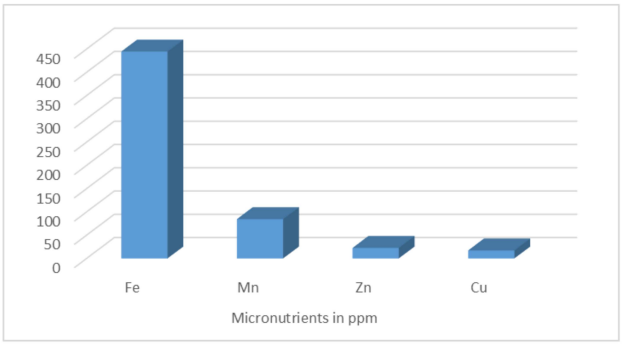

Figure 9. Micronutrients of *Ipomoea staphylina* Roem. & Schult. Leaf samples in percentage

Discussion

Soxhlet extraction

Soxhlet extraction is a common procedure to extract phytoconstituents which is essential to mankind. The leaf sample (700 grams) of *Ipomoea staphylina* Roem. & Schult. yields more percentage of extact in ethanol (87.28%) when compared to other solvents like petroleum ether (3.95%) and with chloroform (8.76%) so it is revealed that, the plant leaf sample is having more alcohol soluble extractive than other solvents which is more essential in extraction of good phytoconstituent (Table 1; Figure 3).

Preliminary phytochemical analysis

The preliminary phytochemical analysis of *Ipomoea staphylina* Roem. & Schult. leaf extracts also revealed the presence of more phytoconstituent in the ethanolic extracts like alkaloids, saponins, flavonoids, steroids, glycosides, phenols and sterols when compared to petroleum ether extract which only confirms the presence of soponins, steroids, glycosides and sterols, similarly chloroform extracts confirms only the presence of saponins, steroids, sterols. So, we took only ethanolic extract for Gas Chromatography and Mass Spectroscopic (GC-MS) analysis for confirmation of different constituents (Table 2).

GC-MS analysis

GC-MS analysis of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract was analysed in the instrument GC Model: Thermo Trace GC Ultra, MS Model: Thermo DSQ II, Ionization: Electron Impact Ionisation (EI), Chemical

Ionisation (CI), Mass Range: 1 - 1074 m/z and obtained spectra was analysed, revealed the presence of 79 compounds in that 24 compounds were unknown and 55 compounds were known for its medicinal properties. Major percentage of compound is Dodecanoic acid, 3-hydroxy- (10.41%) used in the treatment of Fatty Acid Oxidation disorder and it also intermediate of liver fatty acid metabolism (Jones et al., 2000), followed by 9-Hexadecen-1-ol (9.52%) used as Cosmetics, anti-hair fall agent (PubChem- 9-Hexadecen-1-ol/ $C_{16}H_{32}O$), 9-Octadecen-1-ol (8.56%), Hydroperoxide, 1-ethylbutyl (5.88%) etc. and the least percentage is 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol (0.06%).

Eighteen compounds were antimicrobial agents such as Butyrolactone; 4H-Pyran-4-one, 2,3 - dihydro - 3, 5 - dihydroxy - 6- methyl-; 1,2-Benzenediol;2 Furancarboxaldehyde, 5-(hydroxymethyl)-; 2-Methoxy-4-vinylphenol; 3,4-Altrosan; Caryophyllene; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Oleic Acid; 1-Heptadecanol; Phytol; 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-; 3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol; Squalene; gamma.-Sitosterol; Lupeol; 2-Propenoic acid, 3-(4-hydroxyphenyl)-.

Fourteen compounds have anticancer properties such as Butyrolactone; Caryophyllene; alpha.-Caryophyllene; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; Oleic acid; 9,12-Octadecadienoic acid (Z,Z)- ; 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-; gamma.-Tocopherol; Vitamin E; beta.-Sitosterol; Stigmasterol; Lupeol; 2-Propenoic acid, 3-(4-hydroxyphenyl)-; 2-Methoxy-4-vinylphenol.

Fourteen compounds have Anti-hypercholesterolemic properties such as 2-Propenoic acid, 3-(4-hydroxyphenyl)-; Lupeol; gamma.-Sitosterol; Stigmasterol; beta.-Sitosterol; Campesterol; Vitamin E; gamma.-Tocopherol; 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester; 9,12-Octadecadienoic acid (Z,Z)-; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Octadecanoic acid; Hexadecanoic acid, ethyl ester; Dodecanoic acid, 3-hydroxy-.

Sixteen compounds were used as food additive and flavoring agent such as 2-Furanmethanol; 2-Propanone, 1,3-dihydroxy-; Butyrolactone; 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one; Diglycerol; Pentanoic acid, 4-oxo-; 1,2-Benzenediol ; 2-Furancarboxaldehyde, 5-(hydroxymethyl)-; 1,2,3-Propanetriol, monoacetate; 2-Methoxy-4-vinylphenol; Sucrose; Oleic Acid; Hexadecanoic acid, ethyl ester; Octadecanoic acid; Octadecanoic acid, 2,3-dihydroxypropyl ester; 2-Propenoic acid, 3-(4-hydroxyphenyl)-.

Fifeteen compounds have antioxidant properties such as 2-Furanmethanol; 4H-Pyran-4 - one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 1,2-Benzenediol; 2-Methoxy-4-vinylphenol; Caryophyllene; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; Hexadecanoic acid, ethyl ester; 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-; gamma.-Tocopherol; Vitamin E; beta.-Sitosterol; Campesterol; Squalene; gamma.-Sitosterol; Lupeol.

Twelve compounds have anti-inflammatory properties such as 2-Propenoic acid, 3-(4-hydroxyphenyl)-; Vitamin E; gamma.-Tocopherol; 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-; Phytol; 3,7,11,15-Tetramethyl-2-hexadecen-1-ol; 4-((1E)-3-Hydroxy-1-propenyl) -2- methoxyphenol; alpha – Caryophyllene); Caryophyllene; 2-Methoxy-4-vinylphenol; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; Cyclopentane, 1-acetyl-1,2-epoxy-.

Six compounds have hepatoprotective properties such as Pentanoic acid, 4-oxo-; Caryophyllene; 9,12-Octadecadienoic acid (Z,Z)-; 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester; gamma.-Tocopherol; Vitamin E.

Five compounds have antiviral properties such as Cyclopentane, 1-acetyl-1,2-epoxy-; Uracil, 1-methyl-; Stigmasterol; Tetrahydro-4H-pyran-4-ol; Octadecanoic acid, 2,3-dihydroxypropyl ester.

Five compounds have analgesic properties such as Caryophyllene; Benzaldehyde, 2-hydroxy-6-methyl-; alpha.-Caryophyllene; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; Vitamin E. (Table 3; Figure 4-5)

***In vitro* antioxidant properties**

In biological systems most of the free radicals are derivatives of oxygen like superoxide, hydrogen peroxide, hydroxyl radical or derivatives of nitrogen like nitric oxide and peroxynitrite. Reactive Oxygen Species were the major cause for mutagenesis and carcinogenesis. They also induce toxic effects like inactivation of enzymes and alteration of intracellular oxidation-reduction state. It can also generate many types of DNA modifications and chromosome aberrations leading to carcinogenesis. The free radicals damage on the cell/ tissues is neutralized by antioxidants such as á-tocopherol, carotenoids, glutathione, thiols, vitamin C etc., by scavenging and decreasing their formation. In plants several natural compounds exhibit antioxidant and/or radical scavenger properties. They possess low molecular weight and the antioxidant mechanism is very complex (Cai et al., 2004).

In our study it is revealed that *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract showed appreciable antioxidant activity in all tested concentrations which is

almost comparable with the standards used. The antioxidant property of *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract is may be due to the presence of 15 compounds present in it, such as, 2-Furanmethanol; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; 1,2-Benzenediol; 2-Methoxy-4-vinylphenol; Caryophyllene; 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; Hexadecanoic acid, ethyl ester; 2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-; gamma.-Tocopherol; Vitamin E; beta.-Sitosterol; Campesterol; Squalene; gamma.-Sitosterol; Lupeol.

***In vitro* cytotoxic properties**

Many known plant also acts as poison due to overdosage so many medicinal plants also becomes toxic when it is over consumed. So, threshold dosage of phytodrug is necessary to avoid its poisoning.

From our study it is revealed that *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract showed moderate cytotoxicity against DLA and EAC in all tested concentrations which is not with the standard curcumin used. Eventhough 14 known anticancer compounds present in the ethanolic extract failed to suppress the cancer cells in effective way. So, *Ipomoea staphylina* Roem. & Schult. leaf ethanolic extract, neither acts as effective anticancer agent in suppression of cancer cells nor toxic. Which also confirm the traditional use in the consumption of leaves and roots by the tribes of Tamil Nadu.

Elemental composition of *Ipomoea staphylina* Roem. & Schult. leaf

Macronutrient

Potassium (K) was found in normal level in leaf sample. It is commonly growing drought resistance plant so to tolerate the biotic stresses like pathogens and pests, for its survival it has to show defense mechanism, so that disease prone parts like leaf has most potassium accumulation. According to WHO normal human need atleast 4.7 grams of potassium per day to perform normal work. Potassium has very important role in human health like, maintain a normal blood pressure, work as a electrolyte in maintaining the body fluid, helps in the contraction of skeletal, heart and smooth muscles, maintain the kidney health and nerve stability, healps in normal enzyme production in metabolism, maintains normal bone strength (Aaron et al., 2013).

In *Ipomoea staphylina* Roem. & Schult. leaf, nitrogen (N) percentage in normal level. Nitrogen is a main component of DNA, RNA, amino acids, an essential nutrient mainly required for protein synthesis and enzymes, maintains normal growth of cell, messenger element to relaxing the muscles in the body. A healthy adult need 110 miligrams of nitrogen per kg of body weight (Tome et al., 2000).

In *Ipomoea staphylina* Roem. & Schult. leaf, phosphorus (P) was found in normal level. Phosphorus is distributed in all plant parts and easily percolated from one organ to another and mainly present in foliage older to younger leaves, flowers and seeds. it is an essential element participate in photosynthesis, respiration and other metabolic processes. In the human body phosphorus has a vital role in the formation of bone and teeth, helps in the metabolism of corbohydrates and fats, helps in the synthesis of protiens in the human body, triggers tisue repair mechanism, main component of energy rich source called Adinosine tri phosphate (ATP), maintains pH of the blood, maintains heamoglobin structure stability (Calvo et al., 2015).

In *Ipomoea staphylina* Roem. & Schult. leaf, percentage of magnesium (Mg) was found to be normal. Magnesium is an essential nutrient which mainly present in leaf and fruit. In leaf it required for photosynthesis and in fruit it is mainly present to activate sugar producing enzyme. In the human body magnesium is an vital nutrient in normal nerve and muscle function, in maintaining steady heart beat, helps in bone stability, in regulation normal blood glucose level, enzymes normal function, in maintaining normal body fluid, protein synthesis, cell reproduction, transport substances across cell barriers, in the synthesis of ATP, cofactor for more than 300 enzymes (Schwalfenberg et al., 2017).

In *Ipomoea staphylina* Roem. & Schult. leaf, percentage of calcium (Ca) was found to be in normal level. In human being normal function atleast need 1,200 miligrams per day. Bone is mainly made up of calcium supporting muscular body. Calcium also play important role in cell signaling, muscular contraction, blood clotting, nerve function, enzyme activation, cell membrane transport, maintaining regular heartbeat, component of serum (Weaver et al., 2011).

Micronutrient

In *Ipomoea staphylina* Roem. & Schult. leaf, the Copper was found to be normal. Copper is essential for maintainance of brain health, antioxidant defence, main component of neuron communication, essential in healthy skin and connective tissue, essential in body repair mechanism, structural maintainance of heart and blood vessels, proper circulation of blood, formation of white blood cells, in triggering immune response, mitochondrial normal function (Collins et al., 2011).

In *Ipomoea staphylina* Roem. & Schult. leaf, the Iron (Fe) was found to be high. Leaves has highest iron content involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis (ethylene, gibberellic acid, jasmonic acid), Up to 80% of the cellular iron is found in the chloroplasts that is consistent with its major function in photosynthesis. In human body iron is an

essential component of red blood cells (RBC), important components for some proteins, enzymes and also acts as enzyme cofactor, normal function of heamoglobin and myoglobin, DNA synthesis, eletron transport, one of the component of catalyse, xanthine oxidase amd glutathione peroxidase (McDermid et al., 2012).

In *Ipomoea staphylina* Roem. & Schult. leaf, manganese (Mn) was found to be in normal condition. in humans manganese work as mettaloenzymes in the activation of enzyme-substrate reaction, also present in bone, cartilages, connective tissue synthesis,proper functioning of tyroid and sex hormone, regulation of blood sugar level, proper functioning urea cycle, carbohydrate, fat metabolism, amino acid metabolism, blood clotting mechanism and also has antioxidant properties (Aschner et al., 2002).

In *Ipomoea staphylina* Roem. & Schult. leaf, Zinc (Zn) was found to be normal condition. In human body zinc play vital role in proper function of immune system by activation T lymphocytes, activation of atleast 100 enzymes, proper neurophysiological function (Hambidge, 2000).

Conclusion

Currently, the suppression of radical scavegers, suppression of cancer cells is the greatest challenge. As such, new sources of anticancer and antioxidant agents are needed to be discovered and it has become a worldwide challenge. Many scientists from academic institutions and pharmaceutical companies have made an effort to find and discover novel, safe and effective biologically active compound. One of the approaches is by testing the compound derived from the plant origin. Plants are found to be an enormous source for variety of bioactive compounds with diverse molecular structure and function. These molecules are primarily derived from the secondary metabolism of plants and were used to protect it against predation by microorganisms, insects and herbivorous. The use of plant as traditional medicine has been discovered for thousands of years and was passed down from generation to generation all around the world. Nowadays, physicians have been prescribing many drugs that are either directly isolated from plant or are artificially modified version of natural product.

After the present investigation, it can be concluded that leaf ethanolic extract of *Ipomoea staphylina* Roem. & Schult. Showed appreciable antioxidant and moderate anticancer activity due to the presence of secondary metabolites in the plants. It is proved that as the concentration of secondary metabolites increases, the bioactivity will also increase. GC-MS analysis of ethanolic extract revealed the presence of 79 compounds in that 55 compounds were known for its medicinal properties, most of them were Antimicrobial agents followed by food additive and flavoring agents, Antioxidant, Anticancer agents, Anti-hypercholesterolemic compounds, Anti-inflammatory agents, Hepatoprotective agents, Antiviral agents, analgesic, Allergenic, Anesthetic, Antimutagenic, Antispasmodic, Choleretic, Dermatitigenic, Fungicide, Herbicide, Laxative, Pesticide, Lipoxygenase-Inhibitor, Pesticide, Tyrosinase Inhibitor, Vermifuge etc.

Ethanolic extracts is very powerful due to the high efficiency attributed to its intermediate polarity leading to the extraction of polar and non-polar compounds.

Elemental composition of the plant is tested gives positive results for macro as well as micro nutrients in that plant is rich with iron which is essential nutrient for human being.

The overall study on cytotoxic, antioxidant and elemental composition reports that the plant species contains many active compounds which by their synergistic effect is effective in scavenging reactive oxygen species and moderately inhibits the growth of cancer cells. So, It is finally concluded that leaf ethanolic extract of *Ipomoea staphylina* Roem. & Schult. can be explored for potential antimicrobial, antioxidant and anticancerous compounds with rich full of nutrients.

Aknowledgement

The authors thankful to Department of Applied Botany, Kuvempu University and Department of Botany, Tumkur University Karnataka for providing facilities to conduct our experimental work.

Conflict of Interest: None

Author's Contribution

Mrs Pamashree MS and Mr Ashwathanarayana R, has collected the data, conducted the experiment and drafted the article. Dr. Raja Naika, Professor and Dr Roopa B has supervised the experiment reviewed the article.

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