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Isolation and Structure Elucidation of the Bioactive Constituents of Corn Silk (*Zea mays stigma*) Obtained from Abakaliki in Ebonyi State, Nigeria.

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ABSTRACT

The extraction of dried corn silk material was done with different solvents such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether and n- hexane and fractionated by two different solvent systems of n-hexane: acetone: chloroform (3:1:1) and chloroform: methanol: acetic acid (7:3:1) through thin layer chromatography and column chromatography into two components which revealed the presence of two isolates with R_f values 0.85 for isolate A and 0.88 for isolate B respectively. The isolated components were subjected to spectroscopic analyses. The Ultraviolet/Visible spectra (chloroform)(nm) showed the wavelength of maximum absorptions of isolates A and B between 200-800nm. Infrared spectra (KBr) (cm^{-1}) of isolate A showed absorption bands at 3324.8, 2944.6, 1408.9, 1289.7 and 1021.3 while isolate B showed absorption bands at 3339.7 2952.1, 1714.6 and 1021.1. NMR (^{1}H and ^{13}C) analyses revealed the proton and carbon environment of the compounds and finally Gas chromatography-Mass spectroscopy showed molecular weight m/z 554 corresponding to $C_{40}H_{58}O$ and for isolate B m/z 430($C_{27}H_{42}O_4$). Based on the GC-MS spectra isolate A which showed a peak at m/z 554 was probably the molecular weight of Rhodopin, with molecular formula $C_{40}H_{58}O$ while isolate B showed a molecular peak at m/z 430 which suggest the molecular weight for Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost- 8-en-17-yl)- with molecular formula $C_{27}H_{42}O_4$.

Keywords: Bioactive, Constituents, Corn silk, elucidation, Extraction, Isolation, structural.

INTRODUCTION

Extraction is a separation process which involves the separation of natural products from their sources using water or organic solvents [1]. The extraction of natural products progresses through the following stages; the solvent penetrates into the solid matrix, the solute dissolves in the solvents, the solute is diffused out of the solid matrix and the extracted solutes are collected. The purpose of all extractions is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). Its choice and conditions determine the quality and the yield of the constituents. Most herbs contain phytochemicals that provide significant effect to our bodies. In recent time, majority of the world population depends strongly on plants for healing purposes and enhancement of their health status. Medical practices of natives American, Roman, Egyptian, Persian and Hebrew even Nigeria and Africa in general have reported that herbs are used comprehensively to treat nearly every known illness [2]. Like other plant parts, corn silk also has a wide range of traditional usage because of their unique therapeutic properties. Other than healing activities, corn silk could be consumed as tea, used to produce natural antioxidants as well as flavoring agent and food additive [3].

Corn silk has a possible potential usage as a traditional herb to treat diabetes and other diseases. It has diterpenes compound consists of carnosic acid and carnosol. These diterpenes function as activators of glitziness, which is used to treat diabetes [4]. Another study has recommended corn silk to be used as a hypoglycaemic food. Corn silk has also been reported to contain polyphenolic compounds which could act as a herbal drug [5]. Corn silk apart from having proteins, vitamins, carbohydrates, is also an excellent source of fixed and volatile oils, steroid like sitosterol, stigmasterol, alkaloids, saponins and other natural antioxidants like flavonoids. It also contains maizeric acid, resin, sugar, mucilage, fibres that are essential for diet [6] yet it is considered as an agricultural waste or by-product of corn. This recommendation is due to the result of corn silk extract which was able to increase insulin level and heal wounded β-cell [7]. This

research was aimed at isolating and determining the structures of the bioactive compounds in corn silk *(Zea mays stigma*) obtained from Abakaliki in Ebonyi State of Nigeria.

MATERIALS AND METHODS

All weighing was done on JJ600 weighing balance, grinding was done on a heavy duty electric blender, solvents and reagents were of analytical grade and product of BDH company. The UV/ VIS was carried out with HACH DR2000 UV/VIS spectrometer, IR on Agilent FTIR (Cary 630 spectrometer), GC-MS on GC (Agilent 7890N-MS-5975b MSD), NMR on -NMR Nanalysis PRO 60 NMREADY BENCH TOP NMR.

COLLECTION OF PLANT SAMPLE:

Fresh corn silks were obtained from corn fields in Nna street corn farm, EBSU permanent site, Obegu corn farm, Igbegu corn farm and other farm clusters all in Abakaliki, Ebonyi State between May and August 2020 and 2021 AND STORED.

PREPARATION OF PLANT SAMPLE

Drying of the collected sample materials

The fresh corn silks 11200g collected from different corn fields were cleaned from dust, washed and sun dried according to [8] until a constant weight was obtained 1425g. The dried silk was ground to fine powder and sieved through a 100-mesh sieve to a particle size <150 µm. The powdered sample was stored in an air tight and dried polyethene bag during the period of study.

Extraction methods

The corn silk powder was extracted by direct and sequential extraction by a series of solvents with increasing polarity order in terms of their dipole moments (Debye, D) hexane: 0.0, petroleum ether, chloroform: 4.1, ethyl acetate:2.8, ethanol, methanol: 5.1 and water: 9.2 were used for extraction by each method. The extracts were filtered with Whatman filter paper No.1 (185mm) and concentrated under reduced pressure using rotary evaporator in other to recover the solvents. The percentage yield of each extract was calculated in terms of total extractable component (TEC). After drying by evaporation, the extracts were stored in air tight containers.

Yield

Yield is the final result or the amount of product obtained. Its percentage calculation was done based on the final weight % of a product produced by the net weight of the raw materials used by extracts yield of corn silk. It was calculated using the formula in equation 1;

$$\%\text{Yield} = \frac{\text{Extract weight}}{\text{The initial raw material}} \times 100 \quad \text{equation 1}$$

Isolation of components

Thin layer chromatography (TLC) investigation

To isolate the components of the corn silk extracts, different solvent systems were tested in order to obtain a better separation of the extracts. An aliquot of the extracts was dissolved in their solvent of extraction in which they are soluble in such as methanol, ethanol, ethyl acetate, chloroform, petroleum ether and n-hexane. The solutions were spotted on a pre-coated silica gel GF_{254} aluminum plates, a migratory distance of 8 cm with the aid of a capillary tube closed at one end. It was developed using two different solvent systems which include, n-hexane: acetone: chloroform (3:1:1) and chloroform: methanol: acetic acid (7:3:1) which revealed two spots. Elution and reverse elution were carried out in other to ascertain the actual R_f values. The Chromatograms obtained were viewed and visualized with human eyes and iodine vapor. Their Retardation factor (R_f) values were calculated using equation 2 shown below;

$$R_f \text{ value} = \frac{\text{Distance travelled by the component}}{\text{Distance travelled by the solvent}} \quad \text{equation 2}$$

Column chromatography

A series of column chromatography was performed on extracts using columns of 30cm x 3.06cm and 36cm x 2.67cm respectively. Exactly 0.2g of the extracts were collected, dissolved with

their extractable solvents and introduced into the column packed with 60-120 mesh silica gel using wet and dry packing methods. The eluates were exposed to air for some days to obtain the solid crystals. The isolates were subjected to spectroscopic analysis in order to elucidate the structure of the components.

Characterization of the compounds via spectra analysis

The isolated components were subjected to UV, IR, NMR (^{13}C and ^{1}H) and GC-MS spectra analyses in order to determine the probable structures of the active components.

UV analysis

The samples were dissolved in appropriate solvent and run on UV HACH DR2000 spectrophotometer to obtain the concentration at the wavelength of maximum absorption.

GC-MS analysis

2µl of the sample extract was injected into the GC column for analysis. The GC (Agilent 7890N) and MS (5975B MSD) was equipped with DB-5ms capillary column (30 m x 0.25 mm; film thickness 0.25 µm). The initial temperature was set at 40°C which increased to 150°C at the rate of 10°C/min. The temperature was again increased to 230°C at the rate of 5°C/min. The process continued till the temperature reached 280°C at the rate of 20°C/min which was held for 8 minutes. The injector port temperature was 250°C then. Helium was used as the carrier gas with a flow rate of 1ml/min. Split ratio and ionization voltage were 110:1 and 70 eV respectively. To identify the unknown components, present in the extract, their individual mass spectral peak value was compared with the database of National Institute of Science and Technology 2014. Then the Phytochemicals were identified after comparing the unknown peak value and chromatogram from GC-MS against the known chromatogram, peak value from the NIT Library database. Subsequently, the details about their molecular formula, molecular weight, retention time and percentage content were also obtained.

FT-IR analysis

0.5ml of the sample was mixed with ground potassium bromide. This mixture was placed onto the face of a KBr plate and the second window is placed on top. With a gentle circular and back and forth rubbing motion of the two windows, evenly distribute the mixture between the plates. The mixture then appears slightly translucent. The sandwiched plates were then placed in the spectrometer. The Fourier transform infrared spectrum was recorded using Agilent FTIR cary 630 spectrometer in the wavelength range 400-4000 cm^{-1} by potassium bromide pellet technique with a resolution and scanning speed of 4 cm^{-1} and 2 mm/sec respectively.

NMR analysis

The 1H and 13C spectra were obtained using NMR NANALYSIS PRO 60 spectrometer with deuterated chloroform as solvent 60MHz.

Melting point determination

The melting point (MP) of a compound is the temperature at which the solid changes to the liquid state under a pressure of one atmosphere. For pure compounds the melting point is quite sharp. Impure compounds melt over a wide range. The melting point of a compound is a characteristic of the compound as well as a measure of its purity. Samples were introduced into the melting point tube (capillary tube) sealed at one end. The tube was fixed to the thermometer (360°C) by dipping the thermometer into the liquid paraffin contained in a 150ml beaker all clamped to the retort stand. The capillary tube was placed against it side with the bottom of the mercury bulb. The capillary attraction of the film of oil is quite sufficient to hold the tube to thermometer. Having filled up the apparatus the melting point of the compound was determined by heating the beaker with a small Bunsen flame so that the temperature of the oil rises slowly. The melting point was taken at the temperature at which the crystal changes to liquid.

RESULTS

Extraction result

The extraction of the bioactive compounds of corn silk was done with different solvents; ethanol, methanol, ethyl acetate, chloroform, petroleum ether and n- hexane directly and

sequentially. The result recorded as yield and percentage yield is shown in Table 1. The results of yield and percentage yield of the isolate is in Table 2 while the melting point determination of the isolates is shown in Table 3.

Table 1: Yields of Extract from the Extracting Solvents (Direct/ Sequential Extraction)

Direct solvents	Yield (g)	%Yield	Sequential Solvents	Yield(g)	%Yield
Ethanol	4.8	0.048	Ethanol in Pet ether	3.0	0.33
			Ethanol in Chloroform	2.1	0.23
			Ethanol in n-hexane	2.7	0.3
Methanol	7.6	0.076	Methanol in Pet ether	2.1	0.23
			Methanol in Pet ether/n-hexane	0.9	0.11
			Methanol in Chloroform/n-hexane	0.6	0.07
n-hexane	1.5	0.015	Pet ether in n-hexane	0.2	0.02
			n-hexane in Chloroform/Pet ether	0.06	0.007
			n-hexane in Ethanol	0.67	0.074
Chloroform	2.9	0.029	Pet ether in Chloroform	0.5	0.055
			Chloroform in Ethanol	0.3	0.033
			Chloroform in Methanol	0.2	0.022
Petroleum ether	0.9	0.09	Pet ether in n-hexane	0.5	0.055
			Pet ether in Chloroform/n-hexane	0.07	0.008
			Pet ether in Ethanol	0.4	0.044
Ethyl acetate	2.4	0.024	Ethyl acetate in Chloroform,	1.3	0.144
			Ethyl acetate in n-hexane,	1.0	0.111
			Ethyl acetate in Pet ether/n-hexane	0.6	0.071

Table 2: R_f value and yield of the isolated compounds (TLC)

Isolated compound	R_f value	Yield(g)	Yield%
Sample A	0.85	2.00	40
Sample B	0.88	2.70	54

Spectroscopic analyses

i UV/Visible spectra showed absorption maxima at 280nm,530nm,570nm,670nm and 770nm by isolate A while isolate B showed at 260 nm,530nm,570nm,680nm and 770nm.

ii IR spectra (cm^{-1}) of sample A showed absorption at 3324.8 (broad), 2944.6, 2519.7, 1565.5, 1408.9, 1289.7 and 1021.3(sharp) while sample B showed absorption at 3339.7(broad stretched), 2952.1, 1714.6, 1401.5,1267.3 and 1021.1(sharp).

iii ^1H NMR (60HMz $CDCl_3$) spectra of sample A showed chemical shift (δ ppm), at 1.25, 2.5 and 4.75(multiplet) while sample B showed at 0.5-2.5 (doublet), 3.625, 4.32, 7.65 and 8.25. ^{13}C NMR spectra for sample A showed peaks at 5, 12.5, 14, 18, 20, 28, 29,31, 32, 36, 37, 45,46, 51, 52, 53, 54,56,58, 62, 69,72, 76, 77, 79, 80,116, 121, 122, 124, 125, 126,130, 132,139, 142, 144, 156, 166 and 174 while that of sample B showed chemical shift (δ ppm) at 3.6, 4, 8, 11, 12, 18, 24.50, 45, 48, 50, 57,58, 75, 79, 95 ,97, 112, 117 140,147,148, 160, 162, 165, 178,182 and198.

iv GC-MS: For sample A m/z 554 $C_{40}H_{58}O$ for the compound, 18 [M]+, calculated for H_20^+, 28

$(CH_3CH)^+$, 41$(C_3H_5)^+$, 55$(C_4H_7)^+$, 69$(C_5H_9)^+$, 81$(C_6H_9)^+$, 105$(C_7H_5O)^+$, 133$(C_{10}H_{13})^+$, 145 $(C_{11}H_{15})^+$, 157$(C_{10}H_{21}O)^+$, 171$(C_{11}H_{23}O)^+$ 209$(C_{14}H_{25}O)^+$, 430$(C_{32}H_{46})^+$, 536$(C_{40}H_{56})^+$ and 554$(C_{40}H_{58}O)$.

For sample B m/z 430$(C_{27}H_{42}O_4)$ for the compound 29$(C_2H_5)^+$, 43$(C_2H_3O)^+$, 55$(C_3H_3O)^+$, 69$(C_5H_9)^+$, 83$(C_6H_{11})^+$, 121$(C_9H_{13})^+$, 159$(C_9H_{19}O_2)^+$, 173$(C_{10}H_{21}O_2)^+$, 187$(C_{11}H_{23}O_2)^+$, 213 $(C_{13}H_{25}O_2)^+$, 233$(C_{12}H_{25}O_4)^+$, 281$(C_{17}H_{29}O_3)^+$, 355$(C_{23}H_{31}O_3)^+$, 415$(C_{26}H_{39}O_4)^+$ and 430 $(C_{27}H_{42}O_4)$.

Table 3: Melting Point Determination Results

Isolates	Temperature ^oC
Sample A	74
Sample B	173

DISCUSSION

Extraction of corn silk

The extraction was mostly done in organic solvent for easy evaporation for 72hrs and a solid brownish extract was obtained. The organic solvents used were methanol, ethanol, ethyl acetate, chloroform, petroleum ether and n-hexane as recorded in Table 1 which showed that methanol haven the highest yield followed by ethanol thus 300g of the dried ground corn silk was soaked in 1500ml of each of the solvents for 72hrs and sequentially, methanol gave 7.6g which shows 0.076% making methanol the best extracting solvent. Secondly in terms of the yield on the actual extract, when 5g of the extract was eluted in a column, 2g of isolate A and 2.7g of isolate B were obtained which amount to 40% and 54% yield respectively suggesting that the %yield is fair according to [9] yield rating. Based on the extraction yield result rating, corn silk will be best harnessed whole or raw.

Characterization of compound

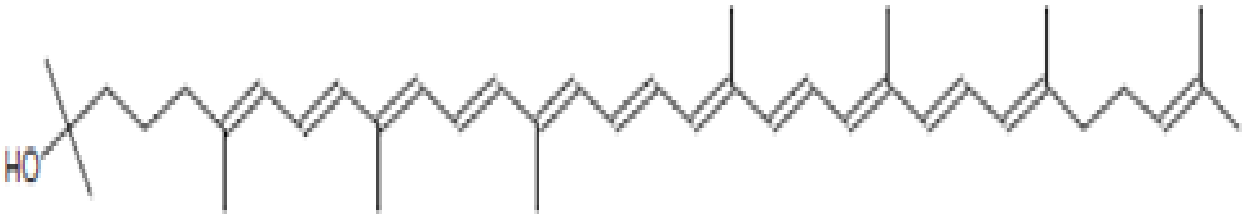
Thin layer chromatogram revealed the presence of two spots and with further chromatographic fractionation, the spots named isolate A and isolate B were obtained. The presence of these compounds justified the uses of this plant for treating different diseases Uv / visible spectra isolate A showed absorption in the Uv region and in the visible region. This is consistent with its milky yellow color while isolate B showed absorption at uv visible region. The FTIR spectra ran on KBr plate for isolate a showed a significant absorption at 3324.8cm^{-1} (broad band), characteristic of a hydroxyl (-OH) group. It also showed a ragged peak at 2944.6 cm^{-1} indicating C-H and at 2519.7 cm^{-1} which signaled extended conjugation. There was also a peak at 1565.5cm^{-1} showing the presence of C=C. The infrared spectra showed a peak at 1408.9cm^{-1} which is associated with methyl (-CH_3) group. A peak appeared at 1289.7cm^{-1} which is a characteristic with C-O and finally there was a strong sharp signal at 1021.3cm^{-1} which confirms C=C while isolate B showed a broad stretched band at 3339.7cm^{-1} which is significant with hydroxyl(-OH) group of carboxylic acid. It also showed significant peaks at 2952.2cm-1 and 2822.8 which indicate the presence of C-H bond. The peak at 1714.6cm^{-1} shows the presence of carbonyl (C=O) group of carboxylic acid. While the peak at 1401.5cm^{-1} is significant with C=C of aliphatic ring. The peaks at 1267.3cm^{-1} and 1110.7$cm^{-1}$are consistent with methyl (-CH_3) group. The peak at 1021.3cm^{-1} predominantly shows the presence of C-O band. The ^1H NMR spectrum ran on chloroform-d at 60MHz for isolate. A showed signals for three different types of protons. The proton signaled at 1.25ppm which appeared doublet which is characteristic of methylene (-CH_2) proton of

unsaturated alkene. The proton peak at 4.625 which appeared multiplet is indicative of adjacent proton of methine (-CH) group of unsaturated hydrocarbons while the peak at 2.25 is a signal for methyl proton group but due to conjugation effect and distortion caused by impurities, it appeared downfield (deshielding effect). ^{13}C NMR spectrum of isolate A showed several peaks of different carbon environments. There is an absorption peak at 124 -130 indicating unsaturated conjugated alkene system which appeared upfield due to shielding effect of the electronegative group -OH. The peaks at 77-79ppm (doublet) could be as a result of a hydroxyl group attached to a tertiary alkanol carbon, causing a shielding effect on the entire structure, which is evident signal characteristic of a carbon single bonded to oxygen (C-O). The methyl carbon at different positions of the compound correspond to the peaks at 5, 12.5, 14, 16, 18, 20, 28, 29 and 31. The peaks at 32, 36, 37, 45, 46,51 and 52 are indicative of methylene carbon atoms. The methine carbons of the compound are showed at peaks 53, 54, 56, 58,62,68,69, 72 and 76. The remaining peaks at 81, 116, 122,124, 130, 166, 174 are as a result of carbons that are non- proton bonded.

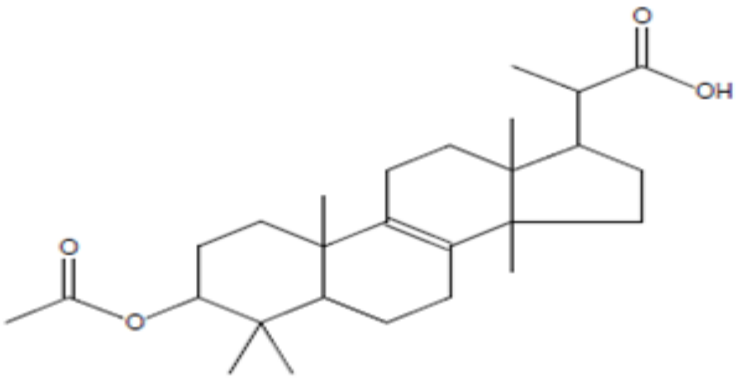
Isolate B proton NMR spectrum showed four signals at different types of proton environments. The peak at 0.5- 2.5ppm (doublet) is characteristic of chemical equivalent protons of methyl group overlapping a methine group which invariably resulted to the split. The signal slightly appeared downfield due to the shielding effect of the electron withdrawing group -COOH. The signal showed at 3.625ppm is characteristic of methyl protons of alkanoate. The proton peak at 4.35 is evident of the presence of methylene proton of an ester. Finally, the weak peaks at 7.6ppm and 8.25 are associated with the presence of a methine protons of the compound. The carbon peaks shown between 75-79ppm are characteristics of C-O of the carboxylic acid and alkanaote groups of the compound. The peak at 198 is characteristic of the carbonyl groups of carboxylic acid and ester groups of the compound. The peaks signalled at 140-162ppm is an indication of the presence of carbon -carbon double bond of a cyclohexene. Each of the carbon -carbon single bond of the cyclo compound showed a peak at 24.50. The peak at 26.5ppm is a signal for the quantenary carbon or non-protonated carbon. The methine carbon showed peaks at 45, 48.50, 57 and 58. The signal peak at 95ppm is as a result of the carbon singly bonded to electronegative oxygen (C-O). Finally, the methyl carbon at different positions of the compound showed peaks at different positions of the spectrum, 8, 11, 12 and 18ppm. With these structural elucidation, the compounds were proposed.

The GC-MS spectrum isolate A showed different fragments of the compound with the highest fragmentation peak at m/z 554 which indicates the molecular weight of Rhodopin or lycopene, 1,2-dihydro-1-hyroxy- and molecular formula $C_{40}H_{58}O$ while compound B (isolate B) spectrum showed the strongest fragmentation peak at m/z 430 which indicates the molecular weight for Propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost- 8-en-17-yl)- or 1,2-(3-Acetoxy-4,4, 10, 13, 14-pentamethyl- 2, 3, 4, 5, 6,7,10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)-propanoic acid and molecular formula $C_{27}H_{42}O_4$. Based on the UV/Visible, FTIR, NMR (^1H and ^{13}C) and GC-MS spectra results, we propose that the two isolates obtained from chromatographic separation of corn silk extracts obtained from the corn cultivated in Abakaliki and its environ are Compound A (isolate A) is rhodopin, a milky yellow crystal with extensive conjugation in its structure which belong to the class of carotenoids while Compound B (isolate B) is propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrost- 8-en-17-yl)- which belong to the class of steroids. The two compounds isolated and characterized in this study are different from the ones isolated by Scholars from various countries. For instance, according to [10] corn silk contains phenolic compounds particularly flavonoid. [11] reported that a flavonoid, 3'-methoxymaysin and reduced derivatives of maysin have been isolated and identified from corn silk of several corn inbreeds. The compound isolated were 2"-O-α-L-rhamnosul-6-C-quinovosylluteolin, 2"-O-α- L-rhamnosyl-6-C-fucosylluteilin and 2"-O-α-L-rhamnosyl-6-C-fucosyl-3'-methoxyluteolin. Five other flavonoids derivatives were also isolated from corn silk ethanol extract (80%) according to [12] and identified as 2"-O-α-L-rhmnosyl-6-C-3"-deoxyglucosyl-3'-methoxyluteolin, 6,4'-

dihydroxy-3'-methoxyflavone-7-O-glucoside, ax'-5'-methane-3'-methoxymaysin, ax-4"-OH-3'methoxymaysin and 7,4'-dihydroxy-3'-methoxyflavone-2"-O-α-L-rhammosyl-6-C-fucoside in corn silk from China. It has also been reported according to [3] that a total of 36 compounds were identified by GC-MS result of dichloromethane extract of Egyptian corn silk in which more than 99%of the volatile compounds obtained in the extract were terpenoids in which the main constituents were cis-α-terpinol, citronellol, 6,11-oxidor-4-ene mostly used in perfume and flavour industries. The difference in type and nature of compounds isolated could be as a result of locality, region and species of corn [12]. The types of solvents and methods of extraction used could be responsible or contributory factors. Above all the results of all the Scholars and researchers have shown that is a valuable asset and should not be treated as a waste agricultural product or by-products.



Isolate A



Isolate B

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