

Gas Chromatographic Mass Spectrometric Analysis of *n*-Hexane Fraction of the Leaves of Sugar Beets (*Beta vulgaris*)

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Summary: The *n*-hexane fraction of the leaves of sugar beet was analyzed qualitatively and quantitatively using GC-MS and GC equipped with FID in separate experiments. The crude was extracted using ethanol and fractionated by *n*-hexane, ethyl acetate and chloroform in the increasing order of polarity. Only the *n*-hexane fraction was analyzed using GC-MS and GC equipped with FID. The nature of products was confirmed using total ion chromatograms as well as fragmentation pattern. The concentration of the major compounds was determined using the peak area. The *n*-hexane fraction was found to contain esters in addition to small quantities of other compounds.

Keywords: *Beta vulgaris* Lamina, Anti-pneumonia oil, *n*-hexane fraction, GC-MS analysis.

Introduction

Identification of the new resources and resource recovery is essential for supporting the growing population of the world. Food, fuels, medicines and life supporting material are under investigations and explorations.

The main focus is on renewable sources which are mainly from the plants/biological system. Among the plants sugar beet and sugarcane are important due to their food and fuel value. These are the major sources of the sugar and source of the biofuel in terms of alcohols as fuel and as raw material of the biodiesel [1-3].

These plants also contain bioactive compounds [4] *i.e.* sugarcane contains antioxidants which are useful for several biochemical processes such as slowing the aging process and inhibition of the intoxication of liver.

Recently the antimicrobial activities of the extracts from the leaves of sugarcane and sugar beet have been reported in literature [5, 6]. Among these, the non-polar fraction of the leaves of sugar beet was found to be very active and is mainly composed of the oils. The compositions of oils are important in order to explain the activity which changes with change in the composition of oil and the nature of microbes.

Essential oils of many plants were found to have antimicrobial activities [7-10]. In many cases these oils act as antimicrobial agents due to the formation of coating on the leaf surface which block some of the essential components of the cells [11]. It

may also be due to the rupture of the cell wall due to chemical interaction.

Based on the high activity of the non-polar fraction, the composition of *n*-hexane fraction of the leaves extract of sugar beet was investigated. This enables us to investigate the active ingredient of this fraction. Attempts were also made for the correlation of the bioactivity with the nature of components.

Results and Discussion:

Qualitative analysis of the *n*-hexane fraction was conducted on a mass spectrometer GC-MS 600 H Jeol. The extract was separated into its components using Agilent 6890 N₂ gas chromatograph equipped with a fused capillary column (HP.5 L = 30 meter I.d.; 0.32 mm film thickness 0.25 μ m) with polydimethylsiloxane as the stationary phase. The carrier gas was helium at a flow rate of 1.8 mL/min. Condition for analysis: injection mode was split. The column was held at 60 °C for 2 min and then heated to 260 °C at the rate of 5 °C per minute while injector temperature was 260 °C. The identification of the components of the oil was confirmed using total ion chromatograms as well as fragmentation pattern. The list of the compounds identified is given in Table-1. The quantitative analysis of the fraction was carried out using the same type of GC and under the same conditions but equipped with FID. The concentration of the components of the oily fraction was determined using the peak area. This was calculated by the software of the Agilent GC. The chromatogram obtained of this analysis is given in Figure-1 and the list of the major compounds and their concentrations are given in Table-1. It can be

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seen from the results that the oil contains higher quantity of the 4-(pent-3-en-1-ynyloxy)but-2-enyl-8-hydroxyoctanoate with a relative concentration of 38.38%, encompasses an ester moiety of long chain carboxylic acid. It also has significant numbers of unsaturated sites which are responsible for its oily nature. The un-saturation indicates that this will be oily rather than wax like in nature. This compound also contains ethereal site which also play significant role in the antimicrobial action [12]. Octadec-1-ene-3,13-diol is the next in concentration having relative concentration of 14.87% Table-2. It also has an ester functionality of long chain carboxylic acid but it is a saturated ester and may behave like a wax. The viscous nature of the extract can be attributed to this compound. It also contributes to the antimicrobial activity of the extract. The rests of compounds of this extract are found to appear in smaller quantity and all of them are unsaturated in nature. It can be seen from the results that major fraction of the extract is composed of esters of long chain carboxylic acids and the rests are either alcohols or ethers both of which are reported with antimicrobial properties.

Experimental

Material and Methods

Plant Material

The work was carried out using the leaves of kawewurma (Germany) variety of the sugar beets which was grown in the Mardan area (Khyber Pakhtun Khwa) of the Pakistan. The leaves were collected in the month of June during the harvesting season. All the leaves were properly cleaned by washing with tap water followed by distilled water. These leaves were placed in the hood for twenty four hours to evaporate the water. These were further dried in the same at the ambient temperature and ventilated conditions outside the hood. It was observed that the rate of drying the midribs is slower than the laminae therefore the midribs were separated from the laminae. The dry laminae/leaves and midribs were powdered separately using laboratory scale grinder.

Table-1: List of the compounds in the *n*-hexane fraction of the extract from the leaves of sugar beet.

Scan No.	Base Peak	M. wt	Name and structure
615	41.0	294.0	$\text{CH}_3-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-(\text{CH}_2)_2-\overset{\text{H}}{\underset{\text{CH}_3}{\text{C}}}-(\text{CH}_2)_7-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_3$ 4-methylheptadeca-12,15-dienoate Methyl
618	43.0	250.0	$\text{H}_2\text{C}=\text{HC}-\text{H}_2\text{C}-\text{O}-(\text{CH}_2)_2-\overset{\text{CH}}{\underset{\text{CH}_3}{\text{C}}}-(\text{CH}_2)_3-\text{C}\equiv\text{C}-\overset{\text{CH}}{\underset{\text{CH}_3}{\text{C}}}-(\text{CH}_2)_2-\text{CH}_3$ 12-(Allyloxy)-4,10-dimethyldodec-5-yne
692	41.0	256.0	$\text{CH}_3-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-(\text{CH}_2)_{13}-\text{CH}_3$ Tetradecyl acetate
697	88.0	284.0	$\text{H}_2\text{C}=\text{CH}-\overset{\text{OH}}{\underset{\text{OH}}{\text{CH}}}-(\text{CH}_2)_9-\overset{\text{OH}}{\underset{\text{OH}}{\text{CH}}}-(\text{CH}_2)_4-\text{CH}_3$ Octadec-1-ene-3,13-diol
756	71.0	196.0	$\text{CH}_3-\text{O}-\overset{\text{O}}{\parallel}{\text{C}}-(\text{CH}_2)_2-\overset{\text{H}}{\text{C}}=\text{C}-(\text{CH})-\overset{\text{O}}{\parallel}{\text{C}}-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_3$ Methyl 6-oxodeca-4,8-dienoate
778	67.0	308.0	$\text{HO}-(\text{CH}_2)_7-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-\overset{\text{H}}{\text{C}}=\text{CH}-\text{CH}_2-\text{O}-\text{C}\equiv\text{C}-\text{CH}=\text{CH}-\text{CH}_3$ 4-(Pent-3-en-1-ynyloxy)but-2-enyl-8-hydroxyoctanoate
782	79.0	306.0	$\text{H}_3\text{C}-(\text{CH}_2)_2-\overset{\text{H}}{\text{C}}=\overset{\text{H}}{\text{C}}-(\text{CH}_2)_2-\overset{\text{O}}{\parallel}{\text{C}}-\text{O}-(\text{CH}_2)_3-\overset{\text{H}}{\text{C}}=\text{CH}-\text{O}-\overset{\text{H}}{\text{C}}=\overset{\text{H}}{\text{C}}-\text{CH}_2-\text{CH}=\text{CH}-\text{CH}_3$ 5-(Hexa-1,4-dienyloxy)pent-4-enyl oct-4-enoate

Table-2: List of the compounds in the *n*-hexane fraction of the extract from the leaves of sugar beet.

Scan No.	Compound	Relative % Concentration
615	Methyl 4-methylheptadeca-12,15-dienoate	5.38
618	12-(Allyloxy)-4,10-dimethyldodec-5-yne	1.12
692	Tetradecyl acetate	13.58
697	Octadec-1-ene-3,13-diol	14.87
756	Methyl 6-oxodeca-4,8-dienoate	3.40
778	4-(Pent-3-en-1-ynyloxy)but-2-enyl-8-hydroxyoctanoate	38.38
782	5-(Hexa-1,4-dienyloxy)pent-4-enyl oct-4-enoate	7.46

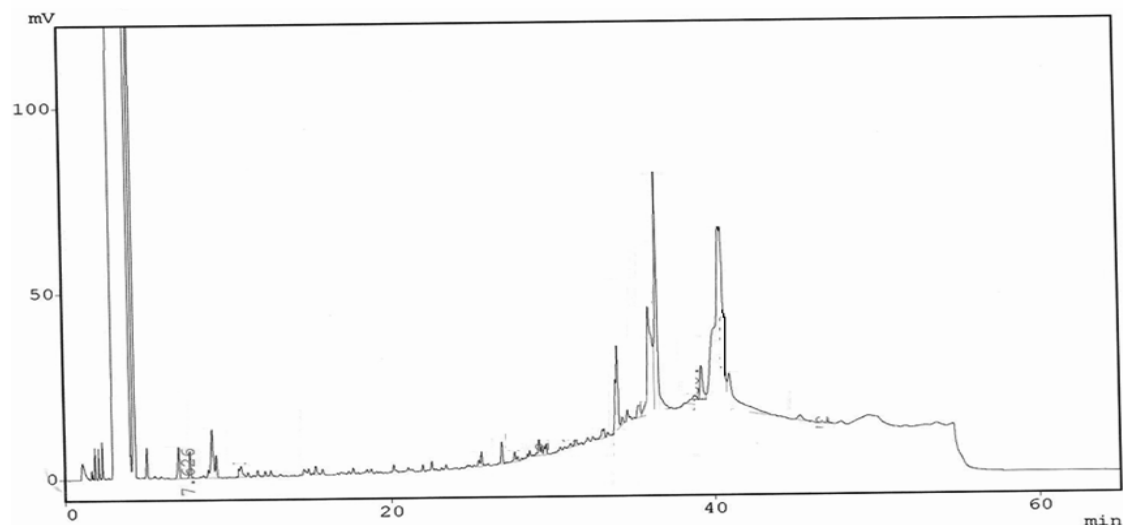


Fig. 1: Gas Chromatographic analysis of the n-hexane fraction of the leaves of Sugar beet.

Table-3: The weight of dry powder of the leaves of sugar beet and extracts in various solvents.

S. No.	Material	Dry weight (g)	Crude (g)	CHCl ₃ Fraction (g)	EtOAc Fraction (g)	n-hexane Fraction (g)
1	Leaves	1500.00	870.00	85.00	58.00	107.00
2	Midribs	1500.00	507.00	73.00	160.00	94.00

Extraction Process

Finely powdered dry laminas/leaves and midribs of the sugar beets were soaked in ethanol for one week in separate containers. Both of these samples were filtered and each filtrate was concentrated under reduced pressure using rotary evaporator. The concentrated crude extracts were treated with various solvents in separate assays. Each of the crude extract was extracted with the solvents in the increasing order of polarity by using *n*-hexane, chloroform and ethyl acetate, respectively. All these fractions were concentrated under reduced pressure using rotary evaporator. The mass of each fraction is given in Table-1. Only the *n*-hexane fraction was considered for analysis and bioassay in this work. The nature of compounds of the *n*-hexane fraction was found the same for both the midrib and leaves. The oils were also extracted by hot extraction using *n*-hexane as solvent in a Soxhlet's apparatus.

Conclusion

The crude extract of the leaves of sugar beet was found to contain higher quantities of the long chain carboxylic acids. It was also found to contain a dihydric alcohol as major component. The oil is also composed of ethers functionality and these compounds are previously reported as antimicrobial agent. The *n*-hexane fraction of sugar beet leaves have significant activity against the selected bacterial strains. It was found to be more effective against the *K. pneumoniae*. Its activity was found almost equal to the ciprofloxacin in both concentrations.

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