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Isolation, Purification and Characterization of Flavor Compounds in the Fruit Pulp of Sysepalum dulcificum

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Abstract
Flavour is an organoleptic responds to parameter commonly described as taste which can be classified as natural, nature-identical or artificial flavouring substances. In this study, cold and hot ethanolic extracts , were obtained from *Synepalum dulcificum* ((Daniell) known locally as ntum in Akaeze dialect of Igbo language of Nigeria and traditionally used as sweetener were evaluated for its flavourant principles. The presence of bioactive components was detected and the result revealed that hot ethanol gave the highest percent extract recoveries of 3.33% while the cold extraction technique yielded 1.81 %. The result of sensory evaluation of purified extract showed that no significant difference was observed between these values except for sample 760 ($p<0.05$). Chromatographic analysis of cold ethanol extract revealed that component E-1 has a melting point of 66-68^{o}C while the GC analysis of E-1showed the eluate of two compounds at different retention times of 12.692 and 15.133 minutes respectively. Spectral Analysis of compounds in E-1(a, b) revealed that they are possible isomers and based on spectral data the structure of compound E-1a was established as 2, 5-dimethyl-2, 4-dihydroxy-3(2H)-furanone while E-1b was established as 3, 5-dihydroxy-6-methyl-2, 3-dihydro-4H-pyranone
Keywords: Flavour, purification, Syepalum dulcificum, bioactive, characterization.

Introduction

Most spices, condiments, teas, and other beverages, owe their individual properties to the pharmacologically active secondary plant metabolites that they contain [1]. Taste is largely due to the reaction with receptor sites on the tongue from molecules, which are mainly non-volatile [2]. Flavour can result from compounds that are divided into two broad classes: those responsible for taste and those responsible for odours [3]. The tongue can detect four distinct flavours and each flavour has dedicated taste buds in different regions of the tongue [4,5,6,7]. However, modern research has shown that, not only are there no distinct regions on the tongue, but a fifth flavour, *umami* (savoury), also exists [5,7].

The mammalian taste gland can respond to a large number of chemical compounds such as sweet, salty, sour, bitter and *umami* taste [8]. *Umami* is not thought to have a flavour of its own, but rather it potentiate other flavours already present [9,5]. Molecular studies have shown that the ligand-binding receptors for both *umami* and sweet taste are from the T1R(transduction receptor 1) family of G-protein–coupled receptors, although there are differences in the heterodimers that constitute the *umami* (T1R1 + 3) and sweet (T1R2 + 3) receptors [10,11].

Less is known about the mechanism of sweet substances and there seems to be no relationship between organic functional groups and sweet flavour [12], as almost every class of compound has a constituent which tastes sweet, yet at the same time the chemistry is very specific [13]. The range and types of compounds that exhibit sweetness are impressive and include peptides, proteins, flavanones, flavonols, dihydrochalcones, isovanillyl, sesquiterpenes, urea compounds, sulfones [14]. Natural flavours are not simply one or two compounds, which could be easily synthesized, but a large number of compounds in a characteristic distribution, called a flavour profile [15]. Volatile chemicals are responsible for the aroma and contribute to the flavour of fresh strawberries and may include aldehydes and furanones [16,17]. 2,5-dimethyl-4-hydroxy-3(2H)-furanone was reported as the main organoleptic principles of pineapple in strawberries [18]. They are known as important aroma chemicals due to their low odour thresholds and attractive flavour properties [19,20].

Synsepalum dulcificum (Daniell) is a promising species but has a poor documented resources [21]. The species are well known for their taste modifying properties which have been traditionally exploited for centuries in Africa [22]. The plant is commonly known as *Ntum* in the Akaeze dialect of Igbo language of Nigeria and is traditionally used as local sweetener. Not much is known of the chemical structure of the compounds in *Synsepalum dulcificum* but the anti-nutritional compositions of the seed has been partially determined. The rich contents of amino acids makes *Synsepalum dulcificum* an excellent raw material for the production of pharmaceuticals and diet supplements [23,24].

MATERIALS AND METHODS

Research duration: This study was carried out at the Chemical Sciences Laboratory, Evangel University Akaeze from 2012- 2015.

Collection and Identifications of Plant Materials: Ripened fruits of *Synsepalum dulcificum* were harvested

from fallow farmland situated in the Southern part of Nigeria during the period. The plant sample was identified by Professor Godwin Udom of the Department of crop production University of Uyo and authenticated by Professor J.C.Okafor and the sample deposited at Evangel University Herbarium.

Sample preparation and extraction procedure: Fresh ripened fruits pulp were ground into fine slurry using a blending machine. The extraction solvent that was employed for the removal of the plant's active principles was ethyl alcohol. The ground plant sample (75 g), was added into 750 mL of the solvent (ethanol 95%) at 4^{o}C in a conical flask. It was covered, shaken every one hour thirty minutes, for 6 hours and thereafter permitted to stand for 48hrs for extraction. The solution at the end of the extraction was shaken and filtered using Whatman filter paper No 4. The filtrate was subsequently evaporated using a vacuum dryer and the percentage yield based on the powdered plant material used was calculated. For the hot solvent extractions the ground plant material (75g) was wrapped in Whatman filter paper, each wrap containing 25 g and placed in a thimble of a Soxhlet extraction apparatus in divided dose. Then 750 mL of the solvent (ethanol 95%) was added into a round bottom flask and the Soxhlet apparatus mounted. The round bottomed flask was heated and extraction of the plant material carried out, and then stopped after several refluxes (one hour thirty minutes) successively. The solvent extract was evaporated to dryness using a rotary evaporator (Model type 34/2; Corning Ltd, England). Percentage yield obtained in relation to the dry material was calculated. The extracts obtained were stored in a refrigerator until required.

Column Chromatography: A 50.0 cm column long with diameter of about 25.0 mm and with a narrow tip was used. The chromatographic column was packed by partially filling it with hexane. A slurry of silica gel in hexane was poured slowly and then rinsed into the column to form a bed of absorbent without holes, channels or bubbles. The sample was introduced and eluted with the fractions obtained from the TLC. Portions with similar R_f values were mixed together and evaporated to dryness.

Sensory evaluation: Twenty panelists were used to perform the sensory evaluation. The Panelists were selected on the basis of their interest and availability. Two training sessions conducted in which the panelists were trained to evaluate sensory attributes of the samples. Sensory quality attributes were evaluated using a 5-point scale for the various sensory parameters with 1 for fairly sweet to 5 for extremely sweet for each attribute. The samples were evaluated for sweetness, mouth feel and general acceptability [25]. The extracts were evaluated within 24 hrs after sample preparation, cooled and stored at refrigeration temperature (~4°C) until subjected to sensory analysis. Randomly coded samples with control were presented to the panelists on a glass cup. Samples were served to panelists in a room with an overhead fluorescent light. Panelists instructed to rinse their mouth with tap water before starting and between sample evaluations.

Melting Point Determination :Melting point determination was carried out to ascertain the level of purity of the fractions. Gallen-kamp melting apparatus and solid block melting point apparatus were used.

Gas Chromatograghy- Mass Spectrophotometric Analysis :Gas chromatograghy- mass spectrophotometric assay of the extracts were carried out using Perkin-Elmer Gas chromatogragh, Clarus 500 system and GC interfaced to a Mass spectrometer fitted with a Elite-I, fused silica capillary column (30mmX0.25mm 1D X 1 µMdf, composed of 100 percent dimethyl polysiloxane). For gas chromatograph-mass spectrometer detection, an electron ionization system of 70 eV ionizing energy were utilized. Helium gas of 99.999 % purity was used as the carrier gas at constant stream rate of 1 mL per minute and a dose volume of 2 micro-liter employed with splitting ratio of 10:1, Injector temperature of 250 °C and ion-source temperature of 280°C were maintained. The oven temperature was set at 110 °C, isothermally for two minutes, with an increase of 10 °C per minute, to 200°C, then 5 °C per minute to 280°C, culminating to a 9 minutes isothermal at 280°C. Mass spectra were carried out at 70 electron volt, at probe interval of 0.5 seconds and fragments from 40 to 450 Dalton atomic unit. Total gas chromatographic running time was determined and the comparative percentage amount of each components assessed by comparing its average peak area to the total areas. Analysis of the mass spectrum GC-MS data was carried out using the database of the National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unidentified component related with the spectrum of the identified components stored in the NIST library. The Names, Molecular weight and structures of the components of the test materials were established.

Infrared (IR) analysis : For GC-FT IR analysis, a Bio-Radiation Digilab FTS-45A spectrometer coupled to a Bio-Radiation Tracer equipped with a liquid Nitrogen cooled narrow-band MCT detector and coupled to a HP 5890 series II gas chromatograph was used. The samples were eluted on a J and W DB-1 column 30 m × 0.25 mm (i.d.)/0.25 μm flick width with helium as stream gas (split injection mode). Deposition tip and transmission line were held above 200 °C. Absorbance spectra were recorded from 4000 to 700 cm^{-1} at a spectral resolution of 1cm^{-1}.

UV-Visible absorption spectroscopy : Double beams Shimadzu ultra violet-2450 Probe ultra violet-visible spectrophotometer was used to measure the absorption spectra over a wavelength series of 200–800 nm which joined with a cell temperature controller. Quartz cuvettes were used for measurements in solution.

Components identification : Components from sample extracts were recognized by comparison of their mass

spectra and linear retention indices with those of the reference standard and their respective structures confirmed by UV-Vis, IR and GC- MS spectra.

RESULTS AND DISCUSSION

Extraction: The result of cold and hot solvent extraction of the fruit of *Synsepalum dulificum* is depicted in Table 1. Ethyl alcohol (C_2H_5OH) hot extraction gave the highest percentage extract recovery of 3.33 % while cold extraction technique yielded 1.81 % respectively.

Table 1: Percentages recovery of cold and hot solvent extracts (75 g) of fruit pulp of *Synsepalum dulcificum*.

Solvent	Crudes recovery (g)		Percent recovery (%)	
	Cold	Hot	Cold	Hot
Ethanol	1.36	2.53	1.81	3.33

Percentage yield = r/y x 100
Where y = weight of sample; r = difference between dried residue and weight of sample.

Sensory evaluation of purified ethanol extracts of *Synsepalum dulcificum* : The mean sensory scores of controls and samples are presented in Table 2. Test samples 397 and 460 had the same score for flavour (4.2 ± 0.27), while sample 222 had a score of 4.1±0.5 with the lowest score of 3.7 ± 0.11 being for sample 760. No significant difference was observed between these values except for sample 760 ($p<0.05$). This observation is comparable to the result of [26]. Also overall acceptability and mouth feel for samples 222, 397 and 460 have same rating except for sample 760 which had a low rating of 1.6±0.47 and 2.8±0.44. These result showed that sample 397 has high sensory attributes comparable to those sweeteners in the market such as monosodium glutamate and aspartame.

Table 2: Sensory profile of purified ethanol extracts of *Synsepalum dulcificum*

Sensory attribute	**Sample 222**	**Sample 397**	**Sample 460**	**Sample 760**
Sweetness	4.1±0.5	4.2±0.27	4.2±0.23	3.7±0.11
Mouth feel	3.4±0.22	3.1±0.31	3.4±0.56	2.8±0.44
General acceptability	4.0±0.01	4.0±0.13	4.0±0.52	1.6±0.47

Monosodium glutamate = 222; acetone extract = 397; aspartame = 460; sucrose = 760

Spectral data of cold ethanol extract of *Synsepalum dulcificum* : Table 3 revealed the spectral data of cold ethanol extract of *Synsepalum dulcificum.* A total of twelve (12) compounds were tentatively identified. Most of the compounds reported were low molecular weight. They include 2,4-dimethyl-2,5-dihydroxy-3(2H)-furanone, 2-hexanone, 3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pran-4-one and four carboxylic acids of molecular formulae $C_{16}H_{32}O_2$, $C_{18}H_{34}O_2$, $C_{19}H_{38}O_2$ and $C_{18}H_{36}O_2$ with retention times of 18.633, 20.561,21.375 and 21.600 minutes respectively .

Table 3: Components detected in cold ethanol extract of *Synsepalum dulcificum*

S/No	Name	Molecular formula	Retention Time(min)	Molecular weight	Base peak (%)
1	2-hexanone	$C_8H_{14}O$	6.467	126	43.00
2	4-methyl-4-hepten-3-one	$C_8H_{14}O$	8.633	126	41.00
3	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	12.692	144	42.95
4	4H-pyran-4-one	$C_6H_8O_4$	15.125	144	43.00
5	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	18.633	256	43.00
6	Methyl n-octadecanoate	$C_{19}H_{38}O_2$	20.567	298	74.05
7	Oleic acid	$C_{18}H_{34}O_2$	21.375	282	55.05
8	Octadecanoic acid	$C_{18}H_{30}O_2$	21.600	284	43.00
9	Methoxyacetic acid	$C_{10}H_{12}O_3$	23.458	180	45.00
10	3,11-Tetradecadien-1-ol	$C_{14}H_{26}O$	24.617	168	55.05
11	2-butyloctyl alcohol	$C_{12}H_{26}O$	24.808	186	57.05
12	1,3-Cyclohexadiene	$C_{11}H_{18}O$	25.700	166	77.05

Table 4: Spectroscopic data of chemical constituents of cold ethanol extract of *Synsepalum dulcificum*

S/No	Name	Spectral data (ms[m/z (%)])
1	2-hexanone	43(100) 55(25) 83(10)
2	4-methyl-4-hepten-3-one	41(100) 97(80) 69(72) 27(44) 38(30)
3	2,4-dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	43(100) 101(73) 73(63) 55(50)
4	4H-pyran-4-one	43(100) 44(73)144(40) 101(30) 55(27) 45(25) 72(21)
5	pentadecane-carbonxylic acid	43(100) 73(90) 60(87) 41(75) 57(62) 55(60) 29(40) 69(32)
6	Methy n – octadecanoate	74(100) 87(60) 43(43) 41(27)
7	(Z)-9- Octadecenoic acid (Oleic acid)	41(100) 55(83) 43(60) 29(52) 69(50) 83(31) 67(25)
8	Octadecanoic acid	43(100) 41(87) 73(80) 60(78) 55(65) 57(61) 29(48) 70(32) 129(30)
9	Methoxyacetic acid	45(100) 152(11) 122(9)
10	3,11-Tetradecadien-1-ol	55(100) 41(85) 67(45) 69(40) 81(35) 43(30)
11	2-butyloctyl alcohol	43(100) 57(99) 41(74) 29(62) 55(60) 71(50) 27(40) 56(35) 69(32) 85(30)
12	1,3-Cyclohexadiene	133(100) 107(92) 105(65) 121(61) 91(60) 43(33) 41(31) 148(30) 166(27) 77(25)

Spectral data of hot ethanol extract of *Synsepalum dulcificum* : Table 5 showed the spectral data of hot ethanol extract of *Synsepalum dulcificum.* A total of seven (7) compounds were tentatively identified. The compounds reported had molecular formulae of $C_7H_8N_2O$, $C_7H_{14}O_5$, $C_{14}H_{28}O_2$ $C_{13}H_{24}O_2$,$C_{16}H_{32}O_2C_{28}H_{56}O_2$ and $C_{20}H_{40}O_2$ with retention times of 13.583,14.750 , 17.417, 20.333, 20.608, 21.233 and 22.317 minutes.

Table 5: Components detected in hot ethanol extract of *Synsepalum dulcificum*

RT(min)	Name	Formula	Basic peak (%)	M. weight
13.583	2-propenyl urea	$C_4H_8N_2O$	57.10	100
14.750	Methyl,alpha-L-fucopyranoside	$C_7H_{14}O_5$	60.05	178
17.417	Methyl tridecanonate	$C_{14}H_{28}O_2$	74.05	228
20.333	Methyl-(E)-9-dodecenonate	$C_{13}H_{24}O_2$	41.10	212
20.608	Methyl heptacosanonate	$C_{28}H_{56}O_2$	74.05	424
21.233	(Z)-11- hexadecenoic acid	$C_{16}H_{32}O_2$	55.10	254
22.317	Decyldecanoate	$C_{20}H_{40}O_2$	43.10	312

Table 6 :Spectroscopic data of chemical constituents of hot ethanol extract of *Synsepalum dulcificum*

Compound	Spectral data (ms [m/z (%)]
1-Propenyl urea	57 (100 (10), 56 (70), 44 (50) 41(40), 58 (30) 100 (10), 84 (0.50), 73 (0.2)
Methyl alpha –L-fucopyranoside	74 (100), 74 (50)43 (40)29 (38) 71 (30)57 (20), 177 (0.5) 147 (0.4) 129 (0.2), 116 (0.1)
Methyl tridecanonate	74(100) 87 (70)42 (45), 41 (40)57 (20) 143(15)185 (10), 129 (5), 197 (20), 101 (0.5), 228 (0.4)
Methyl (9E)-9-dodecenonate	41 (100)50 (90) 74 (60)69 (50)96 (40) 138 (25), 180(20)212 (15)
(Z)-11- hexadecenoic acid	55(100),41(80),69(40),83(35),97 (30) 236 (10),152(5), 192 (0.2)
Decyldecanonate	43 (100), 41 (70),57 (50),173 (30),97(20),155(10),129(5)

Purification of crude ethanol extracts of the fruit pulp of *Synsepalum dulcificum* : The cold and hot ethanol extracts were dissolved sequentially in different solvents to crystallize the extracts. Their melting points, number

of components and their R_F values were noted and tabulated in Tables 7 and 8 respectively. There are two components in each of E-1 and E-2 of Table 7, while E-3 depicts three components. The acetone fraction was poorly resolved yielding more than three components. However, Table 8 showed that all the fractions contains three or more components.

Table 7: Properties of the cold Ethanol Extract

Solvent	Fraction	Description	M.P.(^{O}C)	TLC	
				No. of components	R_f value
Hexane	E-1	off-white crystal	66-68	2	0.87,0.60
Chloroform	E-2	white needle like Crystals	118-122	2	0.41,0.54,
Ethylacetate	E-3	yellow Solid	-	3	0.32,0.39, 0.67
Acetone -		brown solid	-	>3	-

Table 8: Properties of the hot Ethanol Extract

Solvent	Fraction	Description	M.P.(^{O}C)	TLC	
				No. of components	R_f value
Hexane	E-4	dark crystals	109-117	3	0.17, 0.40, 0.32
Chloroform	E-5	yellow slurry	85-102	3	0.41, 0.90,0.44
Ethylacetate	E-6	light brown Solid	-	3	0.32, 0.37, 0.68
Acetone -	brown solid	-		>3	-

Spectral data for the purified ethanol fraction E-1 : Table 9 shows three diagnostic absorption bands in the ir spectrum of fraction E-1, namely 3450 cm^{-1} (OH stretching vibration, 2090 cm^{-1}(C-H stretching) and 1656 cm^{-1}(carbonyl group). A further diagnostic peak at 1450cm^{-1} in the finger print region was also observed. UV absorptions were recorded at 230 nm, 272 nm, 274 nm, 323 nm and 336 but 357nm was indicated as the absorption maxima with GC retention time of 12.683 and 15.133 minutes.

Table 9: Spectral data for the purified ethanol fractions E-1

IR(cm^{-1})(neat run)	UV-Vis(nm)	GC retention Time (min)	Mass of fragment ion (m/z) (% abundance)
3450,2318 2090,1645 1518,1450 1365,1237 1149,1043	357,336 323,274 272, 230	12.683	43(100),101(75),73(60),55(50),144(40)
		15.133	43(100),44(75),144(50),101(45),45(35), 55(30),72(25),73(20),115(0.5)

Spectral data for the purified ethanol fraction E-2 : Table 10 shows three diagnostic absorption bands in the ir spectrum of fraction E-2, namely 3428cm^{-1} (OH stretching vibration, 2933 cm^{-1}(C-H stretching) and 1643cm^{-1}(carbonyl group). A further diagnostic peak at 1449cm^{-1} in the finger print region was also observed. UV absorptions were recorded at 282 nm, 283 nm, 284 nm, 285 nm, 287 nm, 327 nm and 349 nm. However, 358 nm was observed as the absorption maxima and with GC retention time of 21.375 and 21.600 minutes.

Table 10 : Spectral data for the purified ethanol fractions E-2

IR(cm^{-1})(neat run)	UV-Vis(nm)	GC retention Time (min)	Mass of fragment ion (m/z) (% abundance)
3428,2933 2344,2093 1643,1449 1112	358,349 327,287 285,284 283,282	21.375	41(100),55(85),43(60),29(55),69(50)83(30) 67(25)
		21.600	43(100),41(85),73(80)60(75)55(65) 57(60)29(45),70(30),129(25)

Mass spectral data of E-1 fraction obtained from ethanol extract : Table 11 shows the mass spectral data of E-1 fraction obtained from ethanol extract showing an odd number base peak at m/z 43 for a compound whose molecular weight was observed at m/z 144. The identity of this compound was proposed to be 2,5-dimethyl-2,4-

dihydroxy-3(2H)-furanone . The co-elute has a molecular formula of $C_6H_8O_4$, molecular weight 144 , base peaks at m/z 43 and the identity of the compound was proposed to be 3, 5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one.

Table 11: Mass Spectral Data for E-1 fraction from ethanol extract

Retention Time (min)	Molecular mass	Proposed identity	Masses of fragment ion m/z (% abundance)
12.683	$C_6H_8O_4$	2,5-dimethyl-2,4 -dihydroxy-3(2H)-furanone	43(100),101(75),73(60),55(50),144(40)
15.133	$C_6H_8O_4$	3,5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	43(100),44(75),144(50),101(45), 55(30),72(25),73(20),115(0.5)

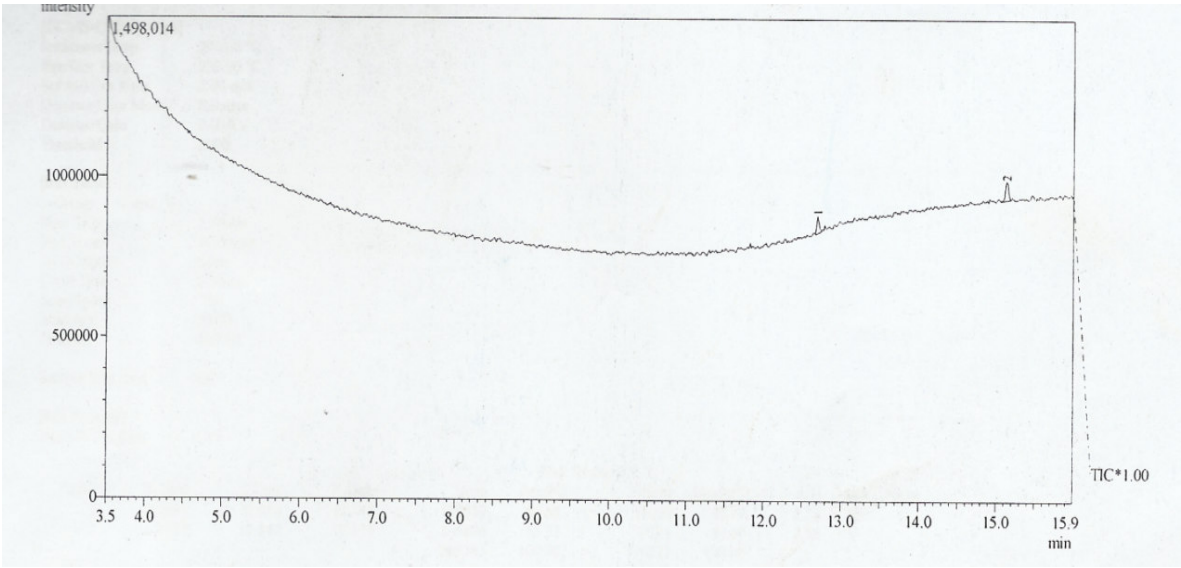


Figure 1:GC-MS chromatogram of purified ethanol E-1 extract of *Synsepalum dulcificum* fruit pulp.

Isolation and purification of flavourant compounds by column chromatography: The crude extract revealed that the presence of target compounds were concentrated in fraction E-1 which were obtained from n-hexane fraction as an off-white powdered solid, with a melting point of 66-68^{o}C while fraction E-2 yielded compounds reported elsewhere to have properties that influence flavour (Table 7). These results emphasize the success of the initial extraction steps in eliminating unwanted compounds. It was established that it is more advantageous to use a shorter column with wider diameter for initial crude separations than a longer column with narrow diameter as was observed by [27].

Spectral Analysis: From the mass spectra (Figure 1 and Scheme 1), it was observed that cleavage of the bond beta to the carbonyl group (ring opening) and subsequent double cleavage resulted to the fragment ion peaks at m/z = 101. This was caused by the elimination of methyl group and carbon monoxide molecule. Further fragmentation resulted to the loss of another carbon monoxide molecule at m/z = 73. This was followed by Mclafferty rearrangement and a loss of an aldehyde group with a mass unit of 30 (HCHO) to yield a high intensity peaks at m/z = 43 which corresponds to the base peak. Most compounds can fragment in a unique pattern and by comparing this fragmentation pattern with standards or by comparing to library database with known spectra, compounds can usually be identified [28]. However, the fragmentation pattern of the co-eluate compound revealed that ring opening and an expulsion of an aldehyde group (CH_2=CHO) yielded a high intense peak at m/z = 101 but the expulsion of carbon monoxide resulted to another prominent fragment ion peaks at m/z = 73, while the elimination of a proton gave a prominent fragment peak at m/z = 72. The peak at m/z = 43 was caused by the removal a of formyl ion (HCO) of mass unit 29 from the fragment ion peak at m/z = 72. From the spectra of these two compounds, fragments 144, 101, 73, 55 and 43 (Figure 2 and Scheme 2) were common to both and appeared to have the similar intensity. Mass peaks at m/z = 72 and 115 had slightly different intensities and may be used as distinguishing fragments. However, by using the linear retention times of 12.692 and 15.133 minutes respectively, these two compounds could be easily distinguished.

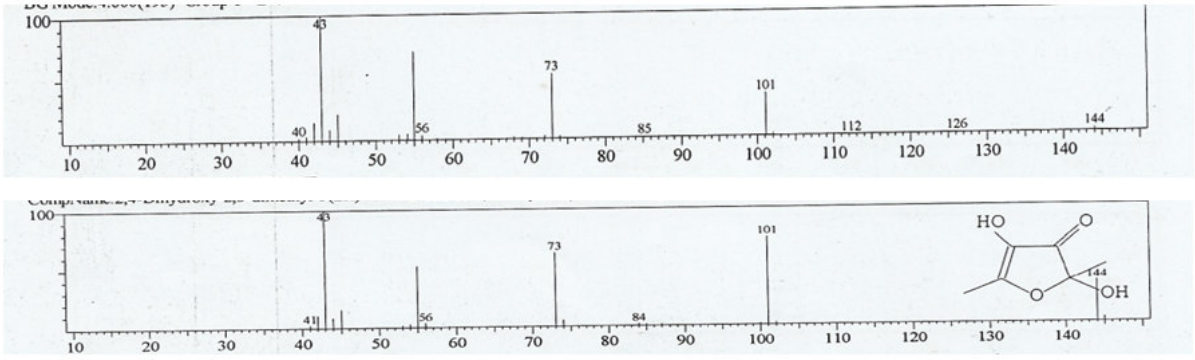


Figure 2: Mass spectrum of 2,4-dimethyl-2,5-dihydroxyl-3 (2H)- furanone(E-1a)

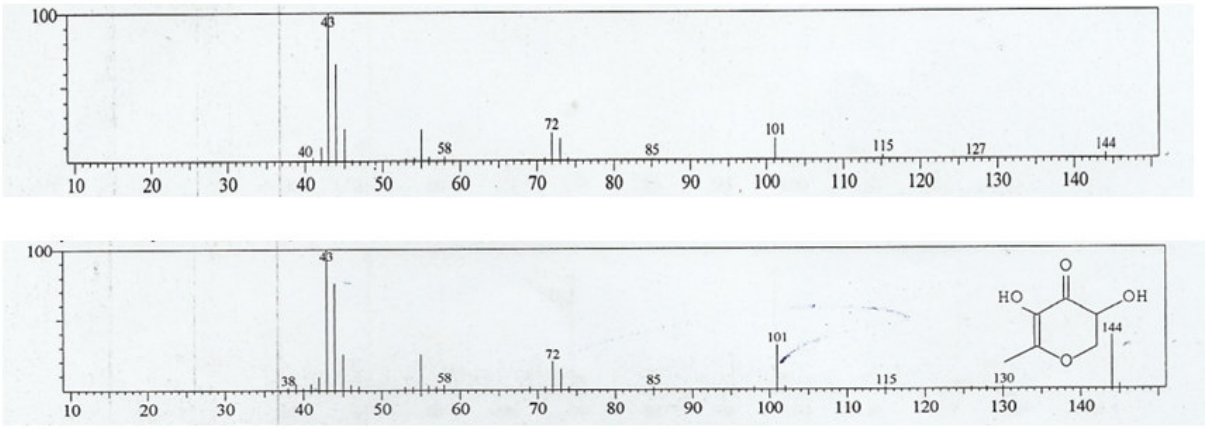
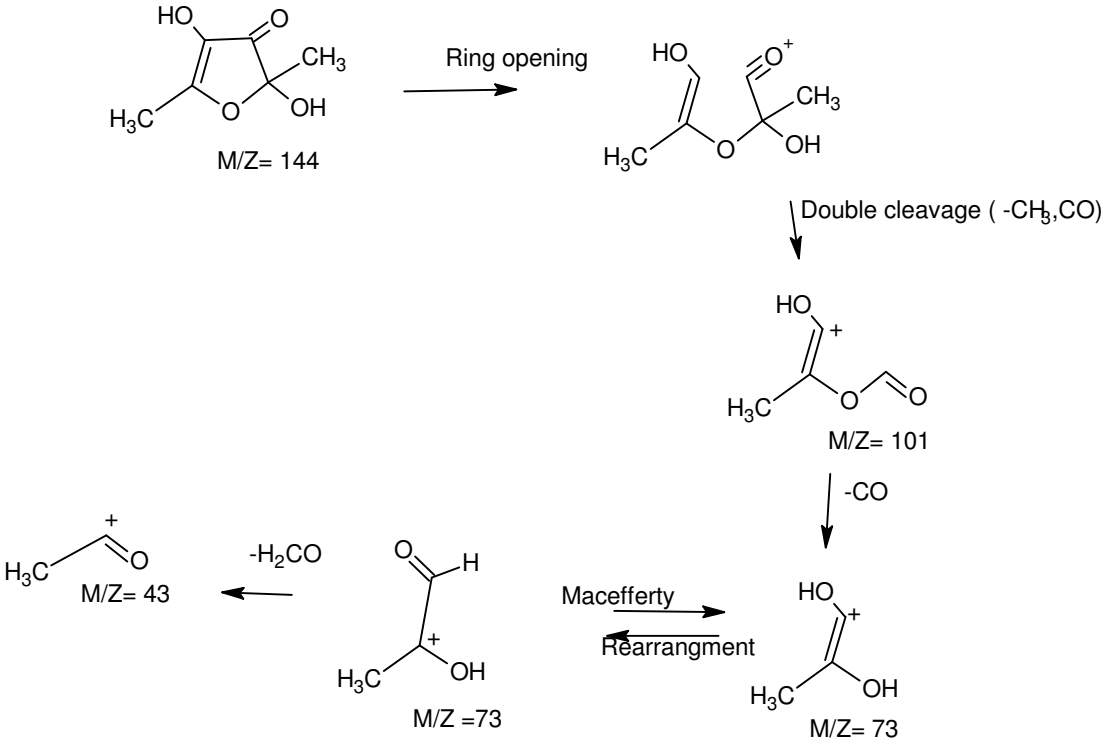
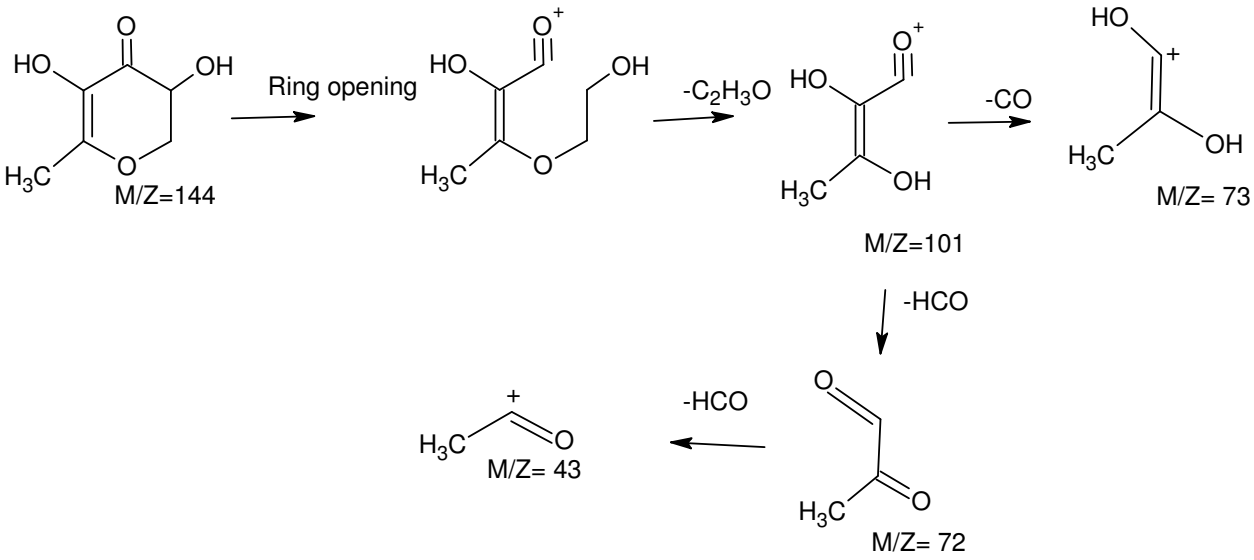


Figure 3: Mass spectrum of 3,5- dihydroxy-6-methyl-2,3-dihydro-4H-pyan-4-one (E-1b)

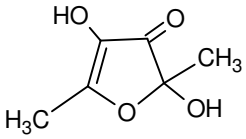


Scheme 1: Fragment ions in 2,5-dimethyl-2,4-dihydroxy-3(2H)-furanone



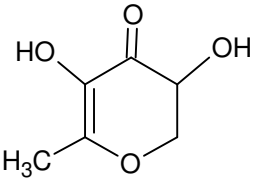
Scheme 2 : 3, 5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one

Structural characterization of compound E-1: The fragmentation pattern in the electron impact-mass spectrometry (EI-MS) was similar to that found in literature, showing strong peak at m/z =101 and was caused by the expulsion of methyl and carbonyl moiety. Further diagnostic fragments at m/z = 75, 101 and 43 originated from the ion m/z = 144 which confirmed the presence of hydroxyl, methyl and carbonyl group in E-1a.



2,5 -dimethyl 2,4 - dihydroxyl - 3 (2H)- furanone

The presence of hydroxyl, carbonyl and gem-dimethyl functional groups were also indicated from the IR spectrum which exhibited strong absorption peaks at 3450 (O-H stretching),1645 (carbonyl), 2990 (C-H stretching), 1450 and1365cm-^{1}(C-H bending). The UV strong absorption at 271-336nm and the IR absorption at 1645cm-^{1} along with GC retention time of 12.892 minutes (Table 11) suggested that compound E-1a is a furanone derivative. The above spectral data were closely related to the spectrum of furanone (2, 4-dimethyl-4-hydroxy- 3(2H)-furanone) a powerful flavouring compound differing from isolated compound in *Synsepalum dulcificum* because it lacks only an additional hydroxyl group. The co-elute (E-1b) has fragmentation pattern similar to that obtained from the library, showing high intensity peaks at m/z = 101 due to the expulsion of an aldehyde group (CH_2=CHO) after ring opening. The peak at m/z = 43 corresponding to peak of highest percentage abundance may be explained as resulting from loss of formyl fragment ion (HCO) at m/z 73. Further diagnostic fragments at m/z = 101, 72 and 43 originated from the ion m/z = 144 which confirmed the presence of hydroxyl, methyl and carbonyl group in E-1b.The presence of different functional groups in E-1b (Table 10) were identified from the IR spectrum, namely carbonyl group (1645 cm-^{1}), C-H stretching (2090cm-^{1}), and hydroxyl group (3450 cm-^{1}). The presence of pyranose (aldehyde) derivative was revealed from the diagnostic absorption bands in UV spectrum at 272-274 nm and IR absorption band at 2090cm-^{1}and 1645cm-^{1}which depict absorptions resulting from aldehydes along with GC retention time of 15.133 minutes suggesting that compound in E-1b is a pyran derivative. The above spectral data was closely related to the spectrum of pyranone a powerful flavouring compound used in food industry. Based on the above information the structure of compound E-1b was established as 3, 5-dihydroxy-6-methyl-2, 3-dihydro-4H-pyranone. Scheme 2 indicates the MS fragmentation pattern of the compound.



3, 5-dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one

Conclusion: Organoleptic guided purification of ethanol (E-1) extract gave 2,5-dimethyl-2,4-dihydroxy-3(2H)-furanone and 3, 5-dihydroxy-6-methyl-2, 3-dihydro-4H-pyranone as the principal flavouring agent. However, 2, 5-dimethyl-4-hydroxy-3(2H)-furanone was reported as the main flavourant principle in pineapple Yoshiro *et al*., 1971. Thus 2,5-dimethyl-2,4-dihydroxy-3(2H)-furanone has a gustatory activity and can be used in food industry as flavourant and also in pharmaceutical industry. The structure of the compounds were established on the basis of IR, UV-Vis and GC-MS spectra.

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