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RESEARCH ARTICLE

Chemical Composition of the Essential Oil of Neem (*Azadirachta indica* A. Juss.) Seeds Harvested in Senegal

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Abstract

Objective: To make better understanding of the neem seed composition (*Azadirachta indica* A. Juss.) particularly in Senegal by the extraction, the proportioning and the determination of the composition of its essential oil. **Methods:** The extraction of essential oil from neem seeds is carried out by hydro-distillation. The Gas Chromatography (GC) and Mass Spectrometry (MS) were used for analysis and identification of components. **Findings:** The yield of essential oil from dry neem seeds (95.7% dry matter) is 0.076%. The major compounds identified are 5,6-dihydro-2,4,6-triethyl- (4H) 1-3,5-dithiazine (39.1%), cis 1,2,4-Trithiolane, 3, 5-diethyl (7.9%), 1-H-indole 2,3-dione (7.9%), trans 1,2,4-Trithiolane, 3,5-diethyl (6.2%), and ethyl thioisobutyrate (4%). The classification of compounds reveals that the essential oil of neem seeds is composed of sulfur and nitrogen compounds (40.1%), sulfur compounds (25.3%), and nitrogen compounds (15.1%), sesquiterpenes (7.4%), Fatty Acid Esters (FAE) (1.5%) and hydrocarbons (0.4%). This composition reveals that neem essential oil is mainly composed of non-terpene compounds. **Novelty:** The method of analysis by GC-MS although being the most widely implemented in the quantification and identification of volatile compounds is not applied in the case of neem seeds of African origin.

Keywords: Chemical Composition; Essential Oil; Neem; Azadirachta indica; Seeds

1 Introduction

Neem (*Azadirachta indica* A. Juss.) is a plant of the Meliaceae family. It originates from the Indian subcontinent. This plant is introduced in many tropical countries such as Senegal. Different parts or extracts of the plant (fruits, leaves, roots, bark, oil, etc.) are used in traditional medicine, in the treatment of many diseases[1–7]. Indeed,

neem extracts have many biological activities such as antibacterial, fungicidal, broad-spectrum of insecticidal, antioxidant, anti-inflammatory, antipyretic, anticancer and hypoglycemic [8–12]. The oil is used in cosmetics in the manufacturing of toilet soaps, anti-dandruff shampoos [2], surfactant oil for hospital textiles [13] and in the production of biofuel [14,15]. Despite the many works dealt with to characterize neem seeds and to guid the valuation of their constituents, the essential oil of these seeds is still unknown. In particular, that of neem seeds from West Africa, particularly in Senegal, has not yet been the subject of study, despite the interest it could have. The extraction of essential oils from plant materials is generally done by hydrodistillation because of its efficiency. For the determination of the composition, the most widely used method is that of Gas Chromatography (GC) analysis coupled with Mass Spectrometry (MS).

This study aims to determine the content using the method of extraction by hydrodistillation and the composition of the essential oil of neem seeds analysis GC-MS.

2 Methodology

2.1 Biological material

The mature and dry neem seeds studied were collected in southern Senegal, dried at sunlight and then in an oven at 40 °C for 7 days. The grinding of these seeds is carried out using an Ika Werke MF 10 basic type knife mill, fitted with a sieve with a 1 mm mesh. And the Dry Matter (DM) content is determined according to the French standard NF V 03-603.

2.2 Extraction of essential oil

The extraction of essential oil from neem seeds is carried out by hydro-distillation. 100 g of seeds are crushed using a blade mill and introduced into the flask of the hydro-distiller containing 1 L of water. After boiling the medium during 6 hours, essential oil obtained is recovered with pentane and then dried by a stream of nitrogen gas according to the method recommended by the French pharmacopoeia.

2.3 Analysis of essential oil

The analysis is carried out by Gas Chromatography (GC) using a VARIAN STAR GC FID HP 5890 chromatograph equipped with a 5ms nonpolar VF column (30 m $\times$ 0.32 mm and a 1 μm film). The temperature of the column is programmed from 40°C to 280 °C at a rate of 5°C/min for a total duration of 48 min. The carrier gas consists of helium with a flow rate of 1.5 mL/min by injection split. A volume of 0.5 μL is injected at a temperature of 200°C and pressure at the top of the column maintained at 15 psi. Quantification is performed by FID at 280°C. The identification of the compounds is carried out by Mass Spectrometry (MS) type AGILENT 5973 under the same analysis conditions as in GC.

3 Results and Discussion

The yield of essential oil from neem seeds, from hydro-distillation after six (6) hours, is equal to 0.076%. This content is higher than that of the essential oil of neem seeds collected in India (0.028%) [16].

This relatively low essential oil content of neem seeds collected in Senegal is higher than those of certain essential oil of certain seeds such as those of longose, *Alpinia zerumbet* (0.04%) [17] but lower than that of other seeds such as common coriander, *Coriandrum sativum* (from 0.31 to 0.8%) [18,19] orblack cumin seeds, *Nigella sativa* (0.68 - 1.28) [20], Celery seeds, *Apium graveolens* (from 1,456 to 1,927%) [21].

In total, 34 compounds have been identified by GC-MS method, a number of compounds greater than that of the essential oil of *Moringa oleifera* (24 compounds) [22] but lower than those of essential oils from *Zingiber roseum* seeds (47 compounds) [23], and Coriander (58 compounds) [19].

The results of the analysis show that the major compound is 5,6-dihydro-2,4,6-triethyl- (4H) 1-3,5-dithiazine also called thialdine (39.1%). It is a heterocyclic compound, consisting of a cycle with 3 carbon atoms, 2 sulfur atoms and a nitrogen atom). Unlike the essential oil of neem seeds, characterized by the preponderance of a non-terpene molecule, other essential oils of seeds such as that of coriander seeds, mainly composed of linalool in very high proportion, from 49 to 52% [18] or that of black cumin seed of which p-cymene (44.6 and 46%) is the major compound [19].

The other compounds in significant proportion are the cis 1,2,4-Trithiolane-3,5-diethyl (7.9%), the 1H-indole 2,3-dione (7.5%), the trans 1,2,4-Trithiolane-3,5-diethyl (6.2%) and the ethyl thioisobutyrate (4%) (Table 1). Indeed, 1,2,4-Trithiolane-3,5-diethyl and trans 1,2,4-Trithiolane-3,5-diethyl are sulfur heterocycles. We can thus notice that the majority compounds of the essential oil of neem seeds are essentially sulfur heterocycles. The sulfur hereocycles are a high proportion (about 56%).

This composition is quite special which sets it apart from other essential oils.

The composition reveals that the essential oil of neem seeds consists essentially of non-terpene compounds (of the order of 92.5% of compounds identified) against only of the order of 7.5% for the terpenic compounds. This low proportion of terpene compounds in this essential oil is quite rare.

Among these terpene compounds, some are quite frequently found in essential seed oils, such as α-Cedrene[24], α-Longipinene[24–27], β-Bisabolene[24,28], δ-Cadinene[24,29,30].

The classification of compounds into categories (Table 2) reveals the dominance of sulfur and nitrogen compounds (40.1%), sulfur compounds (25.3%) and nitrogen compounds, mainly volatile alkaloids (15.1%). These volatile compounds, mainly sulfur or nitrogen, are believed to be responsible for its strong and pungent odor. Terpene compounds are mainly represented by the group of sesquiterpenes (7.4%). The Fatty Acid Esters (FAE) (1.5%) and the hydrocarbons (0.4%) are in small proportions. The very large proportion of sulfur and nitrogen compounds is believed to be responsible for the unpleasant odor of neem oil and its essential oil. But note that the smell of the essential oil is much more pleasant than that of its lipids, in part probably due to the presence of terpene molecules such as italicene, α-bergamotene, valencene, β-bisabolene, commonly found in pleasant smelling essential oils.

This composition of the essential oil of senegalese neem seeds differs from that obtained by Kurose and Yatagai, 2005 on neem seeds collected in India (56 identified compounds) whose major compounds are hexadecanoic acid (34%), oleic acid (15.7%) and 5,6-dihydro-2,4,6-triethyl-(4H)1-3,5-dithiazine (11.7%)[16]. Besides 5,6-dihydro-2,4,6-triethyl-(4H)1-3,5-dithiazine, other non-terpene compounds (Cis 1,2,4-Trithiolane-3,5-diethyl and Trans 1 ,2,4-Trithiolane-3,5-diethyl) and terpene molecules (Italicene, β-Bisabolene, Valencene and Bergamotene) are also found in the essential oil of Indian neem seeds.

This difference in content and composition of between the essential oil of neem seeds collected in Senegal and that of neem seeds collected in India could be explained by the difference in climatic conditions (rainfall, temperature, sunshine, etc.), edaphic and tree genotypes, which are not the same in these two countries.

Table 1. Composition of the essential oil of neem seeds

Compounds	Content (%)	RI[a]	*Th* RI	Identification
2-Methyl-1-D1-aziridine	0.3	665	690	MS, RI
Tiirane methyl	0.3	854	-	MS, RI
3,4-Dimethylthiophene	1.3	887	884	MS, RI
Thiophene, 2,5-dimethyl	0.4	914	884	MS, RI
Disulfide, dipropyl	0.2	1117	1119	MS, RI
Ethyl thioisobutyrate	4	1131	-	MS, RI
2-Methyl-2-ethyl-1,3-dithiolane	0.3	1153	1116	MS, RI
Benzene 1-methyl 2-(propylthio)	0.8	1256	1356	MS, RI
1,1,2-Tri (methylthio) ethene	0.8	1264	1256	MS, RI
N-Formyl-3-methyl-3,4-dihydro-2H-1,4-benzoxazine	0.3	1311	-	MS, RI
Ethane 1,1'-[[(methylthio) methylene] bis (thio)] bis	0.7	1321	1306	MS, RI
Unidentified	0.5	-	-	MS, RI
Unidentified	0.3	-	-	MS, RI
Unidentified	0.3	-	-	MS, RI
Trans 1,2,4-Trithiolane-3,5-diethyl	6.2	1354	1344	MS, RI
Cis 1,2,4-Trithiolane-3,5-diethyl	7.9	1363	1373	MS, RI
β-cubebene	0.6	1387	1388	MS, RI
2-propanamide 1-propene-1(1-methoxy)	2.6	1397		MS, RI
1H-indole 2,3-dione	7.5	1408	1381	MS, RI
(±) 3,5-Diethyl-1,2,4-trithiolane	0.7	1424	1378	MS, RI
Unidentified	1.6	-	-	MS, RI
α-Cedrene	0.6	1432	1411	MS, RI
Unidentified	0.4	-	-	MS, RI
α-Bergamotene	0.5	1438	1435	MS, RI
α-Longipinene	0.7	1450	1403	MS, RI
Bicyclo [4.4.0] dec-1-en 2-isopropyl-5-methyl-9-methylene	0.4	1462	1503	MS, RI
Italicene	0.7	1478	1405	MS, RI
5,6-dihydro-2,4,6-triethyl-(4H)1-3,5-dithiazine	39.1	1498	-	MS, RI
Valencene	1.2	1507	1496	MS, RI
β-Bisabolene	1	1513	1509	MS, RI

Continued on next page

Table 1 continued

δ-Cadinene	0.5	1527	1523	MS, RI
6,10,11,11-Tetramethyl tricyclo [6.3.0 (2,3)] undec-1 (7)-ene	2	1533	-	MS, RI
3H-1,2,4-triazole-3-thione 2,4-dihydro-5-methyl	1	-	-	MS, RI
Trans 3-hexanedioic acid, bis (trimethylsilyl) ester	1.5	1613	1586	MS, RI
1,3,4-Trithiane 2,2,4,4,6,6-hexamethyl	1.4	1619	1547	MS, RI
2-isopropoxyphenyl N-methylcarbamate	2.1	1661	1598	MS, RI
α-cadinol	1.6	1669	1653	MS, RI
Hexanoic acid 4-(1-pyrrolidinylmethyl methyl ester)	1.6	1683	1561	MS, RI
4 (1H)-pyrimidinone 2-amino 6-(methylamino)	1	1774	1552	MS, RI
Total	**94.9**			

RI[a] : MS Retention Indices; ThRI : theoretical Retention Index; RI : Retention Index ; MS : Mass Spectrometry

Table 2. Groups of compounds in the essential oil of neem seeds

Groups of compounds	**%**
Nitrogen and sulfur compounds	40.1
Sulfur compounds	25.3
Nitrogen compounds (volatile alkaloids)	15.1
Sesquiterpenes	7.4
Fatty Acid Ethers (FAE)	1.5
Hydrocarbons	0.4
Identified compounds	89.8

4 Conclusion

Novelty: This study, among others, shows that the essential oil from neem (*Azadirachta indica*) seeds collected in Senegal is composed mainly of non-terpene sulfur and nitrogen compounds (around 80.2%) and few terpene compounds, mainly sesquiterpenes (around 7.5%). This particular composition is different from that of the essential oil of neem seeds harvested in India whose proportion of fatty acids is much higher (more than 50% of the compounds). Note that in this essential oil of Indian seeds, the majority compound is hexadecanoic acid. The proportion of fatty acid ester (about 8%) is higher than that of Senegal but that of 5,6-dihydro-2,4,6-triethyl-(4H)1-3,5-dithiazine (11.68%) is lower than that of senegalese seeds.

Prospects: Valuations of the essential oil of neem seeds can be considered, but it will first be necessary to do the characterization of the essential oil content to assess the antioxidant activity and other biological properties (antimicrobial, antiseptic, etc.) of the neem seed oil.

Recommendations: It would also be important to expand the field of study to other parts of neem (leaves, flowers, roots and bark) and explore their value.

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