

Full Length Research Paper

Evaluation of essential oils composition of methanolic *Allium sativum* extract on *Trypanosoma brucei* infected rats

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The essential oils composition and anti – trypanosomal activity of fermented methanolic *Allium sativum* extract was investigated. 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 thirteen, sixteen and seventeen compounds respectively. The main components were oxygenated hydrocarbon (palmitoleic and steric acid) and n – hydrocarbon (unsaturated).The crude extract show anti- trypanosomal activity on *Trypanosoma brucei* infected rats.

Key words: *Allium sativum*, essential oils, gas chromatographic – mass spectral analysis, retention index (KI, anti- trypanosomal).

INTRODUCTION

Allium sativum L., commonly known as garlic, belongs to family *Alliaceae*. Its close relatives include the onion, the shallot and the leek (McGee, 2004). Garlic has been used throughout recorded history for both culinary and medicinal purposes. It has a characteristics pungent, spicy flavour that mellows and sweetens considerably with cooking (McGee, 2004). It also has been taken as a tonic, a bactericide and a popular remedy for various ailments (Blackwood and Fulder, 1986). More recently, however, it has been recognized as a medicinal plant for the prevention of blood circulatory disorders (Fogarty, 1993; Steiner et al., 1996), cancer (Amagase and Milner, 1993; Nishino et al., 1989; Wargovich, 1986), memory loss (Moriguchi et al., 1994) and anti-trypanosomal (Yusuf and Ekanem, 2010).

It have been reported that garlic bulb contain two classes of antioxidant components namely flavonoids and polyphenol derivatives which are naturally occurring compound of gallic acid. Previous quantitative

phytochemical analysis of fermented methanolic garlic extract shows that the plant contain secondary metabolite with high percentage of glycoside (21.088%), alkaloids (3.570%) and saponins (0.696%), moderate amount of phenol, tannins, flavonoid, steroids, terpenes and anthraquinone and trace amount of phlobatannin (Yusuf and Ekanem, 2010). Several chromatograms of the garlic have been published (Itakura et al., 2001) but so far no analysis of the volatile compounds in garlic that is responsible for its anti-trypanosomal activity has been reported.

Essential oils (EOs) are extremely complex mixtures containing compounds of several different functional-group classes. A specific aromatic profile should be determined by gas-chromatography-mass detection methods, to define their constituent for their safety and efficacy.

EXPERIMENTAL

Plant material

Fresh bulbs of *A. sativum* L., commonly known as garlic were

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Table 1. Compounds present in fraction a of fermented garlic extract.

KI	Compound	%
701	2,3 –Pentanedione	0.77
792	1- octane	1.15
1593	1- hexadecane	2.50
1900	Nonadecane	2.88
1984	Hexadecanoic acid	13.74
2009	Octadecanoic acid	15.92
2800	Octacosane	7.10
2600	Hexacosane	4.80
2456	Tetracosan-1-ol	5.57
	9-Octadecen-18-olide	15.93
	5-Octadecene	3.26
Classification of compounds		
	Unknown compound	4.61
	n-hydrocarbon	21.70
	Oxygenated hydrocarbon	45.50
	Unknown compound	4.61
		71.81

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.

purchased from Minna Central Market, Niger State, Nigeria in the months of March/April 2008 and authentication was carried out at Federal College of Forestry, Ibadan, Oyo state.

Preparation of plant

Garlic bulbs (*A. sativum*) were opened to reveal its fleshy sections called cloves. The cloves were peeled and blended. One hundred gram of *A. sativum* was soaked in 250 ml methanol for 24 h and filtered. The solvent was removed using rotary evaporator. The resulting yield (8.66 g) were subjected to column chromatography using silica gel (60 to 120 mesh) and eluted with n-hexane, n-hexane – ethylacetate, 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 sulphuric acid to give three fractions (A, B and C). The essential oils of the fractions were studied.

Parasite inoculum

Trypanosoma brucei was obtained from the Veterinary and Livestock Studies Department of the Nigerian Institute for Trypanosomiasis Research, VOM, Plateau State of Nigeria. The parasite was maintained by repeated passaging into other rats.

Parasitaemia determination

Parasitaemia count was carried out on infected rats at 24 h interval to monitor infection progress. The counting of the number of parasite was done under the light microscope at X40 magnification

from thin blood smear freshly obtained from the tip of the tail of infected rats.

Administration of crude extract

Infected rats were administered intraperitoneally with 0.5 ml solution of fermented garlic methanolic extract in distilled water containing 300 mg/kg body weight on the first day of sighting parasite in the blood (normally 3 days post infection) of infected rats (Yusuf and Ekanem, 2010). The control group for this experiment was infected untreated rats.

Gas chromatography-mass spectrometry analyses

Agilent 6890 N gas chromatography (GC) was interfaced with a VG analytical 70 – 250 s double-focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 °C. 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.,

Table 2. Compounds present in fraction b of fermented garlic extract.

KI	Compound	%
1065	1- Phenyl ethanone	3.44
1392	1-Tetradecene	1.84
1481	Tridecan-2-one	1.12
1524	2-methoxy-4-(2-propenyl) – phenol acetate	2.71
1676	Methyl-z(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate	4.17
1841	2-Phemethyl benzoate	2.33
1994	1-Eicosene	5.91
2128	Octadecyl acetate	4.65
2195	1-Docosene	5.43
2370	9-Tetracosene	11.05
2400	Tetracosane	4.75
2800	Octacosane	3.68
2848	Hexacosanol	2.91
2852	Hexacosan-1-ol	8.63
	2S,3S-Methyl-2-amino-3-methyl pentanoate	1.07
	5-Octadecene	9.06
Classification of compounds		
	n-hydrocarbon	41.70
	Oxygenated hydrocarbon	24.10
	Aromatic hydrocarbon	6.90
		72.70

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.

al., 2001).

RESULTS AND DISCUSSION

The oil extracted was amber in colour. The analysis of the fractions A of extract showed the presence of sixteen compounds corresponding to 71.80% of the total fraction (Table 1). The compound comprises of n- hydrocarbon (21.70%), oxygenated hydrocarbon (45.50%) and unknown compound (4.60%). The prominent compound are oxygenated hydrocarbon (Octadecanoic acid (15.92%), Hexadecanoic acid (13.74%) and 9-Octadecen -8-olide (12.47%) (Table 1).

Also, the determination of compounds in fraction B identified sixteen compounds corresponding to 72.70% of the total fraction (Table 2). The compound comprises of n- hydrocarbon (41.70%), oxygenated hydrocarbon (24.10%) and aromatic hydrocarbon 6.90%). The prominent among the n- hydrocarbon are: 9- Tetracosene (11.05%) (Table 2).

While, the fractions C garlic extract showed the presence of seventeen compounds corresponding to 87.04% of the total fraction (Table 3). The compound

comprises of n- hydrocarbon (42.640%), oxygenated hydrocarbon (27.50%), aromatic hydrocarbon (10.30%) and nitrogen containing hydrocarbon (6.60%). The prominent among the n-hydrocarbon are: 1-Docosene (14.31%) and 1-Octadene (11.90%) (Table 3).

It is worth mentioning that compounds such as octadecanoic acid, hexadecanoic acid and 9-Octadecen-18-olide which were detected in the fermented extract are systematic name for monoenoic fatty acid (Palmitoleic, oleic and linoleic acid) belonging to omega 6 and omega 7. The parasitaemia of infected treated with garlic bulbs extract oil showed a decrease in the proliferation of parasite and extension of surviving days of rats from 8 days of the control (infected untreated) to 17 days for infected garlic treated rats (Figure 1). The oil could be useful in the management of African trypanosomiasis.

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Table 3. Compounds present in fraction c of fermented garlic extract.

KI	Compound	%
2600	Hexacosane	6.02
2402	Integerrimine	2.67
2470	Senkirikine	3.88
2800	Octacosane	4.55
2500	Pentacosane	3.08
2456	Tetracosan-1-ol	3.61
2195	1-Docosene	14.31
2009	Hexadecyl acetate	6.55
1793	1-Octadene	11.90
1676	Methyl-z(1R,2S)-3-oxo-2-(z)-pent-2-cyclopentyl-acetate	6.55
1524	2-methoxy-4-(2-propenyl) – phenol acetate	3.74
1383	E-(3,7-Dimethyl-2,6-Octadienyl acetate	2.94
1116	Tetradecane	1.60
1094	Methyl-2-butenate	1.74
642	Benzene	0.94
	Serkirkine acetate	4.81
	9-Octadecen-18-olide	7.62
Classification of compounds		
	n-hydrocarbon	42.64
	Oxygenated hydrocarbon	27.50
	Aromatic hydrocarbon	10.30
	Nitrogen containing hydrocarbon	6.60
		87.04

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.

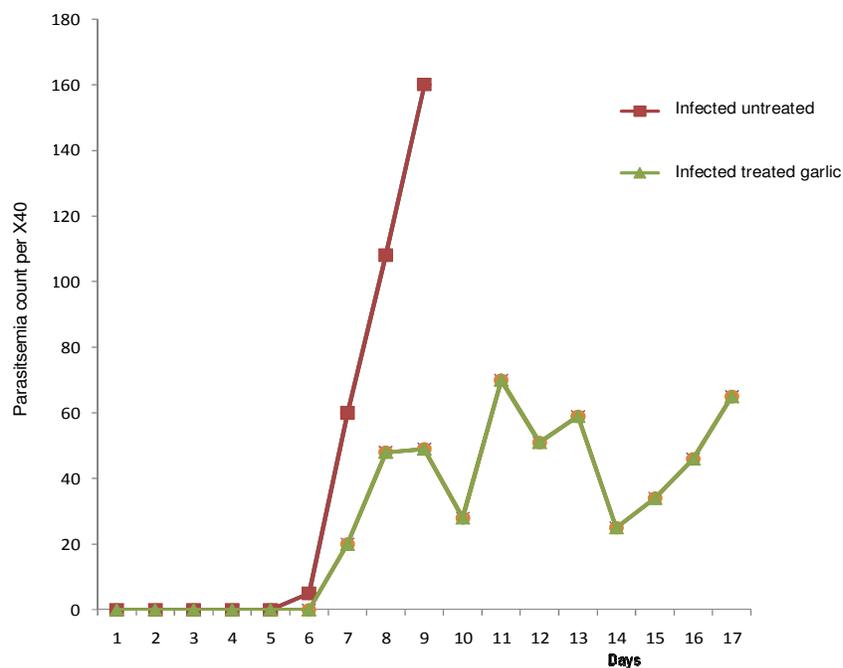


Figure 1. Parasitaemia count of rats infected with trypanosomiasis and treated with fermented menthanolic garlic extract.

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