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Isolation and characterization of active components derived from whole plant of *Saccharum spontaneum* (Linn.)

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ABSTRACT

The aim of the present investigation was isolation and characterization of active components derived from whole plant of Saccharum spontaneum. The plant was extracted with ethanolic solvent. The preliminary phytochemical results revealed that alkaloids, carbohydrates and glycosides, phenolic compounds, saponins, tannins, protein and amino acids, coumarins & flavonoids as active constituents in ethanolic extract of Saccharum spontaneum. The ethanolic extract of Saccharum spontaneum was undergone column chromatography with different solvent fractions. Despite, three compounds were isolated from ethanolic extract of Saccharum spontaneum with the compound 1 was eluted with pet. ether: benzene 50:50, v/v and compound 2 were eluted with ethyl acetate: ethanol 90:10, v/v and then compound 3 were eluted with ethyl acetate: ethanol, 80:20, v/v. The structures of the three isolated compounds were characterized by using FT-IR, NMR and Mass spectrophotometric methods. Thus, the compound 1 was characterized as 3,3',4',5-Tetra hydroxy-6,8-dimethoxy flavone ($C_{17}H_{14}O_8$), the compound 2 was characterized as 3,5-Dihydroxy-4'-methoxy-7- oxyglucopyronoside flavone ($C_{22}H_{22}O_{11}$) and the compound 3 was characterized as 3,3',4',5,7- Penta hydroxy flavone ($C_{15}H_{10}O_7$). Furthermore, pharmacological studies required for the isolated compounds.

Key words: *Saccharum spontaneum*, Isolation, Column chromatography, FT-IR, NMR.

INTRODUCTION

Saccharum spontaneum Linn. belongs to the family Gramineae, commonly known as kaich or kasa is a traditional herb, it has excellence medicinal value; has been advocated in the treatment gynaecological troubles, respiratory disease. It is a tall erect reed-like perennial grass with plume like inflorescence, grows in marshes in Chittagong and other areas. It is distribute throughout India[1] and tropical Asia[2]. Lignin, carbohydrates, proteins and amino acids are present in leaves and stalks[3]. Starch and polyphenolic compounds are present in roots and root-stocks. Aerial parts possess laxative and aphrodisiac properties, and are useful in burning sensations, strangury, phthisis, vesical calculi, blood diseases, biliousness and haemorrhagic diathesis[4]. The stems (culm) are useful in vitiated conditions of pitta and vata burning sensation strongly, renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility[5,6]. Roots are used as galactagogue and diuretic[3] and in ayurveda system roots are also used as astringent, emollient, refrigerant, diuretic, purgative, tonic, and aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles and sexual weakness[7]. The anti diarrhoeal and CNS depressant activity of methanolic extract of *Saccharum spontaneum* has been reported[8]. It have been reported mild anti psychotic activity by amnesic effect (Loss of Memory) in stem extract[6] and antiurolithiatic activity in ethanolic root extract[9]. Therefore, the objective of the present investigation was isolation and characterization of active components derived from whole plant of *Saccharum spontaneum* by using FT-IR, NMR and mass spectrophotometric methods.

MATERIALS AND METHODS

Plant material

The whole plants of *Saccharum spontaneum* (Linn), were collected from Cheranmahadevi, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Saccharum spontaneum* (Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Extraction

The powdered plant materials were successively extracted with ethanol (60-80^{0}C) by hot continuous percolation method in Soxhlet apparatus[10] for 24 hours. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained. The ethanolic extract was stored in screw cap vial at 4^{0}C until further use.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The ethanolic extract of *Saccharum spontaneum* was subjected to the following chemical tests such as tests for alkaloids[11], test for carbohydrates[11], tests of glycosides[11], tests for phytosterol[12], test for coumarins[12], test for flavonoids[13,14], test for tannins and phenolic compounds[15], tests for proteins and amino acids[11], test for saponins[11], test for fixed oils[11].

TLC characterization of ethanolic extract of *Saccharum spontaneum*

The chromatography principle of separation is either partition or adsorption. The constituent which is having more affinity for mobile phase moves with it, while the constituent which is having more affinity for stationary phase gets adsorbed on it. This way various compounds appear as a band on the TLC plate, having different R_f values. The ethanolic extract of *Saccharum spontaneum* was subjected to thin layer and high performance thin layer chromatographic studies for the separation and identification of their components.

Preparation of plates

100g of silica gel G was weighed and made into a homogenous suspension with 200 mL of distilled water to form slurry. The slurry was poured into a TLC applicator, which was adjusted to 0.25 mm thickness on flat glass plate of different dimensions (10X2, 10X5, 30X5, 20X10 cm etc.). The coated plates were allowed to dry in air, followed by heating at 100-105°C for 1 hour, cooled and protected from moisture. Before using, the plates were activated at 110°C for 10 minutes.

Separation of components

The ethanolic extract of *Saccharum spontaneum* was dissolved in ethanol separately and spotted using a capillary tube on TLC plates 2 cm above from the bottom of the plate. The selection of solvent systems were based on increasing the order of polarity. The different spots developed in each system were detected by means of iodine staining.

Isolation of ethanolic extract of *Saccharum spontaneum* by using column chromatography

The 20gms of ethanolic extract of *Saccharum spontaneum* was admixed with 20gms silica gel (60/120 meshes) to get uniform mixing. 200gms of silica gel (70/325 meshes) was taken in a suitable column and packed very carefully without air bubbles using petroleum ether as filling solvent. The column was kept aside for 1 hour and allowed for close packing. Admixture was then added at the top of the stationary phase and started separation of compounds by the eluting with various solvent mixtures with increasing order of polarity. All the column fractions were collected separately and concentrated under reduced pressure. Finally the column was washed with hexane, ethyl acetate and ethanol.

Characterization of isolated Compounds

FT-IR

IR spectra of the compounds isolated from the ethanolic extract of *Saccharum spontaneum* were recorded using a Nicolet 170SX. The spectral resolution for the Nicolet 170SX was 0.25cm^{-1}, and the spectral data were stored in the database at intervals of 0.5 cm^{-1} at 4000-2000 cm^{-1}, and of 0.25 cm^{-1} at 2000-400 cm^{-1}. The solid samples were measured by using KBr disc methods.

^{1}H NMR

^{1}H NMR spectra of the compounds isolated from the ethanolic extract of *Saccharum spontaneum* were recorded using a JEOL AL-400 (399.65 MHz). The measuring conditions for the most of the spectra were as follows: flip

angle of 22.5-30.0 degrees, pulse repetition time of 30s. The long pulse repetition time and small flip angle was used to ensure precise relative intensities. The ^{1}H NMR chemical shifts were referred to TMS in organic solvents.

^{13}C NMR

^{13}C NMR spectra of the compounds isolated from the ethanolic extract of *Saccharum spontaneum* were recorded with a Bruker AC-200 (50.323 MHz). The measuring conditions for the most of the spectra were as follows: a pulse flips angle of 22.45-45 degrees, a pulse repetition time of 4-7 seconds, and a resolution of 0.025-0.045 ppm. The spectra whose spectral codes started with "CDS" were reconstructed from peak positions, intensities, and line widths by assuming all resonance peaks were Lorenz lines. The chemical shift was referred to a TMS for all solvents.

Mass Spectrum

Mass spectra of the compounds isolated from the ethanolic extract of *Saccharum spontaneum* was recorded with JEOL JMS-700 by the electron impact method where an electron is accelerating voltage 75eV and an ion accelerating voltage of 8-10nV. The reservoir inlet systems were used. The dynamic range for the peak intensities were 3 digits and the accuracy of the mass number was 0.5.

RESULTS AND DISCUSSION

The ethanolic extract of *Saccharum spontaneum* (Linn.) was subjected to screening for its phytochemical constituents. The phytochemical screening results are shown in Table 1. The ethanolic extracts containing alkaloids, carbohydrates and glycosides, phenolic compounds, saponins, tannins, protein and amino acids, coumarins & flavonoids.

Table 1 - Phytochemical analysis of ethanolic extract of whole plant of *Saccharum spontaneum* (Linn.)

S.NO.	TEST	ETHANOLIC EXTRACT
I	ALKALOIDS	+
II	CARBOHYDRATES AND GLYCOSIDES	+
III	PHYTOSTEROLS	+
IV	FIXED OIL AND FATS	-
V	SAPONINS	+
VI	PHENOLIC COMPOUNDS AND TANNINS	+
VII	PROTEIN AND AMINO ACID	+
VIII	COUMARINS	+
IX	FLAVONOIDS	+

+ Positive; - Negative

The ethanolic extract of *Saccharum spontaneum* was subjected to the TLC chromatographic profile and column chromatographic separation. The ethanolic extract of *Saccharum spontaneum* dissolved in their mother solvent was taken in a capillary tube and spotted on TLC plates 2cm above its bottom. Most of the sample for application were between 0.1 – 1%. The applied spots were of equal size as far as possible and diameter ranging from 2-3mm. The solvent system for ethanolic extract was developed by trial and error method using various solvents which were differing in polarities.

Table 2 - TLC profiles of ethanolic extract of *Saccharum spontaneum*.

S.NO	SOLVENT SYSTEM	NO. OF SPOT	RF VALUE
1.	PET. ETHER : BENZENE (50:50)	2	0.63, 0.42
2.	PET. ETHER : BENZENE (80:20)	2	0.69, 0.23
3.	ETHYL ACETATE: ETHANOL (90:10)	2	0.31, 0.40
4.	ETHYL ACETATE: ETHANOL (80:20)	2	0.65,0.49

The ethanolic extract of *Saccharum spontaneum* was subjected to column chromatographic separation using normal phase silica gel column. The dark brown solid (20 g ethanolic extract of *Saccharum spontaneum*) was adsorbed on silica gel (20 g) and transferred to a column of silica gel (200g equilibrated with pet. ether). Despite, three compounds were isolated in column chromatography with different solvents. Obviously, the compound 1 (190 mg) was eluted with pet.ether: benzene 50:50, v/v, compound 2 (215mg) was eluted with ethyl acetate: ethanol, 90:10 v/v and compound 3 (110mg) was eluted with ethyl acetate: ethanol, 80:20, v/v. This active fraction was used to identify the chemical tests showed the presence of phytosterols, flavonoids and amino acid as active compounds. The actual compounds were isolated from column chromatography as mentioned in the experimental section. The spectra (IR, ^{1}H & ^{13}CNMR and Mass) of these compounds as mentioned in the experimental section.

Characterization of compound 1

The spectral data IR, ^{1}HNMR & ^{13}CNMR and Mass of the compound 1 are good in agreement with the structure proposed for the compound. The melting point of the compound 1 was found as 201ºC. The IR spectrum of the compound 1 was analysed from the IR data. The absorption at 3475-3257cm^{-1} indicates the presence of phenolic –OH group. The presence of –C=O group in this compound was revealed by the strong absorption at 1661cm^{-1}. The strong absorption at 1039cm^{-1} indicates the presence of –OCH_3 group which has the ether linkage.

The ^{1}HNMR chemical shift values the chemical shift values at δ,12.93ppm indicates the presence of phenolic -OH group at 3rd position. The hump at δ, 10.80,9.20 and singlets at 8.16ppm were due to -OH group at $C_{5,}$ $C_{4'}$ and $C_{3'}$ carbon atom respectively. The chemical shifts as singlets at δ,6.85,6.58 and doublets at δ, 6.39-6.38, 6.23-6.22 due to presence of proton at $C_{2'}$, C_7 and $C_{5'}$,$C_{6'}$. The chemical shifts as singlet at δ, 3.71 and 3.63ppm were due to the presence of methoxy group at C_8 and C_6. The ^{1}HNMR & ^{13}CNMR chemical shift values and the corresponding assignments are given in fig.1 and 2 respectively. The mass spectral analysis of compound 1 led to the molecular peak *m/z* 346(M+3), which indicated the molecular formula $C_{17}H_{14}O_8$. Thus, the compound 1 was characterized as 3,3',4',5-Tetra hydroxy-6,8-dimethoxy flavone is given in Fig 3. The molecular formula of the compound 1 was deduced as $C_{17}H_{14}O_8$. This is the first report of occurrence of this compound in nature as well as the alkaloids in this plant.

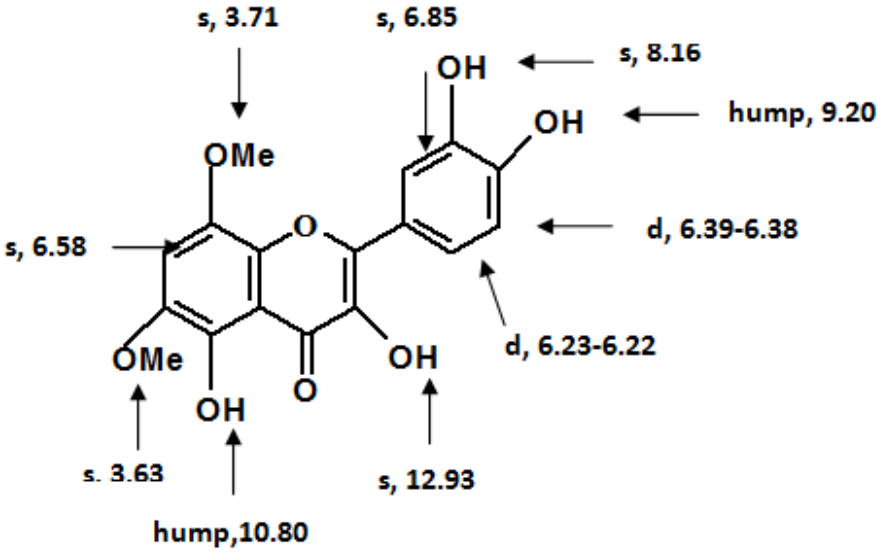

Fig 1: ^{1}HNMR spectral data of compound 1 and corresponding assignments

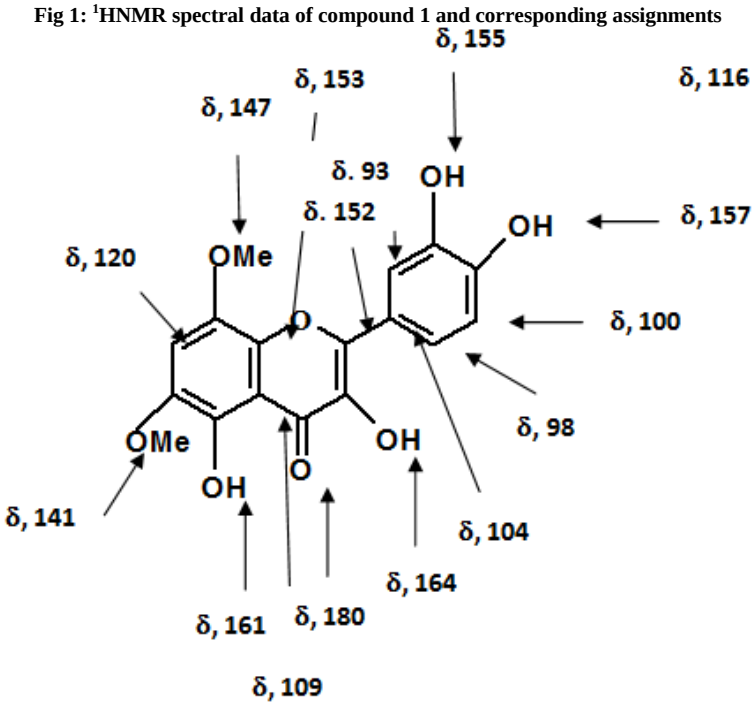

Fig 2: ^{13}CNMR spectral data of compound 1and corresponding assignments

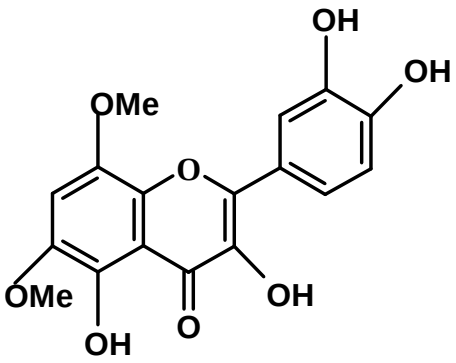

Fig 3: Structure of Compound 1(3,3',4',5-Tetra hydroxy-6,8-dimethoxy flavone)

Characterization of compound 2

The spectral data IR, ^{1}HNMR & ^{13}CNMR and Mass of the compound 2 are good in agreement with the structure proposed for the compound. The melting point of the compound 2 was found as 210ºC. The IR spectrum of the compound 2 was analysed from the IR data. The presence of –OH group known from the absorption at the range 3422cm^{-1}. A strong band at the range 1654cm^{-1} was due to the presence of –C=O group. The absorption at1600 and 1503cm^{-1} prove the aromatic nuclear part in the compound. The presence of –C-O-C- ether linkage indicates in the absorption at 1063cm^{-1}. The ^{1}HNMR and ^{13}CNMR chemical shift values of the compound 2 was found to be 3,5-Dihydroxy-4'-methoxy-7-oxyglucopyronoside flavone (Fig 4&5). The mass spectral analysis of compound 2 led to the molecular peak *m/z* 462 (M+), which indicated the molecular formula $C_{22}H_{22}O_{11}$. Thus, the compound 2 was characterized as 3,5-Dihydroxy-4'-methoxy-7-oxyglucopyronoside flavone is given in Fig 6. The Molecular Formula of the compound 2 was deduced as $C_{22}H_{22}O_{11}$. This is the first report of occurrence of this compound in nature as well as the alkaloids in this plant.

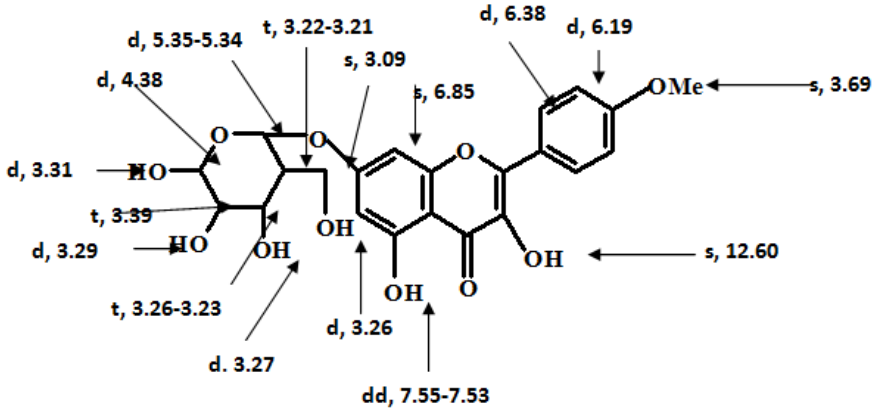

Fig 4: ^{1}HNMR spectral data of compound 2 and corresponding assignments

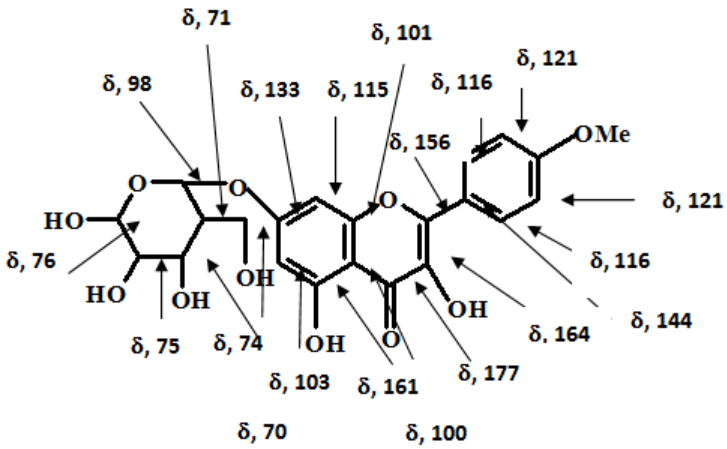

Fig 5: ^{13}CNMR spectral data of compound 2 and corresponding assignments

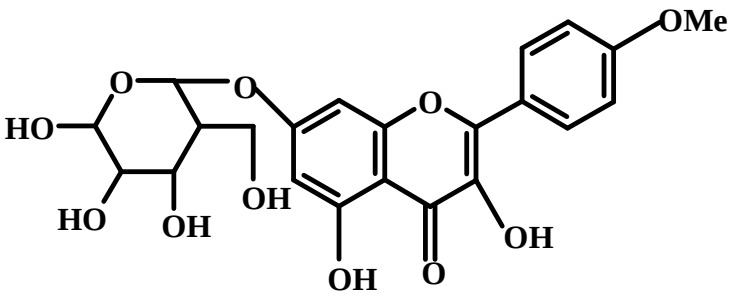

Fig 6: Structure of Compound 2(3,5-Dihydroxy-4'-methoxy-7-oxyglucopyronoside flavone)

Characterization of compound 3

The spectral data IR, ^{1}HNMR & ^{13}CNMR and Mass of the compound 3 are good in agreement with the structure proposed for the compound. The melting point of the compound 3 was found as 315ºC. The IR spectrum of the compound 3 was analysed from the IR data. Absorption at 3314cm^{-1} shows the presence of –OH group, whereas the strong band at 1666cm^{-1} indicates the presence of carbonyl group. The absorption at 1609, 1561, 1552, 1456 and 1380cm^{-1} reveals the presence of aromatic C=C stretching and bending vibrations. The presence of C-O-C ether linkage in the absorption at 1008cm^{-1}. The ^{1}HNMR and ^{13}CNMR chemical shift values of the compound 3 was found to be 3,3',4',5,7- Penta hydroxy flavone (Fig 7&8). The mass spectral analysis of compound 3 led to the molecular peak *m/z* 300(M^{+}-2), which indicated the molecular formula $C_{15}H_{10}O_7$. Thus, the compound 3 was characterized as 3,3',4',5,7- Penta hydroxy flavone is given in Fig 9. The Molecular Formula of the compound 3 was deduced as $C_{15}H_{10}O_7$.

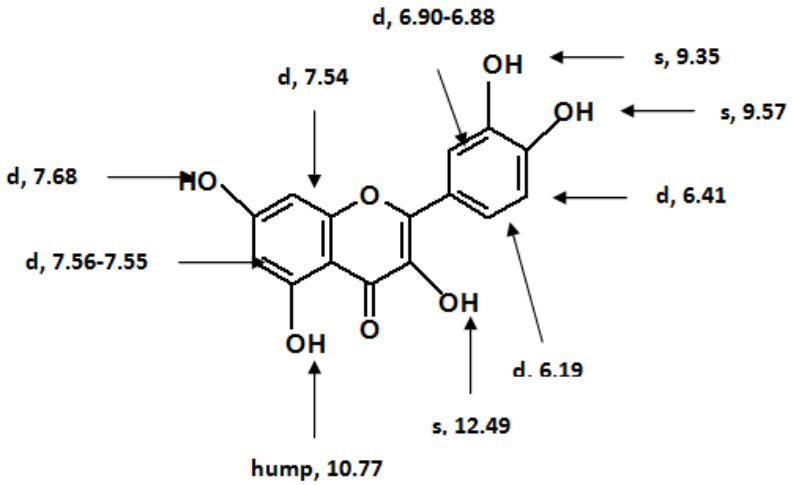

Fig 7: ^{1}HNMR spectral data of compound 3 and corresponding assignments

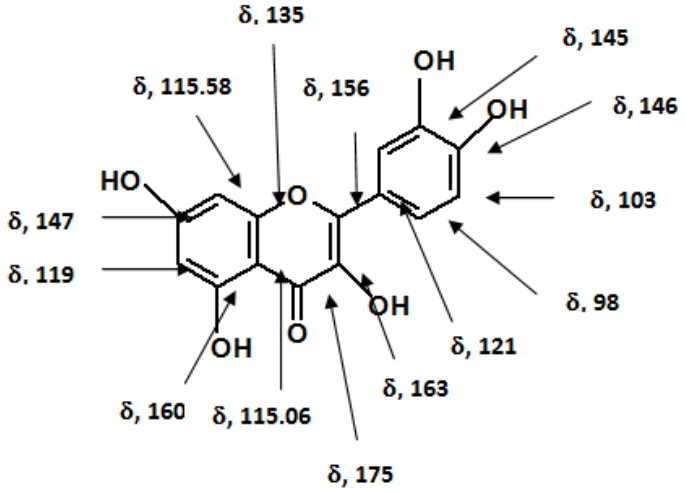

Fig 8: ^{13}CNMR spectral data of compound 3 and corresponding assignments

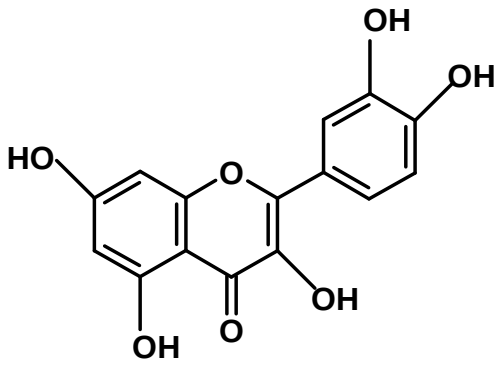

Fig 9: Structure of Compound 3 (3,3',4',5,7- Penta hydroxy flavone)

CONCLUSION

From the above reports, three compounds were isolated from ethanolic extract of *Saccharum spontaneum* (Linn.) such as 3,3',4',5-Tetra hydroxy-6,8-dimethoxy flavone ($C_{17}H_{14}O_8$), 3,5-Dihydroxy-4'-methoxy-7-oxyglucopyronoside flavone($C_{22}H_{22}O_{11}$)and 3,3',4',5,7- Penta hydroxy flavone ($C_{15}H_{10}O_7$). However, this is the first report of occurrence of 3,3',4',5-Tetra hydroxy-6,8-dimethoxy flavone ($C_{17}H_{14}O_8$) in nature as well as the alkaloid in this plant. Therefore, further in-depth biological investigations need for the isolated compounds.

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