GC-MS Analysis of Ethyl Acetate Fraction of the whole Plant Extract of *Dyschoriste perrottetii* (*Acanthaceae*)

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ABSTRACT

The present investigation was carried out to determine the bioactive components in the ethyl acetate extract of D. perrotiettii (Acanthaceae) using GC-MS analysis. The compounds identified in the ethyl acetate fraction were 9-Octadecenal (53.78%), Hexadecanoic acid (22.82%), Octadecanioc acid or Stearic acid (10.68%), 2-methylnonane (3.42%) and 9, 12-Octadecadenoic acid or Linoleic acid (2.84%). FTIR analysis of the compound showed two strongly adsorbing peaks at about 3000 and 2850 cm^{-1} corresponding to CH of an alkane, OH stretch of carboxylic acid respectively. The peak at 1740.98 cm^{-1} corresponds to C=O stretching frequency for carboxylic acids. The peak at 1662.4 cm^{-1} is assigned for carbonyl group (C=O) in carboxylic acid and the weak peak at 1155.97 cm^{-1} corresponds to the C-O stretch of an alcohol or carboxylic acid.

Key words: *GCMS, Dyschoriste perrottetii, Hexadecanoic acid, FTIR and Linoleic acid*

INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs[1,2]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations[3]. Plants synthesize primary metabolites such as proteins, fats, nucleic acids and carbohydrates from substances such as water, carbon dioxide, nitrogen and a number of inorganic salts in small amounts. These primary metabolites are transformed into secondary metabolites (alkaloids, steroids, terpenoids, saponins, flavonoids) that are used as drugs[4].

Dyschoriste perrottetii is a shrub of about half a meter high, with branches and square woody stem rooting at lower nodes. It is widely distributed in the tropics, frequently in temperate and completely absent in artistic region. In Nigeria among the Hausa and Fulani communities, it is commonly known as *fidda hakukuwa, momodil* and *bidi-diyan*. The plant is used in traditional medicine for easy labour in pregnancy and in treatment of

yellow fever and measles. The seeds are used for the removal of foreign materials from the the eyes. A decoction of the aerial parts of this plant is used in traditional medicine in Burkina Faso for the treatment of childhood malaria. Sawadogo *et al.* (2006)[5] reported its use in malaria fever and diarrhoea. An infusion of the leaves is taken orally for primary and secondary infertility[6]. In Giwa Local Government Area of Kaduna state, Nigeria, the plant is boiled and taken orally for the treatment of chicken pox[7]. Also the leaf of *D. radicans* is crushed and mixed with honey and applied to the affected part for treating anthrax. The leaves are also used as infusion for skin diseases, wounds and eye infections[8]. A decoction of the whole plant is used to treat tooth ache by the Zay people of Ethiopia[9]. *Dyschoriste litoralis* is considered a very efficacious remedy for all sorts of coughs, when administered with ginger. The leaves are used for rheumatism and are dried, made in to cigarettes and smoked in asthma. The plant juice is used in the treatment of diarrhoea and dysentery[10]. Pharmacological activities so far reported are antifungal, antidiabetic, antimicrobial, anti-inflammatory, antipyretic, antioxidant and insecticidal activity[11, 12].

MATERIALS AND METHODS

All chemicals and reagents used were of analytical grade and manufactured by BDH. Column chromatography was carried out on silica gel (Merck 60-120 mesh). TLC was carried out on plate coated with silica gel (Merck TLC grade, with gypsum binder and fluorescent indicator) and viewed under UV-lamps at 254 and 365nm. Agilient gas chromatograph (6890N) coupled to 5973 mass selective detector was used for GC-MS analysis.

Plant sample collection and handling

The whole plant, *Dyschoriste perrottetii* was collected fresh at a village at Mando, in Kaduna on 20th may, 2017 in Nigeria. The fresh *Dyschoriste perrottetii* were identified and authenticated at the Herbarium section of the Department of Botany, Ahmadu Bellow University, Zaria. *Dyschoriste perrottetii* Voucher No is 1186. The fresh sample collected were properly washed and dried at room temperature for one week and the whole plant pulverized to fine powder using mortar and pestle. It was sieved to remove coarse plant materials and was weighed using electronic weighing balance and stored in a clean dry container.

Extraction of D. perrottetii

Soxhlet extraction method was employed for the extraction of the plant samples. 100 g of the dried and pulverized plant materials were weighed using electronic weighing balance and was transfered into the thimble of the soxhlet apparatus. Absorbent cotton wool was used to cover the extraction thimbles. 500 ml of ethyl acetate was accurately measured using a measuring cylinder and transferred into the flat bottom flask of the Soxhlet extractor and heated on a mantle. The sample was heated until the solvent was clear of the initial green colour. The solvent was collected and concentrated *in-vacuo* using rotary evaporator at 40^{o}C.

Thin layer chromatography

Thin layer chromatography was used to determine the number of chemical components in the ethyl acetate extract. Suitable mobile phase for good resolution as described by the Association of Official Analytical Chemist (AOAC), 1984 was used to determine the suitable solvent systems. The plant extract was dissolved in minimum amount of ethyl acetate and spotted at the base of the TLC plate and developed using n-hexane and ethyl acetate in different ratios. The resulting chromatogram was viewed under UV lamp at both short wave length of 254 nm and long wave length of 360 nm. Mobile phase of ratio 8:2 of n-hexane and ethyl acetate was found to be the most suitable solvent system that provided excellent resolution on the TLC plate.

Vacuum liquid chromatography (VLC)

The ethyl acetate extract was subjected to VLC using step gradient of n-hexane and ethyl acetate to obtain various fractions. Thirty fractions labelled 1-30 were collected in vials of 30 ml each. All fractions collected were studied by TLC, fractions with similar chromatogram were pooled together and a yellow sticky compound was obtained and labelled DPE-1.

GC-MS analysis of DPE-1

The GC-MS analysis was carried out at the National Research Institute of Chemical Technology (NARICT) in Zaria. The dried yellow sticky compound was dissolved in n-hexane and analyzed using GC-MS model QP2010 plus Shimadzu equipped with detector and slit injection system. The initial temperature was maintained at 60^{O}C for 3 minutes and was gradually increased to 250^{O}C. 1.6µL of solution was injected for analysis and the sample injection temperature was maintained at 250^{O}C throughout the period. The carrier gas, Helium flow was 2ml per minute. The identification of the compound was based on comparison of the mass spectra with those present in the Natural Institute for Standard Technology computer data bank (NIST, 2009 Lib.). Prediction of the bioactivity of compound was done using Dr. Duke's phytochemical and Ethnobotanical database. The relative percentage amount of each phyto-compound was calculated by comparing the average peak area to the total area. The name, molecular weight and molecular formula were recorded.

RESULTS AND DISCUSIONS

The compounds present in the fraction DPE-1 were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) are presented in Table 1.

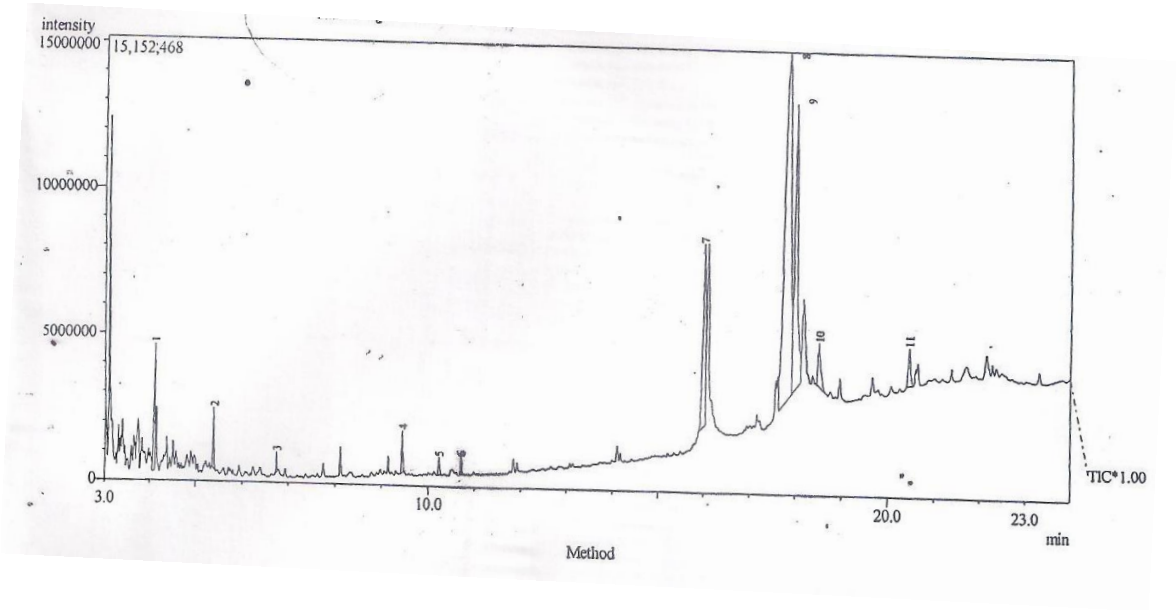

Figure1. GCMS chromatogram of the ethyl acetate fraction DPE-1 of *D. perrottettii*

GC-MS is one of the most precise methods to identify various secondary metabolites present in plant extracts[13]. The isolated ethyl acetate fraction of *D. perrotettii* was analyzed by GC-MS to identify the various compounds in the extract, with the help of NIST library Mass Spectrometry Data Centre. Eleven compounds were identified and listed in Table 1. The chromatograph showed 11 peaks for 11 individual compounds (Figure 1). The major constituents identified in the fraction were 9-Octadecenal (53.78%), Hexadecanoic acid (22.82%), Octadecanioc acid or Stearic acid (10.68%), 2-methylnonane (3.42%) and 9, 12-Octadecadenoic acid or Linoleic acid (2.84%). Among the identified phyto constituents, hexadecanoic acid has the property of antioxidant, antibacterial and antifungal activity[14]. 9, 12- Octadecadienoic acid has anti-inflammatory and antibacterial activity, it is also used in beauty and skin care products[15]. 2-methyldecane and 2-methylnonane are antimicrobial, anti-inflammatory and have wound healing abilities.

Table 1. Phytoconstituents of ethyl acetate fraction of the whole plant extract of *D. perrottetii*

S/N	RT	Peak area	M.Formula	M.Weight	Name of compound
1.	4.083	3.42	$C_{10}H_{22}$	146	2-methylnonane
2.	5.367	1.65	$C_{11}H_{24}$	156	n-undecane
3.	6.738	0.55	$C_{11}H_{24}$	156	2-methyldecane
4.	9.439	1.27	$C_{14}H_{30}$	198	n-Tetradecane
5.	10.236	0.57	$C_{14}H_{30}$	198	2,3,5,8- Tetramethyl decane
6.	10.708	0.50	$C_{12}H_{26}O$	186	5-(Hydroxylmethyl) undecane
7.	16.000	10.68	$C_{18}H_{36}O_2$	284	Octadecanoic acid
8.	17.833	53.78	$C_{18}H_{34}O$	266	9-Octadecenal
9.	18.000	22.82	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
10.	18.500	2.84	$C_{18}H_{32}O_2$	280	9,12- Octadecadienoic
11.	20.458	1.88	$C_{22}H_{42}O_2$	338	13-Docosenoicacid

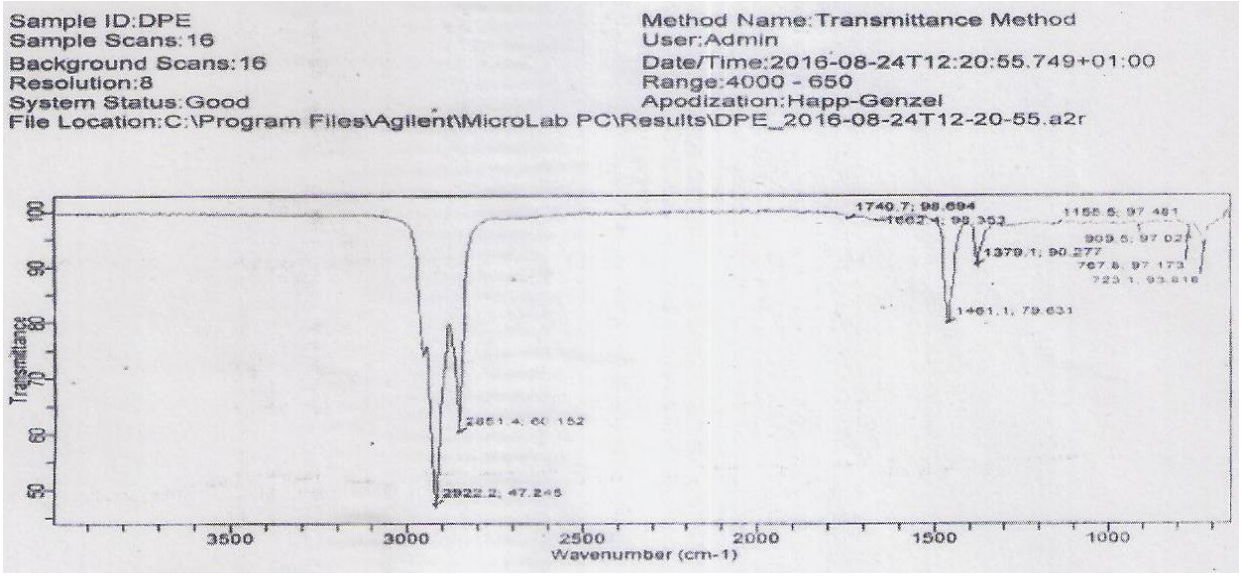

Figure 2: FTIR Spectrum of the compound DPE-1

The FTIR spectrum (Figure 2) shows two strongly adsorbing peaks at about 3000 and 2850 cm^{-1} for CH alkane and OH stretch of carboxylic acid respectively. The peak at 1740.98 cm^{-1} corresponds to C=O stretching frequency for carboxylic acids and the peak at 1662.4 cm^{-1} is assigned for the carbonyl group (C=O) in carboxylic acid. The weak peak at 1155.97 cm^{-1} corresponds to C-O stretch of an alcohol or carboxylic acid.

CONCLUSION

From this study, it is concluded that DPE-1 is a mixture of bioactive principles. The various constituents detected in *Dyschoriste perrottettii* extract justify the use of the plant in traditional healthcare practices. Other possible bioactive compounds of the plant are being investigated in other solvents.

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