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Full Length Research Paper

GC-MS of volatile components of fermented wheat germ extract

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Wheat has been used as food and folk medicine. We have earlier reported that the administration of ethyl acetate extract of ferrmented wheat at 300 mg/kg body weight have anti-trypanosomal activity. To better understand the active constituent for anti-trypanosomal properties, the crude extract was partially purified using column chromatography to give fractions A, B and C, which were further characterized by gas chromatographic – mass spectral (GC/MS) analysis. The fractions identified 12, 14 and 23 compounds respectively. The major volatile compounds were classified into oxygenated hydrocarbon (manool, hexadecyl acetate and butyl dodecanoate) and n-hydrocarbon (1- eicosene).

Key words: Wheat seeds, volatile compound, GC-MS, Retention index (KI).

INTRODUCTION

As source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition of using medicinal plants. About 25% of prescribed medicines in industrialized countries derived directly or indirectly from plants (Raskin et al., 2002). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethanomedicinal plants may still be needed. It is obvious that these phytochemicals will find their way in the antimicrobial drugs prescribed by physicians (Cowan, 1999). Notably in West Africa, new drugs are not often affordable. Wheat (*Triticum* spp) is a world -wide cultivated grass from the area of Middle East. Globally after maize, wheat grain is a staple food used to make flour for leavened, flat and steamed breads, cookies, cakes, pasta and for fermentation to make beer (Palmer and John, 2001). Wheat kernel contains 2 to 4% germ and most nutrients with the exception of starch are concentrated in the germ, the germ is the richest known natural source of tocopherol, abundant in B-group vitamins and protein of high biological value (Tsen, 1985) and its oil of favourable fatty acid pattern (Paul et al., 1987), because of their beneficial nutritional values, wheat germ and wheat bran are frequently used in human food supplements.

Therefore, they are part of the regular western diet. Fermented wheat germ extract called avemer has been reported to control cell growth and proliferation mainly by inhibiting ribonucleotide reductase needed to make new DNA to support replication (Sukkar and Edoardo, 2004). It had also been reported that avemer limit the access to glucose, needed to make the ribose sugar for DNA and RNA for new cancer cells (Boros et al., 2002; Boros et al., 1997). We have earlier reported that the administration of ethylacetate extract of wheat seed at 300 mg/kg body weight to *Trypanosoma brucei*-infected rats was able to reduce parasitaemia and extended the life span of infected treated rats when compared with infected untreated rats (Yusuf and Ekanem, 2010), but the actual volatile constitute has not been fully studied. In this study, the volatile constituent of fermented wheat seeds extract was characterized using GC-MS analysis. GC-MS analysis has been proved to be of great utility in the analysis of compound.

EXPERIMENTAL

Collection of plant material

Wheat seeds were collected from Minna Central Market in the month of March/April 2008 and authentication was carried out at the

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Table 1. Compounds present in fraction A of fermented wheat extract.

KI	Compound	%
642	Benzene	5.64
1329	1,3- Benzodioxole-5- carbaldehyde	1.27
1224	5- hydroxymethyl-2-furancarboxaldehyde	1.09
1494	Ethyl undecanoate	3.64
1676	Methyl-z(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate	8.55
1786	Butyl dodecanoate	1.27
1994	1-Eicosene	13.77
2009	Hexadecyl acetate	12.19
2500	Pentacosane	4.91
2900	Nonacosane	9.10
2370	9-Tetracosene	3.46
2082	1-Octadecan-1-ol	3.64
	Classification of compounds	
	n-hydrocarbon	36.90
	Oxygenated hydrocarbon	19.50
	Aromatic hydrocarbon	8.50
	Total	64.90

The compounds were identified by the combination of both the mass spectra and retention indices on DB – 5 capillary coated column. Values (%) represent percentage composition, KI represent retention index.

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Plant preparation

Wheat germ powder of 70 g was fermented using 30 g of *Saccharomyces cerevisiae* (baker's yeast) for 48 h and the paste was extracted using 250 ml ethyl acetate. The filtrate was concentrated using rotary evaporator and stored at room temperature. The resulting extract was subjected to column chromatography using silical gel (60 to 120 mesh), eluting with a step gradient of n-hexane, n-hexane – ethyl acetate, ethyl acetate, ethyl acetate – methanol and methanol. Thin layer chromatography was performed with precoated silica gel GF- 25- UV 254 plates and detection was done by spraying with iodine to give three (3) fractions (A, B and C). The essential oils of the fractions were studied.

Gas chromatography-mass spectrometry analyses

Agilent 6890N GC was interfaced with a VG Analytical 70 - 250s double-focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: Ionization voltage 70 eV, ion source 250℃. The GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of the GC analysis. The percentage compositions of the oil were computed in each case from GC peak areas and are shown in Table 1. Retention indices for all the compounds were determined according to the Kovats method relative to the n-alkanes series. The identification of the compounds was done by comparison of retention indices and by matching their fragmentation patterns in mass spectra with those of published mass spectra data (Jennings and Shibamoto, 1980; Adams, 1995; Joulain et al., 1998; Koenig et al., 2004). In a few cases, identification of components was carried out by means of commercial libraries (Wiley, NIST05 and Hochmuth) (Itakura et al., 2001).

RESULTS AND DISCUSSION

The results of Gas chromatography/ Mass spectroscopy (GC/MS) analysis of the various fractions of wheat extract are presented in Tables 1 to 3 representing fraction A, B and C respectively.

Fraction A

The result of GC/MS analysis of the fractions A of wheat extract showed the presence of twelve (12) compounds corresponding to 64.90% of the total fraction (Table 1). The compound comprises of n- hydrocarbon (36.90%), oxygenated hydrocarbon (19.50%), aromatic hydrocarbon (8.50%). The major compound: Novacosane (9.10%), 1-eicosene (13.77%) and benzene (5.64%). The prominent among the oxygenated hydrocarbons are hexadecyl acetate (12.19%), ethyl undecanoate (3.64%) and octadecan-1-ol (3.64%). While that of the aromatic hydrocarbon is methyl-z-(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate (8.50%) (Table 1).

Fraction B

The GC/MS analysis of the fractions B of wheat extract

Table 2. Compounds present in fraction B of fermented wheat extract.

KI	Compound	%
1494	Ethyl undecanoate	4.24
1676	Methyl-z(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate	5.03
1922	Dibutyl phthalate	5.32
1786	Butyl dodecanoate	10.15
1994	1-Eicosene	5.62
2056	Manool	15.05
2200	Octadec-9-enoic acid	7.98
2456	Tetracosan-1-ol	5.41
2370	9-Tetracosene	5.03
2195	1-Docosene	3.15
2900	Nonacosane	7.19
2830	10- Demethyl squalene	1.28
2852	Hexacosan-1-ol	1.28
	5- Octadene	5.75
	Classification of compounds	
	n-hydrocarbon	28.10
	Oxygenated hydrocarbon	49.50
	Aromatic hydrocarbon	5.00
	Total	82.60

The compounds were identified by the combination of both the mass spectra and retention indices on DB – 5 capillary coated column. Values (%) represent percentage composition, KI represent retention index.

revealed the presence of fourteen (14) compounds corresponding to 82.60% of the total fraction (Table 2). The compound comprises of n-hydrocarbon (28.10%), oxygenated hydrocarbon (49.50%), aromatic hydrocarbon (5.00%). The major compound 9-novacosane (7.19%), 1-eicosene (5.62%) and 5-octadene (5.75%). The prominent among the oxygenated hydrocarbon are: Manool (15.05%), butyl dodecanoate (10.15%) and octadec -9-enoic acid (7.98%). While that of aromatic hydrocarbon is methyl-z-(1R, 2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate (5.03%) (Table 2).

Fraction C

The GC/MS analysis of the fractions C of wheat extract showed the presence of twenty three (23) compounds corresponding to 72.10% of the total fraction (Table 3). The compound comprises of n-hydrocarbon (35.70%), oxygenated hydrocarbon (29.60%), aromatic hydrocarbon (5.20%) and nitrogen containing hydrocarbon (1.60%). The major compound 1-eicosene (6.03%), pentacosane(5.535) and tetracosene (4.79%). The prominent among the oxygenated hydrocarbon are butyl dodecanoate (5.78%) octadecanoic acid (4.98%) and 5-tetradecencyl acetate (3.49%). While that of aromatic hydrocarbon is methyl-z(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate (5.19%) and nitrogen containing hydrocarbon is 2- phenyl acetonitrile (1.60%) (Table 3).

It worth mentioning that, a major compound such as manool, which was detected in ethyl acetate wheat extract has been previously reported as part of the oil constituent of Pinus contorta bark and Tsuga heterophylla wood (Conner and Rowe, 1976); Salvia stenophylla (Jequier et al., 1980) and Picea ajanensis (Caeov et al., 1981). Manool is a labdane alcohol group of diterpenoids.

Diterpenes from many species are well known for their biological activity. Labdane derivatives have been reported to show significant antileishmanial activity (Kayser and Kiderlen, 1998) Also, compounds such as hexadecyl acetate, and butyl dodecanoate were previously detected in Frieseomelitta varia and Frieseomelitta silvestrii (Cruz et al., 2002) and old plum (Vele et al., 2005). These are ester and retroester derivatives.

Endogenous fatty acid amides and esters are the subject of growing interest in pharmacology, since the discovery of anandamide, the ethanolamide of arachidonic acid (Se´verine et al., 2003). Also, 1- eicosene can be classified as simple aliphatic. Simple aliphatic have been previously reported to show significant suppression of Typanosoma parasitaemia in vivo with daily (Perez et al., 1994). Therefore, it could be concluded that the volatile compound of ethyl acetate extract of fermented wheat can be useful in the management of African trypanosomiasis.

Table 3. Compounds present in fraction C of fermented wheat extract.

KI	Compound	%
642	Benzene	3.29
1065	Phenyl ethanone	5.00
1197	1,2-Benzenediol	1.20
1140	2- Phenyl acetonitrile	1.60
1224	5- hydroxymethyl-2-furancarboxaldehyde	0.80
1274	1-(4-ethylphenyl)-ethanone	1.40
1392	1-Tetradecene	2.80
1494	Ethyl undecanoate	3.29
1676	Methyl-z(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate -	5.19
1790	(z)- 5- Tetradecenyl acetate	3.49
1786	Butyl dodecanoate	5.78
1793	1- Octadene	4.59
1994	1- Eicosene	6.03
2200	Octadecanoic acid	4.89
2370	9-Tetracosene	4.09
2400	Tetracosene	4.77
2900	Nonacosane	3.59
2862	Hexacosan-1-ol	0.47
2456	Tetracosan-1-ol	1.70
2600	Hexacosane	1.00
2500	Pentacosane	5.53
	4-hdroxy-2,5-dimethylfuran-3(H) one	0.47
	3,4,5-Trimethoxybenzaldehyde	0.98
	Classification of compounds	
	n-hydrocarbon	35.70
	Oxygenated hydrocarbon	29.60
	Aromatic hydrocarbon	5.20
	Nitrogen containing hydrocarbon	1.60
	Total	72.10

The compounds were identified by the combination of both the mass spectra and retention indices on DB – 5 capillary coated column. Values (%) represent percentage composition, KI represent retention index.

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