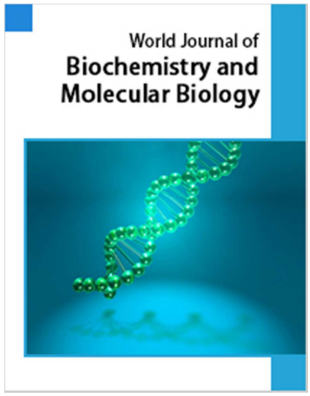
World Journal of Biochemistry and Molecular Biology
2017; 2(5): 29-33
http://www.aascit.org/journal/wjbmb

American Association for Science and Technology



Keywords
Essential Oil,
Afrostyrax lepidophyllus,
Hydrodistillation,
Fungicidal Activity

Received: April 19, 2017
Accepted: September 1, 2017
Published: September 26, 2017

Characterization and Determination of Antifugal Activities of Essential Oil Extracted from the Bark of *Afrostyrax lepidophyllus* "Country Onion or Shirum"

Fai Fredrick Yirankinyuki[1], Wilson Lamayi Danbature[1], Tatah Verwiyeh Silas[2, *], Aisha Poloma[1]

[1]Department of Chemistry, Gombe State University, Gombe, Nigeria
[2]Department of Biochemistry, Federal University, Wukari, Nigeria

Email address

tatah.silas@fuwukari.edu.ng (T. V. Silas)
[*]Corresponding author

Citation

Fai Fredrick Yirankinyuki, Wilson Lamayi Danbature, Tatah Verwiyeh Silas, Aisha Poloma. Characterization and Determination of Antifugal Activities of Essential Oil Extracted from the Bark of *Afrostyrax lepidophyllus* "Country Onion or Shirum". *World Journal of Biochemistry and Molecular Biology.* Vol. 2, No. 5, 2017, pp. 29-33.

Abstract

Food safety remains an increasingly important public health issue due to increasing microbial food poisoning. This study focused on the Hydrodistillation extraction of essential oil from *Afrostyrax lepidophyllus* bark and determination of its biological activities as an antifungal agent. Essential oil was extracted from the bark of *Afrostyrax lepidophyllus* by hydrodistillation method. The GC-MS analysis showed that seven volatile compounds were identified Viz; Dimethyl trisulfide (2.80%), Disulfide, methyl (methylthio) methyl (23.32%), 2,4-dithiapentane Bis(methyl thio)methane (13.40%), Methane (methylsulfinyl) methyl thio (36.04%), Methylthiosulfide Tris (methylthio) methane (4.43%), Tetradecanoic acid (2.75%), 9,12-octadecadien-1-ol (17.26%). The test of fungicidal activity of essential oil extracted from *Afrostyrax lepidophyllus* bark revealed that the essential oil showed good inhibitory activity against mucor and could be recommended for industrial application as an antifugal agent in food processing industries.

1. Introduction

World health organization [1] reported increasing microbial food poisoning during the last decades and stressed that food safety remains an increasingly indispensible public health issue. It has been estimated that as many as 30% of people in industrialized countries suffer from a food borne disease each year and in 2000 at least two million people died from diarrhea disease worldwide [1]. Conventional methods have proven to be expensive and ineffective in food security. It's therefore imperative to search for new methods that reduce or eliminate food borne pathogens possibly in combination with existing methods, [2]. One of such possibility is the use of essential oil as food additives that can act as antibacterial and antifungal additives. Essential oils are odorous and volatile products of various plants and animal species [3]. Essential oils have tendencies to evaporate on exposure to air even at ambient conditions hence is referred to as volatile oil or ethereal oils. They mostly contribute to the odoriferous constituents or essences of the aromatic plants that are used abundantly in enhancing the aroma of some spices [4].

Essential oils are either secreted directly by the plants protoplasm or by the hydrolysis of some glycosides and structures associated with the secretion of essential oils include grandular hairs (Lamiaceae e.g Lavandulan angustifolia) Askun, [5] and Angelini, [6], evaluated some antimicrobial activity parameters viz: mycelia growth inhibition, minimum inhibitory concentration (MIC) and minimum fungicidial concentration (MFC) of six essential oils against *Asperigillus niger, As fungal pergillusterreus, Chaetomium globosium, Penicillium chrysogenum, Penicillium pinophilum, Trichoderma harzianum and Trichoderma Viride*. The antimicrobial activity of essential oils was monitored by the macro dilution technique. In the last two decades there has been a considerable increase in the incidence of life threatening systematic infections. The challenge has been to develop strong strategies for treating fungal diseases, to treat opportunistic fungal infections in human immunodeficiency virus positive patients and others who are immune-comprised due to cancer chemotherapy or the indiscriminate use of antibiotics [7]. Most clinically used antifungal drugs have various drawbacks, they are pretty toxic, they have a low efficacy and high cost, furthermore, their frequent use has produced resistant strains, [8]. The changing face of fungal infections in health care settings, therefore, has generated a great need for news antifungals drugs that can selectively work on new targets with fewer side effects [6]. Strong in vitro evidence indicates that some essential oils like thymus schimperi Ronniger essential oil can act as antibacterial agents against a wide spectrum of pathogenic fungal isolates including *Penicillium chrysogenum, verticilluim, Aspergillus tubingenesis, Aspergillus minutus, Beauveria bassiana and Microsporumgypseum* [9, 10].

Afrostyrax lepidophyllus is commonly known as country onion in west Africa pidgin is a little studied tree native to rain forest of the west central Africa only found in the rumpi hill of south west Cameroon [11]. The seeds and barks is easily found on sale in Cameroonian markets and are used as species in the traditional African cuisine according to [12]. *Afrostyrax lepidophyllus* is a tree commonly grown in the forest and traditionally used as a remedy for child cough, heart rate, worms, constipation, hernia abscesses and boil according to report [13, 14].

The aim of this research was to carry out Hydrodistillation extraction of essential oil from *Afrostyrax lepidophyllus* "country onion or shirum" and determine the bioactivity of the extracted oil on fungi.

2. Material and Method

2.1. Sample Collection and Processing

The *Afrostyrax lepidophyllus* bark samples were harvested from the Rumpi mountain in Cameroon by an agriculturist during dry season and were dried in shade and were stored in a polythene bag and were transported to Gombe state were they were processed. The sample was then sorted out and crushed with an iron mortar and pestle into fine powder for analysis.

2.2. Extraction

Clevenger [15] method of hydrodistillation of essential oil was adopted and modified. 53.45g of Afrostyrax lepidophyllus powdered bark was weighed and placed into 5000m distillation flask and distilled water was added. The flask and it content were mounted on a heating mantle coupled with a Clevenger type apparatus connected to a condenser. Then n-hexane was injected into the Clevenger type apparatus as the solvent. The flask was heated slowly at temperature of 100°C for 30 minutes after which the temperature was reduced to 30-40°C. The extraction was carried out for the period of 3hours. The extracted essential oil was collected into a pre-weighed 1millilitre glass specimen bottles.

2.3. GC-MS Analysis

The GC-MS analysis was carried out in National Research Institute College of Technology (NARICT) Zaria. The GC-MS analysis was carried out on type QP2010 plus shimadzu, Japan. The injection temperature 250°C and injection mode was split. The oven temperature was programmed initially at 60°c for 1 minute then increased at 180°C for 3 minutes and 280°C for 2 minutes. The flow control mode is linear velocity, pressure 56.2kpa, the column flow is 0.99ml/minute. The purge flow is 3.0ml/minute. The total flow is 45.0ml/minute and the split ratio is 41.6. The data analysis and peak measurement was recorded

2.4. Determination of Fungicidal Activity

The fungicidal activity of *Afrostyrax lepidophyllus* essential oil was carried out in microbiology laboratory of Gombe State University.

2.4.1. Preparation of Media and Serial Dilution

9.50g of potato dextrose agar was weighed and placed into a 250ml of conical flask containing distilled water and one tablet of 250mg chloramphenicol was added and the content of the flask was well mixed and made up to the mark with distilled water and mixed again. The mixture was sterilized in an autoclave at 120°C for 15 minutes. The media was kept in a refrigerator while the various concentrations of the extract; 1:10, 1:15, 1:20, 1:30, 1:40, 1:60, 1:80 and 1:100 were prepared with n-hexane as a diluent.

2.4.2. Bioassays

The isolates of *Mucor fungi (Family: Mucoraceae, Genus: Rhizopus, Species: stolonifer*) was collected from Microbiology Laboratory in Gombe State University. The prepared media was then heated and cooled a bit then it was poured into the eight petri dishes containing the various concentrations: 1:10, 1:15, 1:20, 1:30, 1:40, 1:60, 1:80 and

1:100. In each petri dish mucor was streaked into it using a wire loop which was heated before inoculation of the mucor. Then it was placed in an incubator at 37°C and observed between 24-48 hours and the morphological features were monitored.

In the control, one petri dish contained 10ml of n-hexane and the prepared media containing chloramphenicol only and the second petri dish contained n-hexane, the prepared media containing chloramphenicol and fluconazole to inhibit growth of fungi. Both petri dishes were streaked with mucor and placed in an incubator under 37°C and then observed under 24-48 hours. The result was recorded.

The zone of inhibition was calculated using the formula:

$$\frac{Lenght + Width\ of\ zone\ of\ Inhibition}{2}$$

3. Results and Discussions

3.1. GC-MS Results

The GC-MS analysis gave a total of 7 volatile compounds identified in this plant as shown on table 1. The total of 7 components were identified in the essential oil.

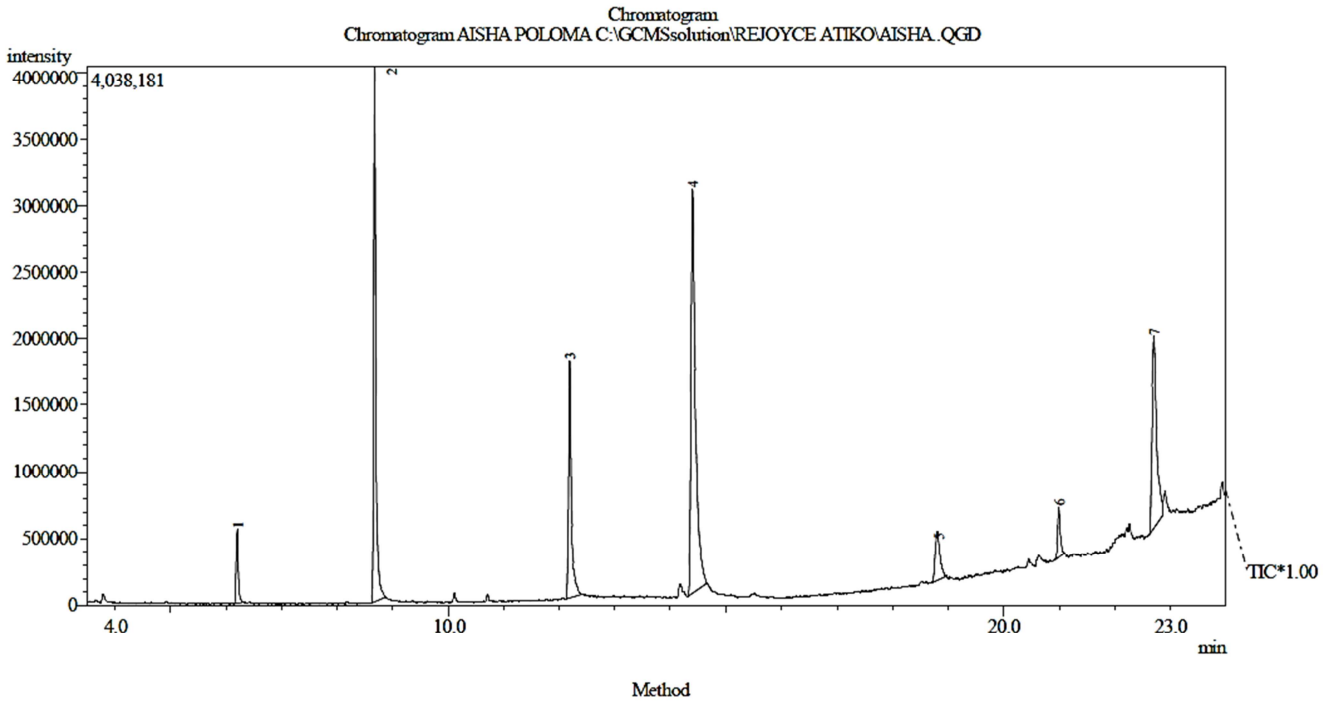
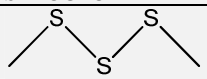
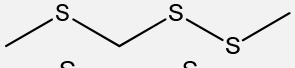
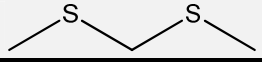


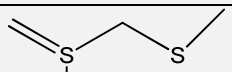
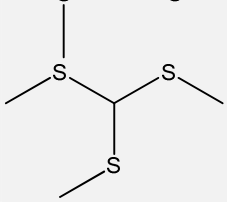
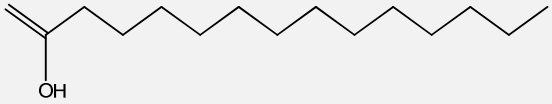
Figure 1. *GC-MS Spectra.*

Table 1. *GC-MS Analysis Result.*

	Composition of *Afrostyrax lepidophyllus* essential oil retention index		
S/N	**Constituents**	**Retention Index**	**Area (%)**
1.	Dimethyl trisulfide	972	2.80
2.	Disulfide	1072	23.32
3.	2,4-Dithiapentane	821	13.40
4.	Methane	1042	36.04
5.	Methyl thiosulfide	1107	4.43
6.	Tetradecanoic acid	1769	2.77
7.	9, 12-Octadecadien-1-ol	2069	17.26
			100

Table 2. *Structural Composition Essential Oils Detected by GC-MS.*

S/N	**CONSTITUENT**	**STRUCTURE**
1.	Dimethyl trisulfide	
2.	Disulfide	
3.	2,4-Dithiapentane	

S/N	CONSTITUENT	STRUCTURE
4.	Methane	
5.	Methyl thiosulfide	
6.	Tetradecanoic acid	
7.	9, 12-Octadecadien-1-ol	

3.2. Fungicidal Activity Result

The result of the fungicidal activity of *Afrostyrax lepidophyllus* on mucor is shown in table 3. The essential oil showed good inhibitory activity against the mucor.

Table 3. *Fungicidal activity of Afrostyrax lepidophyllus on mucor.*

Concentrations	Activity	Inhibition
1:10	+	Total inhibition
1:15	+	Total inhibition
1:20	+	Total inhibition
1:30	+	Total inhibition
1:40	++	Partial inhibition
1:60	++	Partial inhibition
1:80	+++	Partial inhibition
1:100	+++	Partial inhibition

Key: (+) high activity, (++) Moderate activity, (+++) low activity.

$$\text{Zone of inhibition} = \frac{Length + Width}{2}$$

Concentration
For concentration of 1:40: 5.5 +2 ÷ 2 =4mm
For concentration of 1:60: 6.5+2 ÷ 2 =4.5mm
For concentration of 1:80: 8+2÷ 2 =5mm
For concentration of 1:100: 10+2÷ 2=6mm

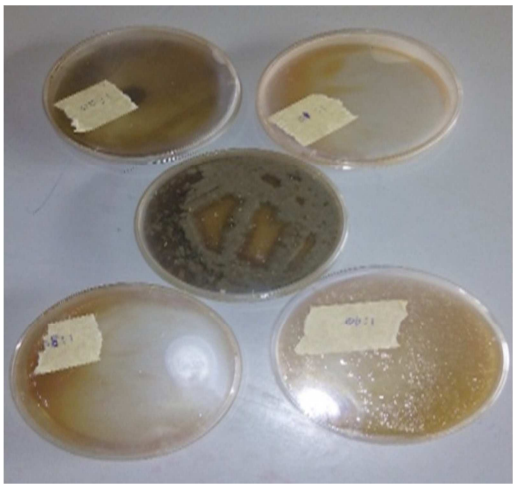


Figure 2. *Plate A Before inhibition and B after inhibition.*

3.3. Discusssion

The colour of the essential oil of *Afrostyrax lepidophyllus* obtained was colourless just like the one extracted from the seed of *Afrostyrax lepidophyllus* [16]. Seven compounds were identified in the essential oil of the bark of *Afrostyrax lepidophyllus* (Table 1) unlike 22 volatile compounds found in the essential oil from the seed of *Afrosterax lepidophyllus* by Hervert *et al*., [17], representing 98.2% of the total composition. The essential oil was characterized mostly by sulphur-containing compounds similar to Hervert *et al*., (2014). The major constituents were, dimethyl trisulfide, methyl thiosulfide, 2,4-dithiopentane methane similar to the result of Kudi and Fai [18]. The essential oil was devoid of monoterpene hydrocarbons. Hervert *et al*., [17] also identified components which are not found in the current analysis VIZ; Methanamine (1.07%), Dimethyl trisulfide (16.7%), Furan (0.79%), 1,2-Benzenediol (1.53%), Disulfide (52.3%), 1,2,4-trithiolane (0.40%), 2,4-dimethyl-3-nitrobicyclo[3.2.1] (2.85%), 2-pyridinemethanamine (0.66%), Bis(2-sulfhydrylethyl) (16.7%), Benzene methanol (0.28%), 2,2-dimethylpropanoic acid (0.50%), 1,2-bis(trimethylsily) benzene (0.27%), cyclotrisiloxane (0.28%),

silane (0.27%), Tetrasiloxane (0.68%), Methyltris (trimethylsiloxane) silane (1.00%), silicic acid (2.15%) and 1,4-phenylenebis[trimethyl] (1.53%). The minor peaks of the (figure 1) spectra were ignored by the library search and as such the common essential oil component like terpenes and terpenoids were not captured.

The results obtained from the fungicidal test on mucor showed that the essential oil extract of *Afrostyrax lepidophyllus* showed good inhibitory activity as also reported by Toumnou *et al*., [19]. From Table 3, it could be seen that, at higher concentration there is total inhibition due to high concentration of the essential oil extract (1:10, 1:15, 1:20, 1:30 and 1:40) than (1:60, 1:80, 1:100) which contained lower concentration of the essential oil extract. The results on table 3 show that the minimum fungi inhibition concentration (MFIC) was 1:30. The diameter of the inhibition zones were 4mm, 4.5mm, 5mm and 6mm for 1:40, 1:60, 1:80 and 1:100 respectively. These results shows that all the concentrations of the essential oil extract of *Afrostyrax lepidophyllus* back had some inhibition effect on the mucor fungi.

3.4. Conclusion

Based on the GC-MS analysis the major constituents of *Afrostyrax lepidophyllus bark essential oil* contained more sulphur compounds than those obtained in the literature. The results obtained in the fungicidal test showed that the essential oil extract of *Afrostyrax lepidophyllus* was effective in the inhibition of fungal growth on bread and other cooked food and thus could be recommended for use as an antifungal agent in food processing industries.

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