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Research Article

NOVEL COMPOUNDS WITH ANTIULCEROGENIC POTENTIALS UNVEILED IN PLANTAIN LEAVES ISOLATES (MUSA PARADISCIACA)

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ABSTRACT

Ethanolic extract of plantain leaves was tested on forty eight ethanolic induced gastric ulcer rats for 28 days. The extract was found to posses potent antiulcerogenic effects. The isolates of such was analysed with Nuclear magnetic resonance (NMR) equipment and found to have antiulcerogenic compounds. The compounds have two carbon atoms without hydrogen bonding at ^{13}C NMR with 29.430 PPM and at 62.097PPM of isolate A and B respectively. The isolates displayed same characteristics in hydrogen and carbon atoms indicating that they may be the same compound. This characterization has shown a structure of a compound that has not been characterized before and the new potent herbal drug for the treatment of gastric ulcer.

Key Words: Plantain leaves isolates, new compounds and gastric ulcer.

INTRODUCTION

Plantain plants belong to the family musa ceae; genus, musa and species; *Musa paradisciaca*. The fruits are of economic importance, eaten as unripe and ripe. The unripe fruits posses antidiabetic properties, (Jimmy, 2012). The phytodemical contents of the plants include phenolic glycosides acids, terpenoids, sterols, alkaloidal amine, tannin etc, (Trease, 1996). The leaves of plantain are traditionally used for the treatment of diabetes, diarrhoea, hypertension, gastric ulcer, kidney disease etc. There is no scientific documentary proof of the use of the leaves extract for the treatment of gastric ulcer. Gastric ulcer is the disease of the gastrointestinal system mainly affecting the stomach. The stomach is J-shape structure, located between the esophagus and the duodenum. Its main functions are storage, mixing and digestion of food, (Guyton, 2011). The digestion of food e.g protein requires the presence of hydrochloric acid needed for the break down of pepsinogen to pepsin to enable pepsin to act on protein. The secretion of the acid is done by parietal cells at the canalicular system involving the ejection of positive hydrogen ions into the canal for its composition with chloride ion, (Oyebola, 2002). The release of acids needs the presence of food, gastrin and histamine and their actions on cholescystokinin (CCK) and histamine receptors. Increase presence of these secretions affects the mucosal linings of the stomach and will cause a break into the mucosal barrier resulting in ulceration of mucosa, hence ulcer. About 400 million people suffer from peptic ulcer annually in the USA (Harvey, 1981) the prevalence rate is 5-15% globally (Sung 2009) and in Nigeria is about 5%, (Smith 2002). There is no 100% potent drug for the treatment of peptic ulcer for now but there are many ulcerogenic substances, foods, and drugs in our environment. The morbidity and mortality rate of this disease in increasing particularly the associated bleeding. (Sonnenberg 1997). This study was carried out with a view to finding a potent herbal drug for the treatment of gastric ulcer using plantain leaves extract, isolates with antiulcerogenic related structurally analysed compounds.

MATERIALS AND METHODS

The plantain leaves extraction: Two kilograms (2kg) of the fresh plantain leaves were washed, peeled and chopped into small pieces, air dried for one week, and made into powder form using mortar and pestle. The powder form (600g) obtained was dissolved in 70% ethanol and kept for 72 hours for extractive ingredient to be obtained. The liquid extract obtained by filtration was concentrated to dryness in a vacuo at 40^0C to yield dry ethanol extract, methods of Trease and Evans, 1996 and Ross 1977 were used.

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Chromatography of the Extract

Three chromatography methods were used; partition, column and thin layer in the purification of the extract and isolates of plantain leaves and separation of the active ingredients from other compounds, (Arun 1993). The methods are based on the principle of affinity of the compounds to the solvent, mobile phases, stationary solid phase, solubility to the solvent and the polarity of the solvent relative to the compounds to be extracted.

Partition Chromatography

This was done according to methods of Trease and Evans 1996. Plantain leaves extract was placed in 500ml flask with a tap and clamped on a clamp stand.

Four solvents, N-hexane, chloroform, butanol and ethyl acetate each of 200ml were used in the extraction. Solvents were added based on their degree of polarity, first was N-hexane, and its fraction was obtained after 15mins, followed by chloroform and its fraction obtained after 15mins, then butanol and its fraction obtained after 15mins and finally ethyl acetate and fraction obtained after 15mins.

Air Drying of the Fraction

The fraction of N-hexane, chloroform, butanol, and ethyl acetate were air dried at room temperature to rid of the solvents.

Application of Fractions in thin layer Chromatography

The essence of thin layer chromatography in the study was to resolve the presence of the different fractions in the extract and also to confirm the results of the partition chromatography indicated by the dissolution of the different fractions in the very solvent the fractions were extracted.

Preparation

The fractions were air dried to rid of solvents. A capillary pipette was used in collecting small quantity of the fractions and spotted on the thin layer chromatography plate with ethanolic extract spotted as original of the fractions.

The spotted TLC plates were placed in beakers of their respective extracting solvent system and allowed to develop for 5-10mins, the different compounds were identified.

Column Chromatography: In this method the most potent of the fractions in the treatment of gastric ulcer i.e n-hexane was used. The principle is based on the fact that the material to be separated is applied in the column and the mobile phase allowed to run through the adsorbent (the silicagel) by the force of gravity and the components of the mixture separated based on their retention by the absorbent polarities and solvent of appropriate elutropic strength.

Methods: The empty column was plugged with cotton wool and tap closed at the bottom. Powdered silica gel was measured $^{2}/_{3}$ of the column N-hexane was added until the silica was finally packed. 1.1g of the n-hexane fraction was added through the column funnel and collected as different aliquots and air dried as isolates.

Analysis of the Structural organic compounds of the Isolates by Nuclear Magnetic Resonance (Aue 1976)

Nuclear magnetic resonance (NMR) was used in the analysis of the organic compounds: It is based on the absorption principles which in a magnetic field the sample(s) applied will absorb electromagnetic radiation in the radio frequency region based on the frequency of the sample applied and the absorption is a fraction of the nuclei in the molecule of the compound.

Methods: Pure isolates A and B obtained from n-hexane fraction were used 20mg of each were analysed using NMR machine.

RESULTS

A spectra chart containing needed peaks representing protons in each compound were obtained, fig.1 & 2. A carbon-13 peaks representing the total number of carbon atoms present in the compounds of the two isolates were recorded. The results showed different hydrogen protons. In carbon ^{13}NMR. In isolate A, there are twenty five (25) carbon atoms with 25 CH_2 and one novel carbon atom without hydrogen bonding. In ^{13}CNMR isolate B, there are 14 carbon atoms with ten CH_2, two (2) CH one (1) CH_3 and one questionable carbon atoms without hydrogen.

TABLE 1: ISOLATES A ^{13}C NMR

	PPM	Carbon/Hydrogen Bond	Peakheight
1.	179.589	CH_2	-13.5
2.	130.002	CH_2	+62.3
3.	129.709	CH_2	+62.3
4.	77.640	CH_2	-49.4
5.	77.003	CH_2	-48.7
6.	68.364	CH_2	+8.4
7.	65.018	CH_2	-12.8
8.	62.097	CH_2	-7.8
9.	34.079	CH_2	-18.0
10.	33.991	CH_2	-33.8
11.	31.897	CH_2	-48.3
12.	29.782	CH_2	-57.0
13.	29.671	CH_2	-86.7
14.	29.671	CH_2	-86.7
15.	29.510	CH_2	-53.4
16.	29.430	-C-	(Novel)
17.	29.306	CH_2	-94.0
18.	29.129	CH_2	-62.8
19.	29.056	CH_2	-70.3
20.	27.204	CH_2	-56.3
21.	27.145	CH_2	-49.9
22.	24.861	CH_2	-22.7
23.	24.671	CH_2	-36.9
24.	22.672	CH_2	-45.3
25.	14.084	CH_2	-144.9

TABLE 2: ISOLATE B ^{13}C NMR

	PPM	Carbon/Hydrogen Bond	Peak Height
1.	130.009	CH_2	+15.8
2.	129.702	CH_2	+11.7
3.	77.633	CH_2	-103.8
4.	77.003	CH_2	-97.3
5.	76.359	CH_2	-100.2
6.	62.097	-C-	no Hydrogen atom (Novel)
7.	31.904	CH	-14.4
8.	29.701	CH	-30.9
9.	29.320	CH_2	-18.0
10.	29.173	CH_3	-34.9
11.	29.108	CH_2	-27.4
12.	27.180	CH_2	-20.4
13.	24.832	CH_2	13.7
14.	22.679	CH_2	?

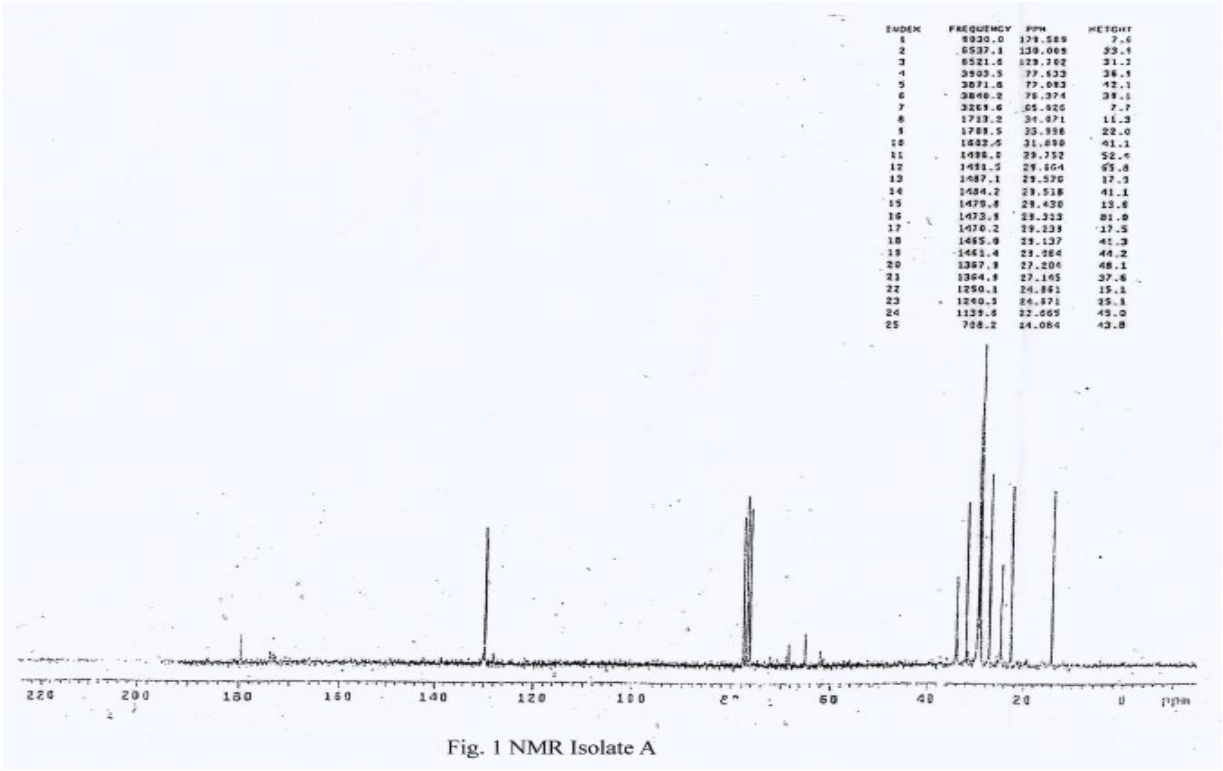

Fig. 1 NMR Isolate A

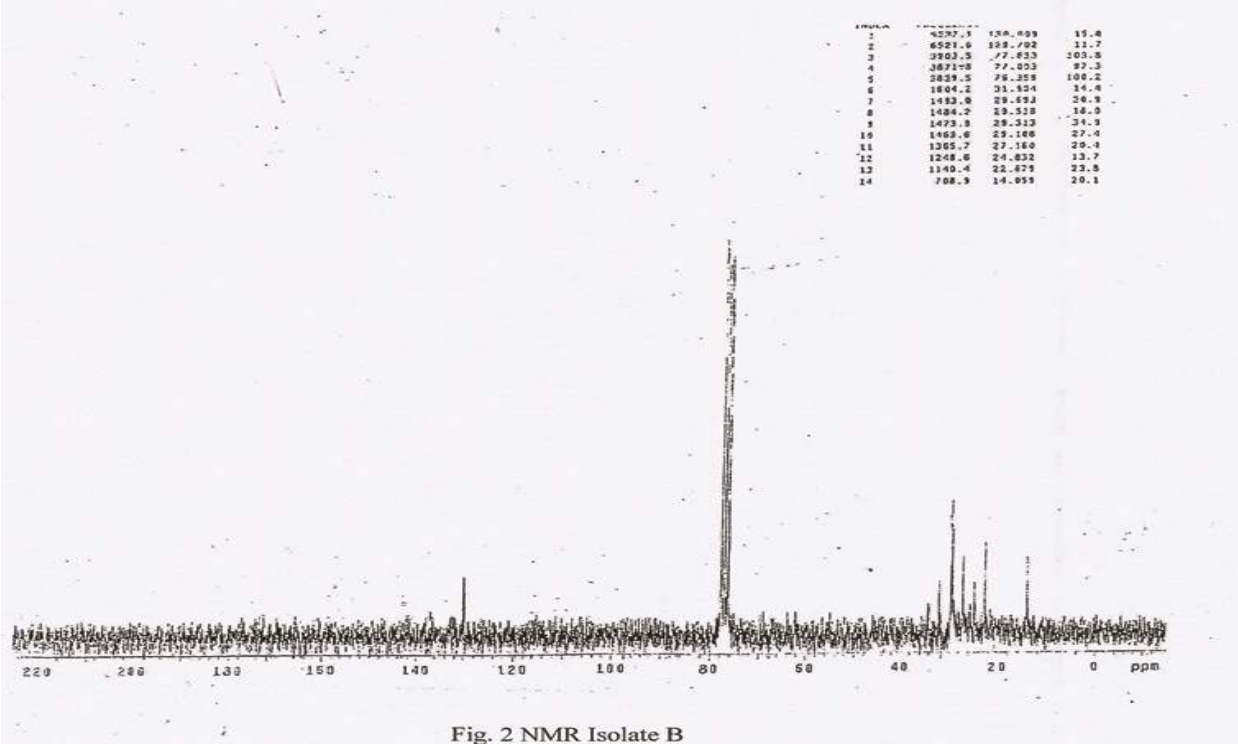

Fig. 2 NMR Isolate B

DISCUSSION

The results have shown that both isolates displayed same characteristics on hydrogen and carbon atom binding indicating that they may be the same compound. The results of this characterization have a shown the necessary structure of the novel compound and the derived potency for herbal anti ulcerogenic drug. The two carbon atoms discovered in the two isolates without hydrogen bonding has confirmed the novelty of the compounds and its

being new. This is the first time a compound of this nature is unveiled. The application of this herbal remedy in the treatment of gastric ulcer has effective potentials in the healing process because of the mechanism of its action. For example; its antioxidant activity, mucosal proliferation stimulation, acid production and secretion inhibition as with the use of proton pump inhibitor (PPI), (Javid 2001) and omeprazole increase mucus production which the gastric need in protecting the gastric mucosa (Lichtenberger, (1995), (Konturex 1991), barrier and ability to inhibit inflammation which this extract possess in its phytochemical analysis.

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