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Original Article

Phytochemical Screening GC-MS and FT-IR Analysis of Sugarcane Juice

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ABSTRACT

The present research reveals the phytochemical examination, GC-MS analysis and FTIR Spectroscopic Analysis in sugarcane juice. Phytochemical analysis revealed the presence of alkaloid, glycosides, phenols, saponins, tannins and carbohydrates. Totally 14 compounds were identified and the chromatograph showed peaks with individual compounds. 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. The FTIR analysis confirmed the presence of N-H, O-H, C=C, C-H, C-O and CH3 functional groups. The result of this study gave an excellent drink of sugarcane juice as alternative for various diseases and it can be used as functional and pharmaceutical food.

Key words: Sugarcane juice, Phytochemical, GC-MS, FT-IR, Functional groups.

1. INTRODUCTION

All over the world, Sugarcane (*Saccharum officinarum)* is the most exclusively cultivated and India is the second largest producer country next to Brazil. It has been used as a sweetening agent for millennia and also mainly in the form of refined sugar[1]. Sugarcane juice is a highly nutritious compound and possesses many medicinal and

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pharmacological properties[2]. The roots and stems of sugar cane are used in medicine to treat skin and urinary tract infections as well as for bronchitis, heart conditions, loss of milk production, cough anemia, constipation. It is also used to treat jaundice and lowering blood pressure[3].

Sugarcane juice is a common beverage drinks and it can be used as nutritional supplement. It is a great preventive and healing source for sore throat, cold and flu. It has a low glycemic index which keeps the body healthy and hydrates the body quickly when exposed to prolong heat and physical activity. It refreshes and energizes the body instantly and can be an excellent substitute for aerated drinks and cola. By using sugarcane juice regularly rapid gain in weight can be achieved, thus can be an effective remedy for thinness[4].

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. Unlike pharmaceutical chemicals, phytochemicals do not have any side effects. Since the phytochemicals can also cure the many of diseases without causing any harmfulness to human beings. Phytochemicals can also be considered as "man- friendly medicines"[5].

An analytical powerful tool was used for identification and determination of phytochemicals compounds by GC-MS and FT-IR. The present study was carried out the bioactive compounds present in sugarcane juice the aid of GC-MS and FT-IR techniques, which may provide an insight in its use of traditional medicine.

2. MATERIAL AND METHODS

2.1 Collection and extraction of sugarcane juice:

Mature stems of sugarcane were cut close to the ground at a plantation in Thanjavur during the period of January, 2017 (Fig. 1). 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. 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 filtered sugarcane juice was used for phytochemical examination, GC-MS analysis and FT-IR Spectroscopic analysis.

2.2 Phytochemical Screening:

Phytochemical examination was carried out for sugarcane juice as per the standard methods[5]. The screening was performed for identification of alkaloid, glycosides, phenols, saponins, tannins and carbohydrates[6,7]. The color intensity or the precipitate formation was used as analytical responses to these tests. The different qualitative chemical tests were performed for establishing the sugarcane juice to detect various phytochemical present in them.

2.2.1Test for Alkaloids:

- **Wagner's Test:** To 2-3 ml extract with few drops Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.
- **Dragendorff's Tests:** To 2-3 ml extract, add few drops Dragendorff's reagent Formation of orange brown precipitate indicates the presence of alkaloids.

2.2.2 Test for Glycosides:

- **Keller-Kiliani Test:** To 2 ml extract, add glacial acetic acid, one drop 5% FeCl 3 and conc. H2SO4. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green indicates the presence of glycosides.
- **Concentrate H2SO4 Test:** To 5ml extract, add 2ml glacial acetic acid, one drop 5% FeCl 3 and conc. H2SO 4. Brown ring appears indicates the presence of glycosides.

2.2.3 Test for Phenols:

- **Ellagic Acid Test:** The test solution was treated with few drops of 5% (w/v) glacial acetic acid and 5% (w/v) NaNO 2 solution. The solution turned muddy or Niger brown precipitate occur

2.2.4 Test for Saponin:

- **Foam Test:** The extract was diluted with 20 ml of distilled water and it was shaken in a graduated cylinder for 15 minutes. A 1 cm. layer of foam indicated the presence of Saponin.
- **Haemolysis Tests:** - Add leaves extract to one drop of blood placed on glass slide. Hemolytic zone appears.

2.2.5 Test for Tannins:

- **Gelatin Test:** To the extract, gelatin (Gelatin dissolves in warm water immediately) solution was added. Formation of white precipitate indicates the presence of tannins.

2.2.6 Test for Carbohydrates:

- **Molisch Test:** Treat extract with few drops of alcoholic alpha-naphthol. Add 0.2 ml conc sulphuric acid slowly along the sides of test tube, purple to violet colour ring appears at junction.
- **Fehling's Test:** Fehling A and Fehling B reagents are mixed and few drops of extract are added and boiled. A brick red coloured precipitate

2.4 GC-MS analysis of Sugarcane juice:

The chemical composition of sugarcane juice was investigated through Gas Chromatography Mass Spectrometry with Electron Ionization (GC-MS/EI) mode. Around 50 ml of 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 concentrated through nitrogen gas flushing up to 1 ml through sample concentrator. The filtrate concentrate was again filtered in the whatmann No.41 filter paper along with sodium sulfate to removal of sediments and also traces of moisture content in the filtrate[8]. This procedure insures precise derivatization time and

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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 (Scion 436-GC Bruker) used in this experiment is coupled with a triple quadruple mass spectrophotometer. The column is a fused silica capillary column of BR-5MS (5% Diphenyl/ 95% Dimethyl poly siloxane) with 30m length, 0.25 mm internal diameter and 0.25µm thickness. The oven program was as follows: 40°C for 2 min, increased to 160°C at the rate of 20°C/min - without hold, again increased to 280°C at the rate of 5°C / min – with no hold, finally to 300°C at the rate of 12°C/min – with 8 min hold. The injector temperature was 280°C and the total GC running time was 41 min. This last increase was to completely elute the compounds of the sample from the column and to free 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 transfer line temperature was set at 280°C and filament source temperature was 250°C. The relative percentage amount of each component was ascertained by comparing the individual peak area to the total peak ions areas. Software adopted to handle mass spectra and chromatograms was MS Workstation 8. The NIST Version 2.0 having more than 2, 00,000 patterns were used for identifying the chemical components. The spectrum of the unknown components idenrtified in the samples were 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 and detailed in the Table. 2[9].

2.5 FT-IR Spectroscopic Analysis:

Fourier transform infrared spectrophotometer (FTIR) is perhaps the most powerful tools for identifying the types of chemical bonds (functional groups) present in compounds[10]. Dried powders of different solvent extracts of each plant material were used for FTIR analysis. The liquid sample of juice was loaded in FTIR Spectroscope (Shimadzu, IR Affinity1, Japan), with a scan range from 4000 to 400 cm-1 with a resolution of $4cm^{-1}$.

3. RESULTS AND DISCUSSION

Plants are very important source of potentially useful bioactive principles for the development of new chemotherapeutic agents[11].The biological and pharmacological properties of many plants are still unknown. World over, the scientists are exploring the potential of utilizing pharmacologically active compounds from medicinal plants[12]. Herbal medicines are used by 80% of the people worldwide due to its high efficiency, cheap cost, non-narcotic nature and fewer side effects[13].

In the present study, the exploration of phytochemical screening of sugarcane juice revealed the presence of alkaloid, glycosides, phenols, saponins, tannins and carbohydrates compounds which are known to have remedial activity against diseases producing pathogen. Therefore it can be used pharmacologically to develop new compounds for health benefit (Table 1).

GC-MS analysis of n- hexane extract obtained from sugarcane juice revealed the presence of 14 phytochemical compounds as depicted by 14 respective peaks for each compound in GC-MS chromatogram (Table 2, Fig.2). 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. These phytochemicals are responsible for various pharmacological actions like Antimicrobial Antioxidant, Antidiabetic, Anti-inflammatory, Anticancer, Antiarthritic, Antiasthmatic, Antitumor etc[14] (Table 3). Sugarcane juice has medicinal value the presence of biological active constituents[2].

The FT-IR spectrum was used to identify the functional groups of the bioactive components present in sugarcane juice based on the peaks values in the region of IR radiation. When the sugarcane juice was passed into the FT-IR, the functional groups of the components were separated based on its peaks ratio. The results of FT-IR analysis confirmed the presence of N-H, C=C and C-O functional groups (Fig. 3 and Table 4). FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

Table 1: Qualitative analysis of Phytochemical constituents present in sugarcane juice

S.NO	Phytochemical Constituents	Sugarcane juice
1.	Test for Alkaloid	+
2.	Test for Phenols	+
3.	Test for Carbohydrates	+
4.	Test for Saponin	+
5.	Test for Glycosides	+
6.	Test for Tannins	+

Present +, Absent -

Table 2: Phytocomponents identified in sugarcane juice by GC-MS

S.No.	RT	Name of the compound	Molecular Formulae	Molecular Weight	Peak Area %
1.	5.21	Dodecane, 1,2-dibromo-	C12H24Br2	326	5.89
2.	6.80	tert-Hexadecanethiol	C16H34S	258	4.18
3.	8.30	Cyclopropyl 4-methoxyphenyl ketone	C11H12O2	176	19.58
4.	10.99	Pentanal, 5-(methylenecyclopropyl)-	C9H14O	138	2.99
5.	12.99	5-Hydroxymethylfurfural	C6H6O3	126	39.56
6.	14.86	Cyclobarbital	C12H16N2O3	236	5.63
7.	22.58	8H-Pyrano[3,4-b]pyrimido[5,4-d]furane,	C12H16N4O2S	280	5.55

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		5,6-dihydro-4-hydrazino-6,6-dimethyl-2-methylthio-			
8.	23.48	Ethanone, 1-(1a,2,3,5,6a,6b-hexahydro-3,3,6a-trimethyloxireno[g]benzofuran-5-yl)-	C13H18O3	222	6.52
9.	25.88	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	366	3.25
10.	28.59	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-	C27H40O4	428	0.47
11.	30.80	Isopropyl linoleate	C21H38O2	322	0.88
12.	31.48	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-	C29H43NO3Si	481	0.05
13.	36.60	9,12,15-Octadecatrienoic acid, 2,3-bis oxy]propyl ester, (Z,Z,Z)-	C27H52O4	496	4.56
14.	38.38	Androst-5-en-17-one, O-(phenylmethyl)oxime, (3β)-	C29H43NO2	465	0.89

Table 3: Biological/Pharmacological Activity of phyto-components identified in sugarcane juice by GC-MS

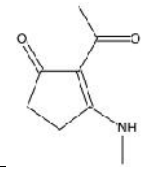
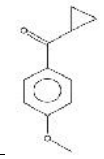
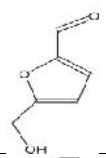
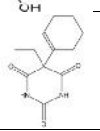
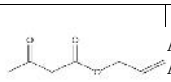
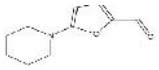
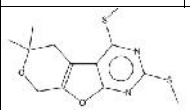
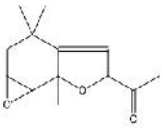
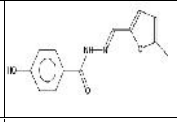
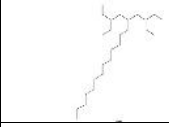
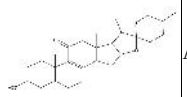
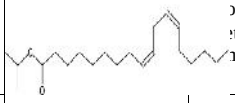
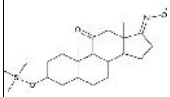
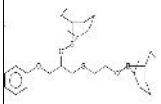
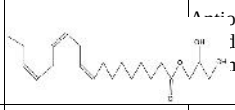
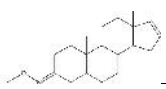
S.No	Compound name	Structure	Biological/Pharmacological activities*
1.	Dodecane, 1,2-dibromo-		nicrobial Activity
2.	2-Cyclopentenone, 2-acetyl-3-methylamino-		Anti-inflammatory
3.	tert-Hexadecanethiol		Enzyme activators
4.	Cyclopropyl 4-methoxyphenyl ketone		Antibacterial, Analgesic
5.	Pentanal, 5-(methylenecyclopropyl)-		
6.	5-Hydroxymethylfurfural		Antioxidant Activity
7.	Cyclobarbital		Antimicrobial and Anti cancerous
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, anti arthritic, anti asthmatic
14.	Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)-		Anticancer
15.	Isopropyl linoleate		xidant, Anti tic, Anti-nmatory
16.	Androstane-11,17-dione, 3-[(trimethylsilyl)oxy]-, 17-[O-(phenylmethyl)oxime], (3α,5α)-		Anticancer, Anti tumour 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 oxy]propyl ester, (Z,Z,Z)-		xidant, liabetic, Anti-nmatory
19.	Androst-5-en-17-one, O-(phenylmethyl)oxime, (3β)-		Antitumour and Antimicrobial Activity

Table 4: FTIR Peak Values and functional groups of sugarcane juice

S.No	Peak Values	Functional Groups
1	3338.18	N-H
2	1638.84	C=C
3	1055.53	C-O

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Fig1: Photograph of Sugarcane (*Saccharum officinarum)*

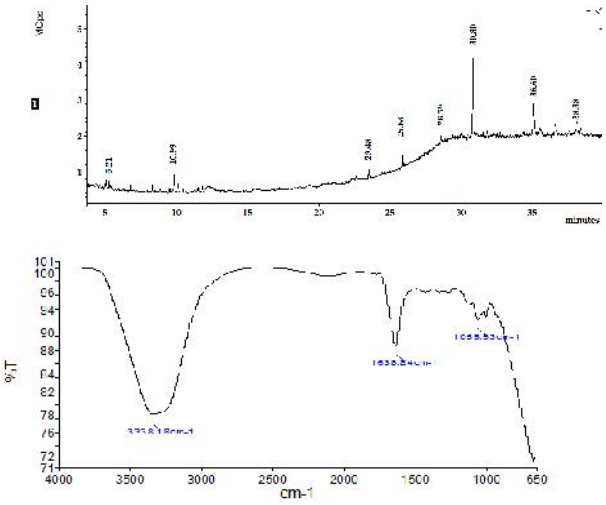

Fig 2: GC-MS chromatogram of Sugarcane juice

Fig 3: *FTIR* analysis of *Sugarcane juice*

4. CONCLUSION

The results were obtained from Phytochemical examination, GC-MS analysis and FTIR Spectroscopic Analysis of sugarcane juice. Further, it can be concluded that besides being sugar (carbon) source the cane juice 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, sugarcane juice can be extended the shelf life of sugarcane juice in refrigerator condition.

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