Research article

Antibacterial properties of *Ipomoea staphylina* Roem & Schult. plant extracts with comparing its preliminary qualitative phytochemical and quantitative GC-MS analysis

Padmashree M. S.¹, Roopa B.¹, Ashwathanarayana R.^{2*} and Raja Naika²

¹Department PG Studies and Research in Botany, Tumkur University, Tumkur-572103, Karnataka, India

²Department PG Studies and Research in Applied Botany, Jnanasahyadri, Kuvempu University, Shankaraghatta, Shimoga-577451, Karnataka, India

*Corresponding Author: ashwinjamadagni497@gmail.com

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Abstract: *Ipomoea staphylina* plant ethanolic extract was subjected to preliminary qualitative phytochemical and quantitative GC-MS analysis with analyzing its antibacterial activity by the standard method. The preliminary qualitative phytochemical analysis is done by standard procedure and quantitative GCMS analysis is done by subjecting it to GC Model: Thermo Trace GC Ultra model instrument. Antibacterial activity is done by the standard agar well diffusion method. Preliminary qualitative phytochemical analysis for pet ether and chloroform extract confirms for fewer phytochemicals but ethanolic extract confirms for the presence of alkaloids, saponins, flavonoids, steroids, glycosides, phenols and sterols. GC-MS analysis of *I. staphylina* 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. The antibacterial experiment revealed that *I. staphylina* plant ethanolic extract has appreciable antibacterial activity in all tested concentrations against selected bacterial pathogens but comparably less with the standard ciprofloxacin used but pet ether and chloroform extract showed negligible antibacterial activity. *I. staphylina* plant could be exploited as a valuable source of antibacterial agent enriching with known antibacterial compounds.

Keywords: *Ipomoea staphylina* - Phytochemical analysis - Antibacterial activity - ciprofloxacin.

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INTRODUCTION

Ipomoea staphylina Roem & Schult. is a climber plant grows near water resources and distributed throughout India, China, and Sri Lanka Deciduous forests. Leaves were stout stragglers. 15 × 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 0.5–1.0 cm; bracts minute; outer sepals 5 × 4 mm, oblong, obtuse, inner obovate with hyaline margins, 6 × 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, subtrigonal, hairy at top. Fruiting is from January–April. (Gamble: *Ipomoea staphylina* Vol- II, 1883; Fig. 1)

PLANT DESCRIPTION

Ipomoea staphylina Roem. & Schult, Syst. Veg. 4: 249 1819.

Ipomoea dichroa Choisy, Prodr. 9: 364 1845.

Common names: Clustered Morning Glory, Kannada: Ugina kodi, Unang kodi, Sunang kodi, Tamil: Onaankodi, Onan Kodi.

Taxonomic Hierarchy

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Asteranae
Order	Solanales
Family	Convolvulaceae
Genus	<i>Ipomoea</i>
Species	<i>Ipomoea staphylina</i> Roem. & Schult

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, Preliminary phytochemical analysis of plant extracts.

Traditional Uses

The plant *I. 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 (Kirtikar & Basu 1999, Sarvalingam *et al.* 2011), anti-diarrheal, gastro protective properties (Suresh *et al.* 2011).

In Tamil Nadu Kanikkars tribal people of Tirunelveli District, used *I. 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 an antidote for snake-bite (Balasubramanian *et al.* 1997, Arinathan *et al.* 2007, Sarvalingam *et al.* 2014), root tubers were rich with starch were eaten in raw (Mohan & Kalidass 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 & Pullaiah 1999).

I. staphylina is used as purgative, dyspepsia, anthelmintic, bronchitis (Savitramma *et al.* 2015) and the *I. staphylina* is used for respiratory disorders (Reddy *et al.* 2013). A literature review reveals anti-inflammatory

activity (Firdous & Koneri 2012a,b), 5-lipoxygenase, α -glucosidase and α -amylase inhibitory activity of *I. staphylina*. Analgesic activity of water and the methanolic extract of *I. staphylina* was also reported against acetic acid-induced writhing test (Kumar *et al.* 2013). Some report proved that *I. staphylina* has anti-ulcer properties (Banerjee & Firdous 2015), Antidiabetic properties (Firdous & Singh 2016), Hepatoprotective and nephroprotective properties (Bag & Mumtaz 2013).

Scientific work done

It has been reported as a analgesic (Nagariya *et al.* 2010, Kumar *et al.* 2013), anti-inflammatory (Kirtikar & Basu 1999, Sarvalingam *et al.* 2011); Firdous & Koneri 2012a,b), anti-diarrheal, gastroprotective effect (Suresh *et al.* 2011), anti-ulcer properties (Banerjee & Firdous 2015), Antidiabetic properties (Firdous & Singh 2016), α -amylase inhibitory activity (Kumar *et al.* 2013), antioxidant (Firdous & Singh 2016), Hepatoprotective and nephroprotective activity (Bag & Mumtaz 2013). Bioactive chemical constituents like Sitosteryl-3-O- β -D-glucoside and chiro deoxy inositol were reported from the leaves of *I. staphylina* (Reddy *et al.* 2013).

The aim of the research topic is to evaluate the antibacterial properties along with the confirmation of phytoconstituents present in it by preliminary qualitative phytochemical and quantitative GC-MS analysis.

MATERIALS AND METHODS

Plant collection and authentication

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

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 & Ayuso 1998). The crude extracts were stored at 4 °C in air-tight plastic containers.

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 (Harborne 1998, Ajaiyeoba 2000). 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⁻¹. The column used is HP-5MS UI (cross-linked 5% methyl phenyl Silox) capillary column (30 m × 0.25 mm) and the film thickness is 0.25 µm. The oven temperature was increased from 50–200 °C at a rate of 10°C min⁻¹. Then, continued 200–300 °C at rate 30 °C min⁻¹. 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⁻¹. Injector and detector temperatures were 250°C. GC-MS was done by injecting 1 µL of sample (0.1 % in absolute methanol).

Antibacterial activity

i. Microorganisms used: Pathogenic bacterial strains like *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604), *Klebsiella pneumonia* (MTCC-7028), *Escherichia coli* (MTCC 1559), *Salmonella typhi* (MTCC-734), *Pseudomonas aeruginosa* (MTCC-1934), *Staphylococcus aureus* (MTCC-902) obtained from the Institution of Microbial Technology (IMTECH), Chandigarh, India were used.

ii. Medium used and composition: Nutrient agar (NA) media used for the culturing of experimental bacterial pathogens. All the ingredients used in the preparation of the nutrient agar medium is of analytical grade.

Composition of nutrient agar media-

Beef extract	3.00 gm
Peptone	5.00 gm
Sodium Chloride (NaCl)	5.00 gm
Agar	15.00 gm
Distilled water	1000 ml
pH	7.4

iii. Preparation of media: Nutrient agar was prepared by adding 3 gm. of beef extract, 5gm of sodium chloride, 15 gm agar was dissolved in 1000 ml of distilled water, pH of the solution was adjusted to 7.4 and then sterilized for 15 min at 15 lbs. pressure in an autoclave.

iv. Preparation of subcultures: One day prior to the experiment, the microorganisms were inoculated into the sterilized tubes containing nutrient broth and incubated at 35°C for 24 hrs.

v. Sterilization of media and glassware: The media used in the present study is nutrient agar and nutrient broth, were sterilized in the conical flask of the suitable capacity of autoclaving at 15 lbs.

vi. Standard drug: Antibacterial drug Ciprofloxacin (1 mg ml⁻¹ of sterile distilled water) were used as a standard antibiotic to compare with the plant crude extracts.

vii. Preparation of bacterial cultures: The test pathogenic bacteria were aseptically inoculated in sterile test tubes using nutrient broth and incubated at 37°C for 24 hours. The plant crude extracts were diluted with 10% DMSO make it to desired concentrations of 12.5, 25, 50 and 100 mg ml⁻¹ respectively. The drug Ciprofloxacin was used as a standard antibiotic (1 mg ml⁻¹ of sterile distilled water) to compare with the plant crude extracts. Nutrient agar plates were uniformly swabbed with respected bacterial strains cultured in nutrient broth. 0.6 cm diameter wells were punched in the inoculated plates using a sterile cork borer. 100 µl of different concentrations of crude extracts and standard (Ciprofloxacin, 1 mg ml⁻¹ of sterile distilled water) and DMSO (10%) were filled into the respectively labeled wells and incubate for 24 hours at 37°C.

RESULTS*Extracts yield of Ipomoea staphylina leaf*

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

Soxhlet extraction of *Ipomoea staphylina* 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%). The result shows that the highest yield is obtained in ethanol followed by chloroform and least is petroleum ether (Table 1; Fig. 3).

Figure 3. Yield of crude extracts in percentage.

Preliminary qualitative phytochemical analysis of Ipomoea staphylina leaf extracts

The preliminary phytochemical analysis of *Ipomoea staphylina* leaf extracts were analyzed results showed that, the analysis of petroleum ether extract reveals the presence of saponins, 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).

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

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

Note: -, Negative results; +, Positive results.

Quantitative GC-MS analysis of Ipomoea staphylina leaf extracts

Due to the less extract yield and less secondary metabolites present in petroleum ether and chloroform extracts, we took only leaf ethanolic extract of *Ipomoea staphylina* for Gas chromatography mass spectroscopy (GC-MS) analysis.

GC-MS analysis of *I. staphylina* 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, 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, Choleric, Dermatitogenic, Fungicide, Herbicide, Laxative, Pesticide, Lipoxigenase-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%) (Table 3; Figs. 4–5).

Table 3. GC-MS analysis of *Ipomoea staphylina* Roem & Schult. ethanolic extract. [%- Percentage in the crude extract]

S.N.	%	Chemical name	Properties	References
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 <i>et al.</i> (2000), Yanagimoto <i>et al.</i> (2002), PubChem- Furfuryl alcohol/C ₅ H ₆ O ₂
2	0.4	Propane, 1-(1-methylethoxy)-	Inhibitors of Hepatitis C Virus, used in the treatment of mental disorders.	PubChem- Propane, 1-(1-methylethoxy)/C ₆ H ₁₄ O
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.	PubChem- Dihydroxyacetone/C ₃ H ₆ O ₃
4	0.06	Butyrolactone	cdc2 and cdk2 kinases inhibitor, anti-cancer activity, antimicrobial, antidepressant, Flavoring Agents.	Giarman & Schmedit (1963), Nishio <i>et al.</i> (1996), Kitagawa <i>et al.</i> (1994), PubChem- Butyrolactone/C ₄ H ₆ O ₂
5	0.23	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	Food-grade flavor ingredients.	PubChem- 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furanone/C ₆ H ₈ O ₄
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 <i>et al.</i> (1989), PubChem- Diglycerin/C ₆ H ₁₄ O ₅
7	0.1	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-	Unknown	
8	0.06	Pentanoic acid, 4-oxo-	Hepatoprotective, Flavoring Agents.	PubChem- Levulinic acid/C ₅ H ₈ O ₃
9	0.86	Cyclopentane, 1-acetyl-1,2-epoxy-	Anti-inflammatory, antiviral and bronchodilatory properties.	Awakan <i>et al.</i> (2018)
10	0.23	Tetrahydro-4H-pyran-4-ol	Hepatitis C virus inhibitors, treatment of respiratory and urinary track disorders.	PubChem- Oxan-4-ol/C ₅ H ₁₀ O ₂
11	0.08	Uracil, 1-methyl-	Antiviral Compounds.	PubChem- 1-Methyluracil/C ₅ H ₆ N ₂ O ₂
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 <i>et al.</i> (1997), Kumar <i>et al.</i> (2010), Yu <i>et al.</i> (2013)
14	0.21	1,2-Benzenediol	Antibacterial, Flavoring Agents, Antioxidants, moderately toxic, treatment of respiratory and urinary track disorders.	Xu <i>et al.</i> (2003), PubChem- Pyrocatechol/C ₆ H ₆ O ₂
15	0.21	2-[2-(5-Norbornenyl)oxy]-tetrahydropyran	Unknown	

16	2.02	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	Antimicrobial, Preservative.	Gopalakrishnan <i>et al.</i> (2011)
17	0.97	1,2,3-Propanetriol, monoacetate	Food Additives	PubChem- 1,2,3-Propanetriol, monoacetate/ C ₅ H ₁₀ O ₄
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 & Jeong (2010), Silici <i>et al.</i> (2005), Jeong <i>et al.</i> (2011), Fukai <i>et al.</i> (2009), Pherobase.com- 2-methoxy-4-vinylphenol; PubChem- 2-Methoxy-4-vinylphenol/ C ₉ H ₁₀ O ₂
21	0.08	3,4-Altrosan	Bacteriostat, Fungicide.	Jadhav <i>et al.</i> (2014), Ghelardini <i>et al.</i> (2001), Tambe <i>et al.</i> (1996), Sabulala <i>et al.</i> (2006), Yang <i>et al.</i> (2000), Huang <i>et al.</i> (2012), Park <i>et al.</i> (2011), Calleja <i>et al.</i> (2013), Dahham <i>et al.</i> (2015), Kumar <i>et al.</i> (2010), PubChem – Caryophyllene/C ₁₅ H ₂₄
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.	Leal <i>et al.</i> (1988), PubChem- 6-Methylsalicylaldehyde / C ₈ H ₈ O ₂
23	0.58	Benzaldehyde, 2-hydroxy-6-methyl-	Pheromone of the Acarid Mite Tyrophagus perniciosus, Collohmanna 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.	
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.	PubChem – Sucrose / C ₁₂ H ₂₂ O ₁₁
25	0.36	alpha.-Caryophyllene	Essential oil present in Humulus lupulus, anti-inflammatory, Antitumor activity, analgesic, anti-inflammatory, antiseptic, immunostimulant, perfumes.	Fernandes <i>et al.</i> (2007), Legault <i>et al.</i> (2003), Legault & Pichette (2007), PubChem- Humulene / C ₁₅ H ₂₄
26	0.47	DL-Arabinitol	Indicator of liver cirrhosis, gastrointestinal candidiasis etc in serum and urine.	Wong <i>et al.</i> (1990)
27	10.41	Dodecanoic acid, 3-hydroxy-	In the treatment of Fatty Acid Oxidation disorder, intermediate of liver fatty acid metabolism.	Jones <i>et al.</i> (2000)

28	0.37	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	Antimicrobial, Antioxidant, Antiinflammatory, Analgesic.	Gopalakrishnan <i>et al.</i> (2011)
29	0.17	Cyclohexanol, 1R-4-trans-acetamido-2,3-trans-epoxy-	Camphorlike odor and are used in making soaps, insecticides, germicides, dry cleaning, and plasticizers.	Cyclohexanols / CTD
30	1.2	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Treatment in asthma, antimicrobial, cancer preventive, Anti-inflammatory.	Ogunlesi <i>et al.</i> (2009), Yu <i>et al.</i> (2013), Ponnamma & Manjunath (2012), Srinivasan <i>et al.</i> (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 <i>et al.</i> (2000), PubChem- Oleic acid/ $C_{18}H_{34}O_2$
34	5.53	Pentadecanoic acid	Adhesives and sealant chemicals, Agricultural chemicals (non-pesticidal), Finishing agents, Lubricants and lubricant additives, Surface active agents.	PubChem- Pentadecanoic acid/ $C_{15}H_{30}O_2$
35	0.15	Hexadecanoic acid, ethyl ester	Antioxidant, lubricant, hypocholesterolemic nematocide, pesticide, anti-androgenic, flavoring agent, hemolytic, 5-Alpha reductase inhibitor.	Kumar <i>et al.</i> (2010), Maruthupandian & Mohan (2011), Dr. Duke's Phytochemical and Ethnobotanical Databases
36	0.12	d-Mannose	Protein quality control in human body.	PubChem- D-Mannose/ $C_6H_{12}O_6$
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.	PubChem- Oleyl alcohol/ $C_{18}H_{36}O$
38	0.12	1-Heptadecanol	Flavoring Agents, Insect sex pheromone, antiacne agents, antibiotic.	Butler & McDonough (1981), Kubo <i>et al.</i> (1994), PubChem- 1-Heptadecanol/ $C_{17}H_{36}O$
39	0.92	Phytol	Antimicrobial, Anti-inflammatory.	Srinivasan <i>et al.</i> (2014)
40	2.3	9,12-Octadecadienoic acid (Z,Z)-	Anticorony, Antialopepic, Antiarteriosclerotic, Antiarthritic, antianaphylactic, Antieczemic, Cancer preventive, antiprostatic, hepatoprotective, Hypocholesterolemic, Metastatic, Nematocide, Insectifuge, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticorony,	Ponnamma & Manjunath (2012), Maruthupandian & Mohan (2011), Kalaivani <i>et al.</i> (2012)

41	3.54	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Anti-inflammatory, Hypocholesterolemic, Antihistaminic.	Srinivasan <i>et al.</i> (2014)
42	1.21	Octadecanoic acid	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Propepic, Suppository.	Ponnamma & Manjunath (2012)
43	8.56	9-Octadecen-1-ol, (E)-	pheromonal component from the sting of the honey bee.	Pickett <i>et al.</i> (1982)
44	0.72	1-Nonadecanol	Unknown	
45	0.68	12-Chlorobicyclo[8.2.0]dodecan-11-one	Unknown	
46	0.15	Z,E-2,13-Octadecadien-1-ol	Insect sex pheromone component.	Schwarz <i>et al.</i> (1983)
47	9.52	9-Hexadecen-1-ol, (Z)-	Cosmetics, anti-hair fall agent.	PubChem-9-Hexadecen-1-ol/ C ₁₆ H ₃₂ O
48	0.44	1-Tetracosanol	Unknown	
49	2.85	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Unknown	
50	0.14	Ethanol, 2-(9-octadecenyl)-, (Z)-	Unknown	
51	0.25	cis-9-Hexadecenal	Insect pheromone.	Berg <i>et al.</i> (2005)
52	1.63	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	hypocholesterolemic, antieczemic, Nematicide, hepatoprotective.	Gnanavel & Saral (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.	PubChem- Glyceryl monostearate/ C ₂₁ H ₄₂ O ₄
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 & Manjunath (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.	Wikipedia - 1-Hexacosanol
59	0.16	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, 3,3,7,11-	Unknown	
60	0.06	Tetramethyltricyclo[5.4.0.0(4,11)]undecan-1-ol	Fungistatic, Ginsenol is found in tea. Ginsenol is isolated from ginseng plant rootlets.	Aleu <i>et al.</i> (2001), PubChem- Ginsenol/ C ₁₅ H ₂₆ O
61	0.34	gamma.-Tocopherol	Anticancer, Antioxidant, Antitumor, Antiinflammatory, Hypocholesterolemic, Cardioprotective.	Ponnamma & Manjunath (2012)
62	0.28	1-Triacontanol	Plant growth stimulator.	Jones <i>et al.</i> (1979), Khandaker <i>et al.</i> (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 & Manjunath (2012), Srinivasan <i>et al.</i> (2014), Kumar <i>et al.</i> (2010)

64	0.15	beta.-Sitosterol	Anti-hypercholesterolemia, Reduces blood levels of cholesterol, antioxidant activity, anticancer.	Kalaivani <i>et al.</i> (2012)
65	1.03	Campesterol	Antioxidant, Hypocholesterolemic.	Ponnamma & Manjunath (2012)
66	1.77	Stigmasterol	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemic.	Ponnamma & Manjunath (2012)
67	0.69	4-Acetoxy cinnamic acid	Plant growth inhibitor.	Hiradate <i>et al.</i> (1999)
68	0.1	Squalene	Antibacterial, Antioxidant, Immunostimulant.	Srinivasan <i>et al.</i> (2014)
69	3.77	gamma.-Sitosterol	used to treat Hyperlipidemias, Antioxidant, antibacterial and prophylactic activities.	Venkata <i>et al.</i> (2012), Akpuaka <i>et al.</i> (2013), Pubchem- gamma-Sitosterol/ C ₂₉ H ₅₀ O
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, Lipoxigenase inhibitor, Pesticide.	Maruthupandian & Mohan (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; Choleric; Dermatitogenic, Fungicide, Herbicide, Laxative, Pesticide, Lipoxigenase-Inhibitor, Pesticide, Tyrosinase Inhibitor, Vermifuge.	Ponnamma & Manjunath (2012), Kumar <i>et al.</i> (2010)
78	2.46	D:A-Friedooleanan-3-ol, (3.alpha.)-	Unknown	
79	0.53	Friedelan-3-one	Unknown	

Antibacterial activity of Ipomoea staphylina leaf extracts

Antibacterial activity of *Ipomoea staphylina* leaf extracts were measured in millimeters and expressed in Mean $\pm$ standard error of mean (SEM) and the experiment is triplicated to know the exact concentration value. Petroleum ether extract of *I. staphylina* leaf showed nil antibacterial activity in all tested concentrations along with the control the standard Ciprofloxacin showed excellent antibacterial activity against all the tested bacterial pathogens. For accurate values the experiment is triplicated and the values were expressed in terms of Mean $\pm$ standard error of mean (SEM). The values obtained from the petroleum ether extract were shown in the table 4.

Chloroform extract of *I. staphylina* leaf showed nil antibacterial activity in all the tested concentrations. The control showed nil activity, the standard Ciprofloxacin showed excellent antibacterial activity against all the tested bacterial pathogens. For accurate values the experiment is triplicated and the values were expressed in terms of Mean $\pm$ standard error of mean (SEM). The values obtained from the antibacterial activity of *I. staphylina* leaf chloroform extract which is showed in the table 5.

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
 Report File :
 Tuning File : C:\GCMSsolution\TUNE\2018\FEB\26.02.18c.qgt
 Modified by : Admin
 Modified : 2/28/2018 10:13:05 AM

Peak#	R.Time	I.Time	F.Time	Area	Area%	Peak Report TIC
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 (i).

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-octadecenyl)-, (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. GC-MS analysis of *Ipomoea staphylina* Roem & Schult., leaf ethanolic extract (ii).Table 4. Antibacterial activity of *Ipomoea staphylina* Roem & Schult. leaf petroleum ether extract.

Petroleum ether extract	Zone of Inhibition in mm (Mean±SEM)					Standard
	12.50 mg ml ⁻¹	25 mg ml ⁻¹	50 mg ml ⁻¹	100 mg ml ⁻¹	Control	
Test Organisms						
<i>Xc</i>	0±0	0±0	0±0	0±0	0±0	30.66±0.66
<i>Ps</i>	0±0	0±0	0±0	0±0	0±0	28.66±0.66
<i>Kp</i>	0±0	0±0	0±0	0±0	0±0	30.33±0.33
<i>Ec</i>	0±0	0±0	0±0	0±0	0±0	32±0.57
<i>St</i>	0±0	0±0	0±0	0±0	0±0	32.33±0.33
<i>Pa</i>	0±0	0±0	0±0	0±0	0±0	31±0.57
<i>Sa</i>	0±0	0±0	0±0	0±0	0±0	33.66±0.33

Note: *Xc*- *Xanthomonas campestris*, *Ps*- *Pseudomonas syringae*, *Kp*- *Klebsiella pneumoniae*, *Ec*- *Escherichia coli*, *St*- *Salmonella typhi*, *Ps*- *Pseudomonas aeruginosa*, *Sa*- *Staphylococcus aureus*.

Table 5. Antibacterial activity of *Ipomoea staphylina* Roem & Schult. leaf chloroform extract.

Chloroform extract	Zone of Inhibition in mm (Mean±SEM)					
Test Organisms	12.50 mg ml ⁻¹	25 mg ml ⁻¹	50 mg ml ⁻¹	100 mg ml ⁻¹	Control	Standard
<i>Xc</i>	0±0	0±0	0±0	0±0	0±0	30.66±0.66
<i>Ps</i>	0±0	0±0	0±0	0±0	0±0	28.66±0.66
<i>Kp</i>	0±0	0±0	0±0	0±0	0±0	30.33±0.33
<i>Ec</i>	0±0	0±0	0±0	0±0	0±0	32±0.57
<i>St</i>	0±0	0±0	0±0	0±0	0±0	32.33±0.33
<i>Pa</i>	0±0	0±0	0±0	0±0	0±0	31±0.57
<i>Sa</i>	0±0	0±0	0±0	0±0	0±0	33.66±0.33

Note: *Xc*- *Xanthomonas campestris*, *Ps*- *Pseudomonas syringae*, *Kp*- *Klebsiella pneumoniae*, *Ec*- *Escherichia coli*, *St*- *Salmonella typhi*, *Pa*- *Pseudomonas aeruginosa*, *Sa*- *Staphylococcus aureus*.

Table 6. Antibacterial activity of *Ipomoea staphylina* Roem & Schult. ethanolic extract.

Ethanolic extract	Zone of Inhibition in mm (Mean±SEM)					
Test Organisms	12.50 mg ml ⁻¹	25 mg ml ⁻¹	50 mg ml ⁻¹	100 mg ml ⁻¹	Control	Standard
<i>Xc</i>	9.66±0.88	14±1.15	14.66±0.88	21.33±0.88	0±0	30.66±0.66
<i>Ps</i>	9±1.15	12.33±1.45	15.33±1.45	17±0.57	0±0	28.66±0.66
<i>Kp</i>	9.33±1.45	10.33±0.88	13.66±0.88	20.33±1.2	0±0	30.33±0.33
<i>Ec</i>	0±0	11.33±1.2	12±1.15	16±0.57	0±0	32±0.57
<i>St</i>	10.33±1.45	17.33±0.88	19.33±1.45	21±1.54	0±0	32.33±0.33
<i>Pa</i>	9.33±0.88	19.33±0.88	21.33±0.88	23.33±0.66	0±0	31±0.57
<i>Sa</i>	10.33±0.88	16.66±0.88	21±0.57	22±1.15	0±0	33.66±0.33

Note: *Xc*- *Xanthomonas campestris*, *Ps*- *Pseudomonas syringae*, *Kp*- *Klebsiella pneumoniae*, *Ec*- *Escherichia coli*, *St*- *Salmonella typhi*, *Pa*- *Pseudomonas aeruginosa*, *Sa*- *Staphylococcus aureus*.

Ethanolic extract of *I. staphylina* leaf showed appreciable antibacterial activity in all tested concentrations (12.50, 25, 50, 100 mg ml⁻¹) which is showed in the table 4.4.3. In 100 mg ml⁻¹ concentration maximum zone of inhibition showed by *Pseudomonas aeruginosa* (23.33±0.66) followed by *Staphylococcus aureus* (22±1.15), *Xanthomonas campestris* (21.33±0.88), *Salmonella typhi* (21±1.54), *Klebsiella pneumoniae* (20.33±1.2), *Pseudomonas syringae* (17±0.57) and least was *Escherichia coli* (16±0.57) (Table 6; Figs. 6 & 8). The control DMSO shows nil zone and standard ciprofloxacin showed maximum zone of inhibition for all the pathogenic bacteria (Table 6; Fig. 7).

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* yields more percentage of extract 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; Fig. 3).

Preliminary phytochemical analysis

The preliminary phytochemical analysis of *I. staphylina* 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 saponins, 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 *I. staphylina* 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%).

Figure 6. Antibacterial activity of *Ipomoea staphylina* Roem & Schult. ethanolic crude extract with: **A**, *Escherichia coli*; **B**, *Klebsiella pneumonia*; **C**, *Pseudomonas aeruginosa*; **D**, *Pseudomonas syringae*; **E**, *Salmonella typhi*; **F**, *Staphylococcus aureus*; **G**, *Xanthomonas campestris*.

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 was found to have anticancer properties, in that compounds 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 were in major percentage

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-.

Figure 7. Antibacterial activity of standard ciprofloxacin and control with: **A**, *Escherichia coli*; **B**, *Klebsiella pneumonia*; **C**, *Pseudomonas aeruginosa*; **D**, *Pseudomonas syringae*; **E**, *Salmonella typhi*; **F**, *Staphylococcus aureus*; **G**, *Xanthomonas campestris*.

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)-.

Fifteen 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; Figs. 4–5).

Antimicrobial properties

Petroleum ether and chloroform extracts of *I. staphylina* leaf showed negligible antibacterial activity against tested bacterial pathogen but ethanolic extract of *I. staphylina* leaf showed appreciable antibacterial activity in all tested concentrations (12.50, 25, 50, 100 mg ml⁻¹) which is due to the phytochemical dissolved in it and antibacterial compounds present in it. In 100 mg ml⁻¹ concentration maximum zone of inhibition showed by *Pseudomonas aeruginosa* followed by *Staphylococcus aureus*, *Xanthomonas campestris*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Pseudomonas syringae* and least was *Escherichia coli*. All the tested pathogens were belongs to plant and animal pathogens, some of them were opportunistic pathogens. In recent years drug resistance bacterial strains were dangerous threat to human beings and new antibacterial drug synthesis is necessary to get rid these drug resistance bacterial strains so, drug which has multiple pathogenic target and safer to consume is appreciable, in that concern plant origin compounds were safety as well as cost effective. Control DMSO shows nil zone inturn confirm the positive effect of ethanolic extract (Table 4–6; Figs. 6–8).

Figure 8. Antibacterial activity of ethanolic crude extract of *Ipomoea staphylina* Roem & Schult. leaf. [ISE - *Ipomoea staphylina* ethanolic extract]

CONCLUSION

After the present investigation, it can be concluded that leaf ethanolic extract of *Ipomoea staphylina* can act as good antibacterial agents. Because the leaf and bark of the plant tested on different organisms and some of the organisms have controlled in high concentration of plants extract, but few showed negative results, this bioactivity is 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, Choleric, Dermatogenic, Fungicide, Herbicide, Laxative, Pesticide, Lipoxigenase-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 an essential nutrient for the human being.

The overall study on antimicrobial reports that the plant species contains many active compounds which by their synergistic effect may reduce or check the growth of microbial colonies. So, it is finally concluded that leaf ethanolic extract of *Ipomoea staphylina* can be explored for potential antimicrobial compounds with rich full of phytoconstituents.

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