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GC-MS Analysis of Phyto-Components in Raw and Treated Sugarcane Juice

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A B S T R A C T

Sugarcane (*Saccharum officinarum*) is the major cash crop of the Indian tropical and sub-tropical region. Mostly, the foremost part of Indian economy is based on sugarcane crop, which is cultivated in major state of India. Phyto-components are the compound that occurred in plant naturally and play important role for biologically activity (antibacterial, anti-fungal, anti-cancer, anti-diabetic etc.), to prevent many diseases by scavenging and chelating the free radicals. Preservation of sugarcane juice was examined to reduce the spoilage and to increase the shelf life using natural preservatives. The preservation of the juice was carried out using ginger, neem, mint and black salt and stored under refrigeration temperature. In the present study, we identified the phyto-components presence in raw sugarcane juice and addition of additives such as ginger, lemon, mint and black salt of sugarcane juice through GC-MS analysis. In the raw sugarcane juice, it was identified that the major compound 5-Hydroxymethylfurfural (39.56%) with retention time 12.99 min and the minor compound was Isopropyl linoleate (0.88%) with 30.80 retention time. When compared to raw sugarcane juice, treated sugarcane juice has 9, 12, 15- Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z) (6.29%), 8H-Pyrano[3,4-b]pyrimido[5,4-d]furane, 5,6-dihydro-4-hydrazino-6,6-dimethyl-2-methylthio(6.01%), Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)(1.43%), with retention time 36.60, 22.58 and 23.48 mins respectively. Significant compounds such as 1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl- (0.16%) and Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)- (0.17%) were also identified. the identified compounds having biological and pharmacological activity such as antimicrobial, antifungal, anticancer, antioxidant, antimutagenic and hypercholesterolemic properties. Hence the developed hurdle technology can be adopted to extend the shelf life of sugarcane juice.

Introduction

Sugarcane (*Saccharum officinarum*) is an important industrial crop cultivated in tropical and subtropical regions of the world. India is the world second largest producer of sugarcane next to Brazil. Sugarcane has been used as a sweetener for millennia and today refined sugar is used in copious quantities to supplement the natural sugar (fructose) found in fruits and vegetables (Phanikumar, 2011). Sugarcane juice is commonly used as a delicious drink in both urban and rural areas. A part of sugarcane juice consumed as expensive and pleasing beverages in India. It possesses therapeutic value (Banerji *et al.*, 1997).

In general sugarcane juice is spoiled quickly by the presence of simple sugars. The sugarcane juice can be introduced as delicious beverages by preventing the spoilage of juice with appropriate method. Biodegradation is caused by microorganisms mainly *Leuconostoc* sp. (*L. mesenteroides* and *L. dextranium*) also takes place (Krishnakumar and Devadas, 2006).

Many commercial juices are filtered to remove fiber or pulp, but high-pulp fresh orange juice is a popular beverage. Common methods for preservation and processing of fruit juices include canning, pasteurization, freezing, evaporation, drying and addition of preservatives (Ashish Khare *et al.,* 2012).

According to research reports, phyto-compounds are proven to have important biological and medicinal properties that may make sugarcane a valuable functional food plant (Iacopini *et al.,* 2008). Additionally, the use of *S. officinarum* in traditional medicine in Nigeria and some parts of Asia especially India for the treatment of diseases such as jaundice and liver-related disorders, dyspepsia, haemorrhoids, menorrhagia, dysentery, agalactia, phthisis and general debility (Kadam *et al.,* 2008; Suresh-Kumar *et al.,* 2010) suggest inherent medicinal phytochemicals.

The role of phyto-components in protecting tissues and cells against destructive effects of free radical has been greatly studied. The market in India for antioxidant rich supplements, fortified drinks and snacks has now advanced well into the mainstream, with products like green tea, antioxidant enriched drinks, health bars, powder drink mixes, etc. The by-product of sugarcane industry, blackstrap molasses, has been recognized for its therapeutic properties. Considering, these aspects it becomes worthwhile to have a deeper insight for antioxidant properties of sugarcane (Manish *et al.,* 2015).

Gas Chromatography (GC) and mass spectrometry (MS) provides a powerful tool for identifying the various compound presences in the sample. GC separate mixture in to individual components and the MS detects components or molecules on the basis of their charged ion and mass to charge ratio. The objective of the present study was to GC-MS analysis of juice sample from the selected plant for the identification of phyto-components and their relation to biological and pharmacological activity.

Therefore, the study evaluated the phyto-components of raw sugarcane juice and treated sugarcane juice *as* well as its antimicrobial properties. Data generated from such studies as this, will contribute to the phyto-components database useful in the assessment of antimicrobial properties, a major prerequisite for solving the problem of food spoilage in developing countries including India. The study will also serve to provide some baseline data necessary for further investigation into the functional properties of sugarcane juice.

Materials and Methods

Collection of plant material

Mature stems of sugarcane were cut close to the ground at a plantation in Thanjavur during the period of July, 2015. Upon arrival at the laboratory, the stems were cleaned, hand-peeled and cut into three portions with equal length (about 50 cm) and used for the experiment.

Preparation and formulation of sugarcane juice

Sugarcane juice was extracted by power operated sugarcane crusher machine. The collected sugarcane juice was filtered through the double sieve and muslin cloth to remove

the extraneous matter. The sugarcane juice was brought to the laboratory for further processing. The process flow chart for sugarcane juice recovery is given in Figure 2. With 100 ml of sugarcane juice, 0.6 ml the ginger extract, 1.5 lemon extract, 0.5 ml of mint extract and 1 gm of black salt were added. Without these additives raw sugarcane juice (RSJ) act as control and addition of additives was designated as treated sugarcane juice (TSJ) respectively.

GC-MS analysis of the sample

The chemical composition of sugarcane juice with and without treatment was investigated through Gas Chromatography Mass Spectrometry with Electron Ionization (GC-MS/EI) mode. Around 50 ml sugarcane juice was soaked in 1:2 ratio of hexane and incubated at shaking incubator overnight at room temperature and then filtered through blotting paper. The filtrate is then concentrated through nitrogen gas flushing up to 1 ml through Sample Concentrator. The concentrate was again filtered in the Whatmann No. 41 filter paper along with Sodium sulfate to remove the sediments and traces of moisture in the filtrate. This procedure insures precise derivatization time and reproducible sample injection. Immediately after extraction and filtration, 2 µl of the sample was injected into an injection port in 1:10 ratio of split mode. The mobile gas helium was set to 1ml min^{-1}.

The GC-MS/MS is a Scion 436-GC Bruker model coupled with a Triple quadruple mass spectrophotometer with fused silica capillary column BR-5MS (5% Diphenyl/ 95% Dimethyl poly siloxane) and Length : 30m; Internal diameter: 0.25mm; Thickness: 0.25µm. The column oven temperature program was as follows: 40°C hold for 2 min, Up to 160°C at the rate of 20°C/min - No hold, Up to 280°C at the rate of 5°C / min - No hold, Up to 300°C at the rate of 12°C/min - 8 min hold, Injector temperature 280°C and total GC running time was 41 min. This last increase was to clean the column from any residues. The mass spectrometer was operated in the positive electron ionization (EI) mode with ionization energy of 70eV. The solvent delay was 0-3.0 min.

A scan interval of 0.5 seconds and fragments from m/z 50 to 500 kilo Daltons was programmed. The inlet temperature was set at 280°C, source temperature 250°C. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was MS Work station 8. The NIST Version 2.0 library database of National Institute Standard and Technology (NIST) having more than 2, 42,466 patterns were used for identifying the chemical components. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

GC-MS analysis of raw sugarcane juice

GC-MS analysis of n- hexane juice extract obtained from raw sugarcane juice (*Saccharum officinarum*) revealed the presence of 14 phytochemical compounds as depicted by 14 respective peaks for each compound in GC-MS chromatogram (Table 2 and Fig. 1). Major compounds identified were 5-Hydroxymethylfurfural (39.56%) and Cyclopropyl 4-methoxyphenyl ketone (19.58%) with retention time 12.99 and 8.30 min respectively. Minor compounds such as Isopropyl linoleate (0.88%) and Pentanal, 5-(methylenecyclopropyl) (2.99%) with

retention time 30.80 and 10.99 min respectively were identified.

GC-MS analysis of treated sugarcane juice

GC-MS analysis of n- hexane juice extract obtained from treated sugarcane juice (*Saccharum officinarum*)with addition of additives revealed the presence of 19 phytochemical compounds as depicted by 19 respective peaks for each compound in GC-MS chromatogram (Table 3, Fig. 3). major compounds were identified 9, 12, 15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z) (6.29%), 8H-Pyrano[3,4-b] pyrimido [5,4-d] furane, 5,6-dihydro-4-hydrazino-6, 6-dimethyl-2-methylthio (6.01%), Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g] benzo furan-5-yl) (1.43%), with retention time 36.60, 22.58 and 23.48 respectively. Lower percentage compound were identified 1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo [3.3.1] non-9-yl)-3- (9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl- (0.16%) and Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl) oxime], (3α,5α)- (0.17%) with retention time 35.09 and 31.48 respectively.

The compounds present were of different classes such as steroids, acids, phytosterols, alkaloids, ketones, ester, etc. Among different compounds identified 9, 12, 15-Octadecatrienoic acid, Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z) 8H-Pyrano[3,4-b] pyrimido [5,4-d] furane, 5,6-dihydro-4-hydrazino-6,6-dimethyl-2-methylthio, Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyl oxireno[g]benzofuran-5-yl) were found to be present in large amount as when compared to phytocompounds of raw sugarcane juice based upon the peak areas of the compounds.

Irrespective of the amount or concentration (high or low) in which these compounds were found to be present, almost all these compounds have been reported to possess some pharmacological or the other biological activity (Table 1).

Kim *et al.*, 2010 Syringol and 4-hydroxydihydro-2(3H)-furanone are known to possess antioxidant activities. Many phytochemical compounds identified such as, Tridemorph, Pentanal, 2-methyl, 4H-Pyran-4-one, 2, 3-, dihydro-3, 5-dihydroxy-6-methyl-, 4-hydroxydihydro-2(3H)-furanone, 2-Furancarboxaldehyde, 5-(hydroxymethyl) - have been reported to be antimicrobial (antibacterial or antifungal) in nature.

Fig.1 GC-MS chromatogram of raw sugarcane juice

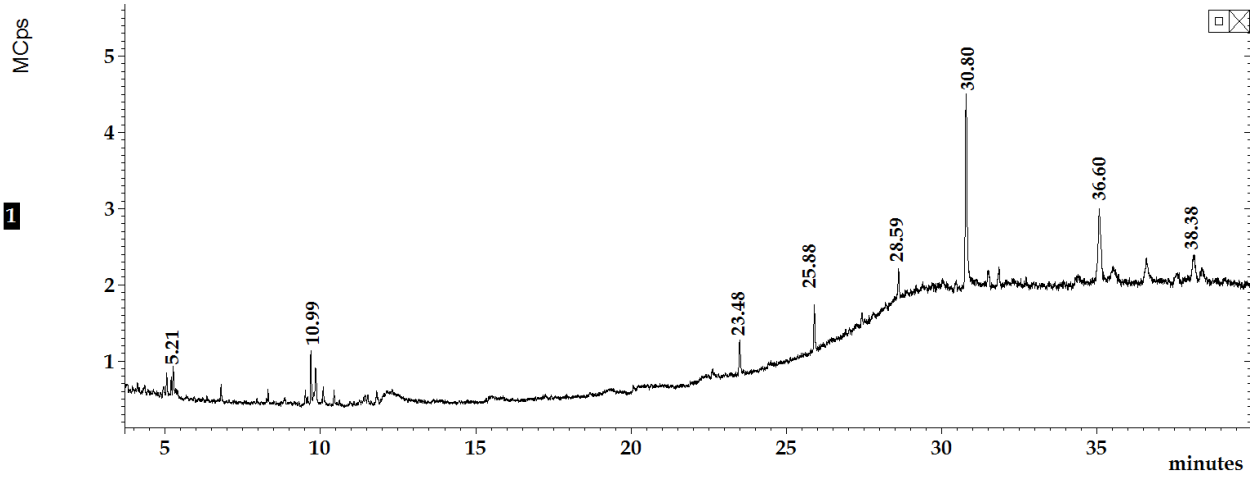


Fig.2 The process flow chart for raw sugarcane juice preparation

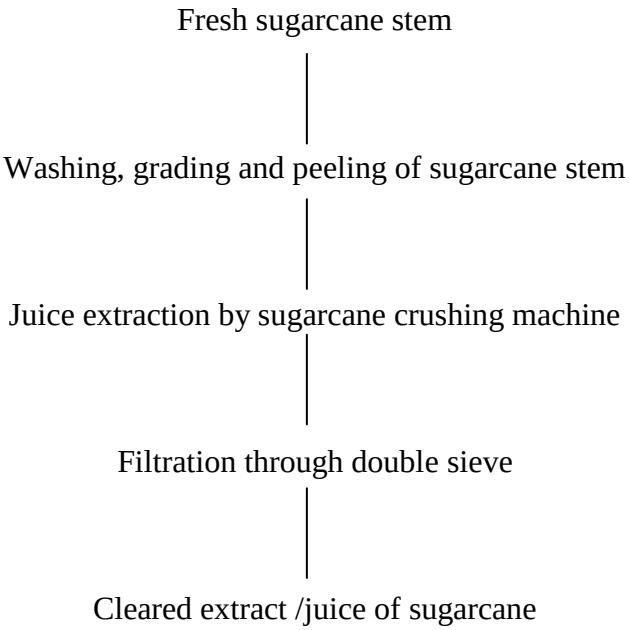


Fig.3 GC-MS Chromatogram of Treated Juice Sample

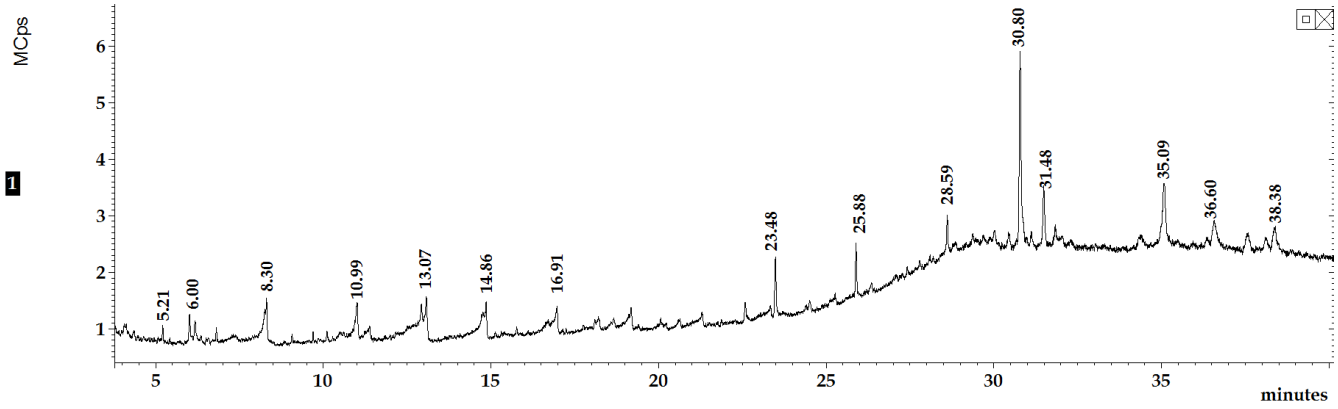
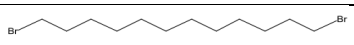
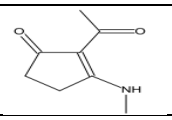
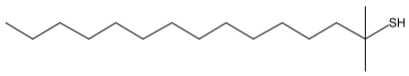
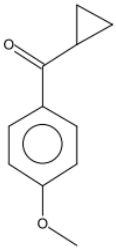
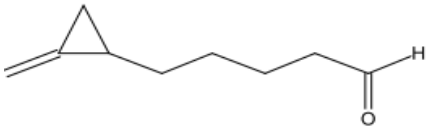
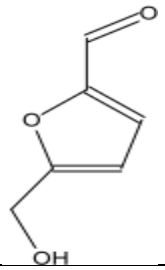
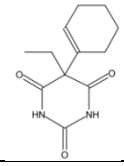
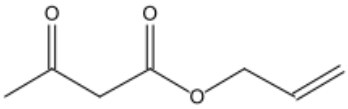
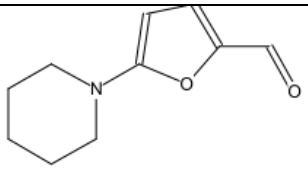
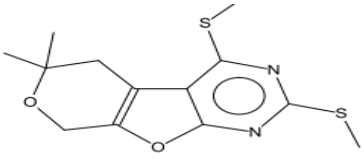
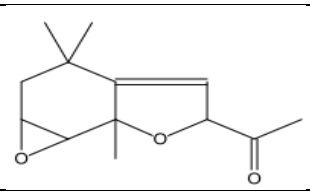
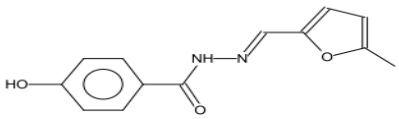
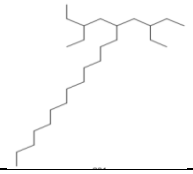
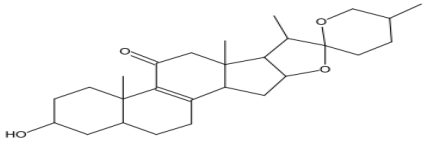
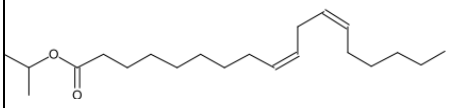
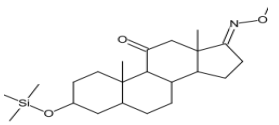
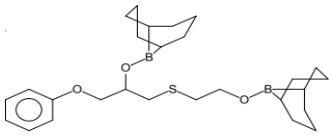
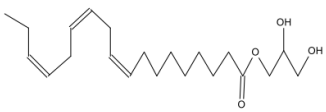
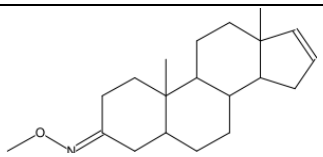


Table.1 Biological activity of identified compound in treated sugarcane juice

S.No	Compound name	Structure	Biological/Pharmacological activities*
1.	Dodecane, 1,2-dibromo-		Antimicrobial Activity
2.	2-Cyclopentenone, 2-acetyl-3-methylamino-		Anti-inflammatory

3.	tert-Hexadecanethiol		Enzyme activators
4.	Cyclopropyl 4-methoxyphenyl ketone		Antibacterial, Analgesic
5.	Pentanal, 5-(methylenecyclopropyl)-		-
6.	5-Hydroxymethylfurfural		Antioxidant Activity
7.	Cyclobarbital		Antimicrobial and Anticancerous
8.	Butanoic acid, 3-oxo-, 2-propenyl ester		Antimicrobial Activity
9.	Furan-2-carboxaldehyde, 5-(1-piperidyl)-		Antioxidant Activity
10.	8H-Pyrano[3,4-b]pyrimido[5,4-d]furane, 5,6-dihydro-4-hydrazino-6,6-dimethyl-2-methylthio-		Antitumor activity

11.	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)-		Antimicrobial Activity
12.	Furfurole, 5-methyl-, 4-hydroxybenzoylhydrazone		Nematicidal and Antimicrobial Activity
13.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-		Anticancer, antiarthritic, antiasthmatic
14.	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-		Anticancer
15.	Isopropyl linoleate		Antioxidant, Antidiabetic, Anti-inflammatory
16.	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-		Anticancer, Antitumour and Antimicrobial Activity
17.	1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-		-
18.	9,12,15-Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-		Antioxidant, Antidiabetic, Anti-inflammatory
19.	Androst-5-en-17-one, O-(phenylmethyl)oxime, (3β)-		Antitumour and Antimicrobial Activity

*Dr.Duke's Phytochemical and Ethnobotanical Databases available at http://www.ars-grin-gov/duke

Table.2 Identified compound, area and retention time of peak of raw sugarcane juice

S. No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	5.21	Dodecane, 1,2-dibromo-	$C_{12}H_{24}Br_2$	326	5.89
2.	6.80	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	4.18
3.	8.30	Cyclopropyl 4-methoxyphenyl ketone	$C_{11}H_{12}O_2$	176	19.58
4.	10.99	Pentanal, 5-(methylenecyclopropyl)-	$C_9H_{14}O$	138	2.99
5.	12.99	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	39.56
6.	14.86	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236	5.63
7.	22.58	8H-Pyrano[3,4-b]pyrimido[5,4-d]furane, 5,6-dihydro-4-hydrazino-6,6-dimethyl-2-methylthio-	$C_{12}H_{16}N_4O_2S$	280	5.55
8.	23.48	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)-	$C_{13}H_{18}O_3$	222	6.52
9.	25.88	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	3.25
10.	28.59	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	$C_{27}H_{40}O_4$	428	0.47
11.	30.80	Isopropyl linoleate	$C_{21}H_{38}O_2$	322	0.88
12.	31.48	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-	$C_{29}H_{43}NO_3Si$	481	0.05
13.	36.60	9,12,15- Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4$	496	4.56
14.	38.38	Androst-5-en-17-one, O-(phenylmethyl)oxime, (3β)-	$C_{29}H_{43}NO_2$	465	0.89

Table.3 Identified compound, area and retention time of peak of treated sugarcane juice

No.	RT*	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	5.21	Dodecane, 1,2-dibromo-	$C_{12}H_{24}Br_2$	326	6.36
2.	6.00	2-Cyclopentenone, 2-acetyl-3-methylamino-	$C_8H_{11}NO_2$	153	7.35
3.	6.80	tert-Hexadecanethiol	$C_{16}H_{34}S$	258	4.75
4.	8.30	Cyclopropyl 4-methoxyphenyl ketone	$C_{11}H_{12}O_2$	176	18.53
5.	10.99	Pentanal, 5-(methylenecyclopropyl)-	$C_9H_{14}O$	138	3.53
6.	13.07	5-Hydroxymethylfurfural	$C_6H_6O_3$	126	29.21
7.	14.86	Cyclobarbital	$C_{12}H_{16}N_2O_3$	236	2.95
8.	16.91	Butanoic acid, 3-oxo-, 2-propenyl ester	$C_7H_{10}O_3$	142	1.78
9.	19.11	Furan-2-carboxaldehyde, 5-(1-piperidyl)-	$C_{10}H_{13}NO_2$	179	2.93
10.	22.58	8H-Pyrano[3,4-b]pyrimido[5,4-d]furane, 5,6-dihydro-4-hydrazino-6,6-dimethyl-2-methylthio-	$C_{12}H_{16}N_4O_2S$	280	6.01
11.	23.48	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)-	$C_{13}H_{18}O_3$	222	1.43
12.	24.52	Furfurole, 5-methyl-, 4-hydroxybenzoylhydrazone	$C_{13}H_{12}N_2O_3$	244	4.79
13.	25.88	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	$C_{26}H_{54}$	366	1.29
14.	28.59	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	$C_{27}H_{40}O_4$	428	0.29
15.	30.80	Isopropyl linoleate	$C_{21}H_{38}O_2$	322	0.52
16.	31.48	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-	$C_{29}H_{43}NO_3Si$	481	0.17
17.	35.09	1,8-Dioxa-5-thiaoctane, 8-(9-borabicyclo[3.3.1]non-9-yl)-3-(9-borabicyclo[3.3.1]non-9-yloxy)-1-phenyl-	$C_{27}H_{42}B_2O_3S$	468	0.16
18.	36.60	9,12,15- Octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	$C_{27}H_{52}O_4$	496	6.29
19.	38.38	Androst-5-en-17-one, O-(phenylmethyl)oxime, (3β)-	$C_{29}H_{43}NO_2$	465	1.67

*RT –Retention Time

Mathur *et al.,* (2011) has been reported to be hypocholesterolemic, nematicide, pesticide, antiandrogenic, hemolytic, 5-alpha reductase inhibitor activities. n- Hexadecanoic acid is a significantly important phytochemical compound, also found to be present in the extract and is known to have been reported to be antimicrobial and antioxidant (Oskoueian *et al.,* 2011).

Oleic has been reported to be effective in treatment of skin papillomas. 2-benzenedicarboxylic acid and Palmitic acid are two other biologically active compounds, which possess anti-tumor and anticancerous properties. Isosorbide dinitrate has been reported to be utilized in vasodilator therapy of heart failure. (Banerjee *et al.,* 1991).

Stearic acid is involved in lowering of plasma cholesterol levels. 1, 2-Benzenediol possesses carcinogenic activity (Klingler and Ebertz, 2005). Levulinic acid is a Precursor to pharmaceuticals, Melamine possesses trypanocidal activity, 1, 2, 3-Propanetriol, 1-acetate is anti-dipogenic in nature (Stewart *et al.,* 2004).

From the results obtained from GC-MS analysis of raw juice of sugarcane and treated juice, it can be concluded that besides being sugar (carbon) source, the plant also exhibits several biological and pharmaceutical properties which provide an insight to the medical value of sugarcane plant which can be further evaluated to optimize how the plant may be utilized to explore its medicinal potential. Further treated sugarcane juice can be extended the shelf life of sugarcane juice in refrigerator condition.

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