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Extraction and chemical characterization of neem seed oil (*Azadirachta indica*)

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INFO

Keyworks
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gas chromatography
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ABSTRACT

The neem tree (*Azadirachta indica* A. Juss) is known for its therapeutic, pesticide, fertilizer and pharmacological properties. Due to these attributes, this tree has economic potential and continues to be used worldwide. For oil extraction, the Sohxlet system was used, for the extraction and characterization of volatiles, the HS-SPME/GC-MS method was used, and the fatty acid profile was characterized by GC-FID. The average yield obtained from the oil was 23%. A total of 34 volatile compounds were detected, of which 9 were identified by Kovat's index: 2-methyldecane, 3-methyldecane, 4-methyldecane, 2-methylundecane, 3-methylundecane, 3,4-dimethylthiophene, 2,5-diol- 3-hexane, 1,2,3-trimethylbenzene and buticyclohexane. In addition to the volatiles in the oil, 7 fatty acids were found; oleic acid, linoleic acid, palmitic acid and stearic acid, with the oil extracted from the seeds having the highest concentration of oleic acid and linoleic acid in commercial neem oil. Given the presence of these chemical compounds in neem oil, it is suggested, for subsequent investigations, the isolation and application of organosulfur compounds as insecticides. The extraction of oil from flowers and neem seeds with solvents that are less harmful to the environment, together with its application, makes it possible to evaluate its pesticide potential. In addition, it is essential to evaluate the influence of neem on the bee population, analyzing cases of mortality and seeking solutions to avoid environmental damage. Additionally, the viability of neem oil as an industrial and hospital cleaning product is highlighted, exploring antimicrobial, antibacterial and biodegradable properties.

Palavras-chaves
Azadirachta indica a. juss
cromatografia gasosa
microextração em headspace de fase sólida
sohxlet

RESUMO

Extração e caracterização química do óleo da semente de nim (Azadirachta indica)
A árvore nim (*Azadirachta indica* A. Juss) é conhecida por suas propriedades terapêuticas, pesticidas, fertilizantes e farmacológicas. Devido a esses atributos, essa árvore tem potencial econômico e continua sendo utilizada mundialmente. Para a extração do óleo foi utilizado o sistema Sohxlet, para a extração e caracterização dos voláteis foi utilizado o método HS-SPME/CG-EM e o perfil de ácidos graxos foi caracterizado por CG-DIC. O rendimento médio obtido do óleo foi de 23%. Foram detectados 34 compostos voláteis, dos quais 9 foram identificados pelo índice de Kovat: 2-metildecano, 3-metildecano, 4-metildecano, 2-metilundecano, 3-metilundecano, 3,4-dimetiltiofeno, 2,5-diol- 3-hexano, 1,2,3-trimetilbenzeno e buticiclohexano. Além dos voláteis no óleo, foram encontrados 7 ácidos graxos; ácido oleico, ácido linoleico, ácido palmítico e ácido esteárico, entre esses a maior concentração encontrada foi de ácido oleico e ácido linoleico. Diante da presença destes compostos químicos no óleo de nim, sugere-se, para investigações subsequentes, o isolamento e aplicação dos compostos organossulfurados como inseticidas. A extração do óleo das flores e sementes de nim com solventes menos prejudiciais ao meio ambiente, juntamente com sua aplicação, possibilita a avaliação de seu potencial pesticida. Além disso, é fundamental avaliar a influência do nim na população de abelhas, analisando casos de mortalidade e buscando soluções para evitar danos ambientais. Adicionalmente, destaca-se a viabilidade do óleo de nim como produto de limpeza industrial e hospitalar, explorando as propriedades antimicrobianas, antibacterianas e propriedades biodegradáveis.

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INTRODUCTION

The neem or neem tree (*Azadirachta indica* A. Juss) originates from Myanmar and the Indian subcontinent region (Islas et al., 2020; Sarkar et al., 2021). In the botanical classification, it belongs to the Meliaceae family, genus Azadirachta and species Indica (Sarkar et al., 2021). The tree has been used for over 2,000 years in India as an agricultural pesticide, in the production of drugs, cosmetics, fertilizers and disinfectants (Perera et al., 2021; Baby et al., 2022; Rana e Saxena, 2023 and Alba et al., 2023). It also has medicinal uses in the treatment of cancer (Jeelani et al., 2023), in the fight against the malaria vector (Ayinde et al., 2020) and is anti-inflammatory and antiviral (Loganathan et al., 2021).

In animals in the control of parasites, such as canine leishmaniasis (Zatelli et al., 2022). Among the plants with pesticidal activity, *Azadirachta indica* has several studies that demonstrate different uses against insects such as insecticide, larvicide (Jeelani et al., 2023), anti-food, reproduction inhibitor, development inhibitor (Kilani-Morakchi et al., 2021) and repellent (Ayinde et al., 2020; Ghosh et al., 2021).

This is due to the large amount of phytochemicals, among which the substance azadirachtin is known as the most efficient for insecticide use, being the main one in biopesticide formulations (Perera et al., 2021; Rana e Saxena, 2023). Azadirachtin is present in varying concentrations in all parts of the plant, but the seeds have the highest concentration of azadirachtin, being more used in the production of oils for insecticide use (Chaudhary et al., 2021; Loganathan et al., 2021; Rana e Saxena, 2023). In view of this, oil extraction is considered the best way to take advantage of the benefits of the plant (Loganathan et al., 2021; Kumar et al., 2022; Rana e Saxena, 2023).

The methods used to extract neem oil are mechanical pressing, soxhlet, supercritical fluid, microwave and ultrasound (Chaudhary et al., 2021; Saha Tchinda et al., 2021). Soxhlet extraction is the most used to obtain bioactive molecules from natural sources (Fagbemi et al., 2021), its advantages are repetition in cycles of the sample solvent in the extraction and not requiring the filtration step after extraction (López-Bascón e Luque de Castro, 2020). On the other hand, the disadvantages are: high extraction time; degradation of thermolabile substances, due to the temperature used being close to the boiling point of the solvent (extractor); the need for another stage of evaporation of the solvent contained in the sample after extraction (Hirondart et al., 2020). In the extraction of neem oil via the Soxhlet system, the solvents that provide the most satisfactory yields are hexane (Chaudhary et al., 2021), as well as mixtures such as hexane/methanol and hexane/ethanol (Barbosa et al., 2023). However, other factors influence the choice of solvent. Selection based on polarity makes it possible to isolate related substances (Alba et al., 2023). In addition, the environmental issue is relevant, as hexane is a pollutant (Kumar et al., 2022). Green solvents such as acetone, isopropanol and ethanol also appear as options, useful for separating compounds and fatty acids of importance in the pharmaceutical and food industry (Saha Tchinda et al., 2021).

Neem seed oil has in its chemical constitution a diversity of volatile organic compounds (VOCs) belonging to different chemical classes such as: alcohols, acids, esters, phenols, flavonoids, sulphates, aldehydes, hydrocarbons and terpenes (Guchhait et al., 2022; Tulashie et al., 2021), which are responsible for the characteristic aroma of olive oil. In addition to the aroma, volatiles have antibiotic, antioxidant, antiviral, antitumor, antibacterial, antifungal properties and are used in the prevention and treatment of various diseases such as diabetes and cancer in humans (Rana e Saxena, 2023; Jeelani et al., 2023). Along with volatiles, neem oil has fatty acids in its composition, which are very important for the normal functioning of the human organism (Mulla et al., 2020; Saha tchinda et al., 2021). These fatty acids are used in pharmaceutical products (Zheng et al., 2021).

Thus, given the importance of the presence and use of these compounds, the general objective in the present study was the extraction of oil from the neem seeds through the soxhlet system, the characterization of the profile of volatile compounds by microextraction in solid phase headspace mode, followed by analysis by gas chromatography coupled with mass spectrometry HS-SPME/GC-MS, and fatty acids by gas chromatography with flame ionization detector GC-FID.

MATERIALS AND METHODS

Collection of the neem seeds

The seeds of neem were collected in the months of November and December 2021, at the Federal University of Northern Tocantins (UFNT), Cimba unit (-7.181285262491843, -48.19801163546441) in Araguaína-Tocantins. The ripe seeds have been pulped using a common nylon sieve, and then dried, under the sun, for about 18 days. Although, the unripe seeds have been separately reserved, for one week, until they became fully ripe to be pulped.

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After all the seeds have been pulped, they were taken to a drying oven with air circulation of 55°C for 72 hours.

Figura 1 - Dry seeds after passing through the drying oven with air circulation

Oil extraction

After drying, the seeds were peeled and macerated in sufficient quantity for extraction, using a mortar (gral) with a porcelain pistil. The mass of the macerated sample was measured, about 11.0 g, on an analytical scale (Model AUY220, Shimadzu brand), then transferred to the paper filter cartridge, and then inserted into the soxhlet extraction system. The solvent used to extract the oil was the n-hexane analytical grade P.A. (quimex brand), where 8 extraction cycles were performed. At the end of the extraction, the sample was taken to the rotary evaporator (brand Lucadema, model Luca-EV01) with a heating bath (brand Fisatom, and model 550). After evaporation of the solvent hexane, the oil was transferred to a beaker and anhydrous sodium sulfate (dynamic brand) was added to remove water remnants in the sample. Then, the sample containing the salt was filtered on filter paper, the residual mass was weighed on an analytical scale and the oil output was calculated, the measured value was 2.7g.

Figura 2 - Extracted neem seed oil

Microextraction solid phase headspace and chromatographic analyses

For the preparation of the sample by HS-SPME, approximately 1.26 g of the neem oil was weighed and transferred to a vial for headspace of 10 mL of transparent glass with a silicone cover. Subsequently, the vial was placed on the magnetic stirrer (Model, IKA C-mag HS 4) for 15 minutes at an approximate temperature of 45°C monitored by an infrared thermometer (model ST-600, brand Incoterm). Then, the needle of the holder was inserted into the vial, exposing the fiber CAR/PDMS (carboxen/polydimethylsiloxane film)

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to the headspace of the vial for 15 minutes to capture the volatiles. After that, the fiber was collected, and the holder was removed from the bottle and transferred to the GC-MS for analysis of the volatiles.

For the separation of volatile substances, it was used the gas chromatograph coupled to the mass spectrometer (GC-MS Agilent Technologies 7890B CG system and MSD 5977B). Before performing the chromatographic race, the fiber was conditioned according to the recommended conditions of the manual. Injector, 250 °C; initial oven temperature 40°C, with an increasing rate of 4 °C/min up to 220°C and after 20°C/min ending at 280 °C, the race had a total time 50 minutes; the temperature of the ionization source was 230 °C and quadrupole 150 °C. The carrier gas was helium (99.99 % purity), injection mode manual split 1:50, the column was the capillary type HP-5MS (5 % phenylmethylpolysiloxane) with dimensions 30 m x 25.0 µm x 0.25 µm, ionization energy of 70 eV.

For the identification of the compounds, it was used the NIST 2014 library present in the program provided by Agilent and the NIST Chemistry Book on the Web, the confirmation occurred by calculation and confirmation by reference to the Kovats index.

For analysis of the oil in the automatic injection mode with solvent, the oil is diluted at the ratio 1/100 in hexane (V/V) using a micropipette 10-100 µL and 100-1000 µL. The injection occurred in 1:50 split mode, and about 1 µL of the sample was injected automatically using a 10 µL glass syringe. The employed chromatographic conditions have been optimized according to the work of (Hossain et al., 2013). The initial temperature of the oven was 45 °C for 3 minutes, with a heating rate of 3 °C / minute, until reaching 150 °C with a variation of 20 °C / minutes ending at 250 °C, the analysis time lasting 48 minutes. Injector temperature, 155 °C and ionization source 230 °C, and quadrupole 150 °C.

Kovats index determination

A mixture of n-alkane pattern (C7-C30) has been used to determine the Kovats index of each compound detected in the oils analysis, through the retention times of n-alkanes, compounds and comparing values in the literature that have the same stationary phase of the capillary column (NIST Chemistry Book on the Web). The parameters of the chromatographic runs used have been the same as the oil analyses, except for the injection of fiber, which occurred in automatic mode.

Gas chromatograph with flame ionization detector (GC-FID)

For chromatographic analysis, 5,0 g of neem oil, 10 mL of methanol, and 50 mg of catalyst were added to a Teflon cup. The cup was introduced into a stainless-steel autoclave which was hermetically sealed and placed in a shaking heater with a temperature of 150 °C for a 4 hour period. After this time, the material was removed from the cup and centrifuged to separate the catalyst from the reaction medium. The excess methanol was evaporated using a rotary evaporator, thus obtaining the methyl esters of fatty acids.

It was weighed 114 mg of the sample and 1 mL of chloroform added. Subsequently, the oil was esterified, and a Gas Chromatograph was used GC – Shimadzu 2010 plus, with Rtx-Wax capillary column, coupled with flame ionization detector (FID), injecting (1 µL) of sample and with a programming column temperature of 210 °C for 50 minutes, the FID temperature of 250 °C and the flows of H2, air and carrier gas (N2) 40, 400 and 1.6 mL min-1, respectively. A seed oil pattern was used to identify fatty acids. A commercial sample of the neem oil, acquired in an agricultural products store located in the city of Teresina-PI, was analyzed under the same conditions for comparison with the oil profile extracted from the seeds.

RESULTS AND DISCUSSION

The average oil output was 23.73 % ± 0.07. In the literature, using the same technique and hexane solvent, it has been found results of 38.8 % (Yami et al., 2020), 40.35 % (Tesfaye et al., 2018), 52.3 % (Saha tchinda et al., 2021) and lower results, 21.51 % (Kaura et al., 1998) and 13.33 % (Ungo-Kore et al., 2019). According to (Keneni e Marchetti, 2017) the grain oil output was 25-45 %, which is within the range presented in the literature. Oil output is related to tree development environment conditions, climate, rainfall, soil characteristics; genotype; conditions for the collection; storage of plant material; particle size (Fernandes et al., 2019; Neves e Carpanezzi, 2008; Saha Tchinda et al., 2021; Beyecha Hundie et al., 2022). The oil yield can even be affected by the amount of sample used in the extraction. This is evidenced in articles where oil yields were cited. Consequently, these variables impacted the result of oil extraction output.

Analysis of volatile substances by GC-MS

In the analysis of volatile substances by GC-MS, 35 compounds were detected, among them those with the highest relative percentage area in direct

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injection were: undecane, 13.44 %; dodecane, 8.65 %; (Z)-2-methylpent-2-eno-1-ol, 4.53 %; 2-methylundecane, 4.05 %; 2-methyldecane, 3.08 % and ethylbenzene 2.93 %. The compounds with the largest percentage area captured by HS-SPME were undecane, 7.57 %; 1, 3-dimethylbenzene, 7.44 %; toluene, 5.35 %; ethylbenzene, 3.94 %; dodecane, 3.41 % and decane, 3.45 %. Table 1 presents the volatile substances found.

About five compounds belonging to the branched hydrocarbon function were identified in the automatic injection with solvent, 2-methyldecane, 3-methyldecane, 4-methylundecane, 2-methylundecane and 3-methylundecane. With the application of SPME fiber, 4 volatile compounds were identified: 3,4-dimethyliophene, 2,5-diol-3-hexane, 1,2,3-trimethylbenzene and bulticyclohexane.

Tabela 1 - Lists the 35 volatile substances detected separated by the injection forms

Injection	Compounds	Molecular Formula	TrX	Area (%)	Ident.	Simi. %
Automatic injection with solvent	2,3,4,5,6,7,8-Heptahydroxyoctanal	$C_8H_{16}O_8$	6,737	0.91	*	8.8
	Ethylbenzene	C_8H_{10}	8,366	2.93	*	43.5
	1,4-Dimethylbenzene	C_8H_{10}	8,675	2.36	*	31.1
	2-Miristinoil panthethein	$C_{25}H_{44}N_2O_5S$	9,933	0.42	*	20.3
	3-Tetradecanol	$C_{14}H_{30}O$	10,365	1.60	*	8.7
	2,4-Dimethyl-3-hexanol	$C_8H_{18}O$	11,099	1.52	*	14.3
	1-Ethylbutylhydroperoxide	$C_6H_{14}O_2$	12,137	1.73	*	24.6
	(Z)-2-methylpent-2-eno-1-ol	$C_6H_{12}O$	13,067	4.53	*	8.2
	Decane	$C_{10}H_{22}$	14,56	2.90	*	27.9
	Undecane	$C_{11}H_{24}$	19,45	13.44	*	34.9
	Dodecane	$C_{12}H_{26}$	24,258	8.65	*	30.6
	2-Methyldecane	$C_{11}H_{24}$	17,696	3.08	I	16.2
	3-Methyldecane	$C_{11}H_{24}$	18,008	2.54	I	10.5
	4-Methylundecane	$C_{12}H_{26}$	22,331	1.91	I	22
	2-Methylundecane	$C_{12}H_{26}$	22,537	4.05	I	14.8
	3-Methylundecane	$C_{12}H_{26}$	22,849	2.86	I	10.1
	DL-cystine	$C_6H_{12}N_2O_4S_2$	39,296	1.62	*	22.1
Manual injection with SPME fiber	Toluene	C_7H_8	4,216	5.35	*	64.4
	Butyl acetate	$C_6H_{12}O_2$	5,403	0.1	*	96.8
	2-Methyl-2-pentenal	$C_6H_{10}O$	5,862	0.45	*	54.6
	Ethylbenzene	C_8H_{10}	6,707	3.94	*	49.3
	1,3-Dimethylbenzene	C_8H_{10}	6,958	7.44	*	36.5
	1,2-Dimethylbenzene	C_8H_{10}	7,708	2.29	*	35.4
	3,4-Dimetilphene	C_6H_8S	8,078	0.42	I	35.7
	2,5-Diol-3-hexane	$C_6H_{12}O_2$	10,405	1.71	I	6.9
	1,2,3-Trimethylbezene	C_9H_{12}	11,266	1.84	I	25.9
	Decane	$C_{10}H_{22}$	11,563	3.25	*	50.9
	Butilcyclohexane	$C_{10}H_{20}$	12,663	1.30	I	28.6
	1-Ethyl-2-propylcyclohexane	$C_{11}H_{22}$	13,187	0.87	*	48.2
	Undecane	$C_{11}H_{24}$	15,288	7.57	*	50.1
	Phytol	$C_{20}H_{40}O$	17,106	1.01	*	22.2
	4-Methylundecane	$C_{12}H_{26}$	17,454	1.60	*	50.5
	Dodecane	$C_{12}H_{26}$	18,901	3.41	*	41.1
	(E)-propenilpropiltrisulfide	$C_6H_{12}S_3$	23,434	0.06	*	79.3
	Di-(1-propenil)-trisulfide	$C_6H_{10}S_3$	23,668	0.07	*	57.9

Identification attempt*; IdentifiedI;

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Figures 3 and 4 show the chromatograms of automatic injection with solvent and manual injection with SPME fiber, respectively. In these figures are listed the volatiles with the highest percentage area and those identified by Kovats index.

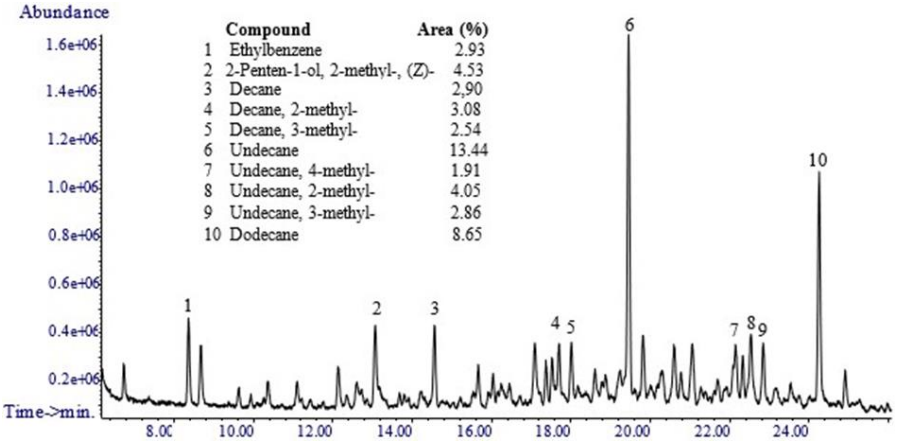

Figura 3 - Chromatogram of neem oil volatiles by direct injection with solvent

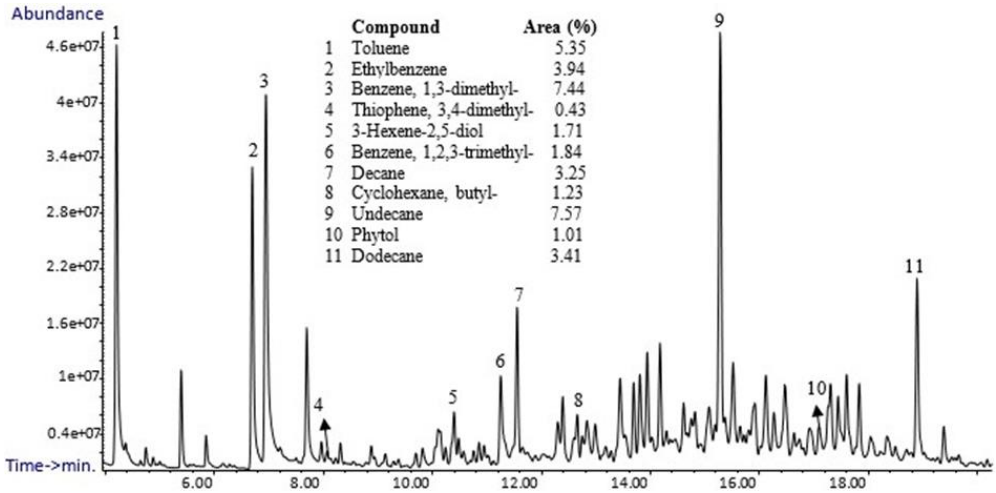

Figura 4 - Neem oil chromatogram by HS-MEFS-CG-EM

Some compounds found in our work, mostly hydrocarbons, have bioactivity. Phytol, for example, is mentioned in several publications, it was researched in the neem oil by (Chinnasamy et al., 2021), and also identified in other oils of Lantana camara, Caulerpa racemosa (Forssk.) J. Agardh, Padina boergesenii Allender & Kraft and Moringa oleifera (Ayalew, 2020; Ragunath et al., 2020; Zhao et al., 2019). The compound phytol is an unsaturated long-chain linear diterpene alcohol (Islam et al., 2018; Olofsson et al., 2014), chlorophyll hydrolyzed in molar proportion 1:1, being one of the most abundant isoprenoids on the planet (Gutbrod et al., 2019). For years, studies have shown that phytol and its derivatives have antimicrobial, antitumor, antibiotic, antioxidant (Islam et al., 2015), anti-inflammatory, anticancer (Shariare et al., 2021) and anti-arthritic agent with the ability to reduce inflammations in joints and spinal cord (Carvalho et al., 2020). In addition, phytol is a precursor of tocopherol (vitamin E), phylloquinol (vitamin K) and fatty acid phytyl ester (Gutbrod et al., 2019).

Analyzed by CG-MS the volatiles of the neem seeds and identified the compounds 2-methyl-2-pentenal, 3,4-dimethyliophene, cis and trans of propenilpropiltrissulfide and di-(1-propenil)-trissulfide (Balandrin et al., 1988). The last compounds that are organosulfurate are part of the plant's defense mechanism against insect attacks. Compound 3,4-dimethyliophene was detected using HS-SPME/GC-MS in volatiles in the neem leaf (Perera et al., 2018) and identified in garlic essence oil (Allium sativum) (Satyal et al., 2017).

The abovementioned volatile and 2-methyl-2-pentenal were identified in neem seeds oil (Mubarak e Kulatilleke, 1990) and in onion samples by SPME (Choi et al., 2017). The 2-

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methyl-2-pentenal had proven antifungal activity against Fusarium oxysporum f. sp. Cubense stipe 4 (Zhang et al., 2013). Performed extraction of yellow onion oil by steam carrier, some volatiles found were 3,4-dimethyliophene and (E)-propenilpropiltrissulfide, one of the compounds with the highest concentration, being one of the main volatiles that collaborates with aroma and flavor of the onion oil (Cantrell et al., 2020). In our work, some sulfur compounds found in the neem seed oil were found in onions (Balandrin et al., 1988; Breu, 1996). This indicates the existence of similar metabolic processes in both species (Balandrin et al., 1988), although having this similar volatile profile, the aroma of the neem does not cause tear effect and loses intensity (Koul, 2004).

Conducted a study of the volatiles of Australian wild rice, identified an aroma similar to plastic, and found that one of the compounds that contribute to this odor is 2-Miristinoil pantethine (Zhao et al., 2022) that was also detected in our study. Toluene is present in the essential oil of the the neem leaf (Ebrahimi et al., 2013), inhibiting the presence of bacteria R. solanacearuam. Other compounds such as ethylbenzene and 1,3-dimethylbenzene obtained excellent antibacterial and antifungal activity (Mohamadpoor et al., 2022; Raza et al., 2016).

The volatile undecane was detected in buchanania lanzan seed oil (Desai et al., 2022) and dodecane compound in leaf oil of Strychnos nux-vomica (Suganthy e Gajendra, 2020), but no articles were found that report these volatiles in the neem oil. Detected dodecane and undecane in Pseudomonas spp. evaluated the effects of volatiles in promoting the growth of Vigna radiata seedlings, the benefits of these two substances in the seedling is to contribute to plant development, induced systemic resistance and antimicrobial action (Jishma et al., 2017).

Hydrocarbons, decane, dodecane, undecane, 2-methyldecane, 3-methyldecane, 2-methylundecane and 4-methylundecane are present in plant extracts with antimicrobial properties (Bukvicki et al., 2013; Kumar Tyagi et al., 2013; Winnett et al., 2017). Thus, it is possible that the volatiles mentioned contribute synergistically to antimicrobial activity with other compounds of the neem oil. The oil extracted in this work was applied in hydroponic lettuce cultivation in another project.

Analysis of the neem oil by GC-FID

It observed by the analysis of the oil extracted from the seeds and the commercial oil of neem, the presence of the same fatty acids: palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, and arachidonic acid. The fatty acids of the oil extracted from the seeds have a higher percentage area, except linolenic, indicating higher concentration in relation to commercial oil. Moreover, a greater number of fatty acids was identified in the fixed oil extracted from the seeds. Figures 3 and 4 show the chromatograms of oil extracted from the seeds and commercial of neem, with the percentage areas of each compound.

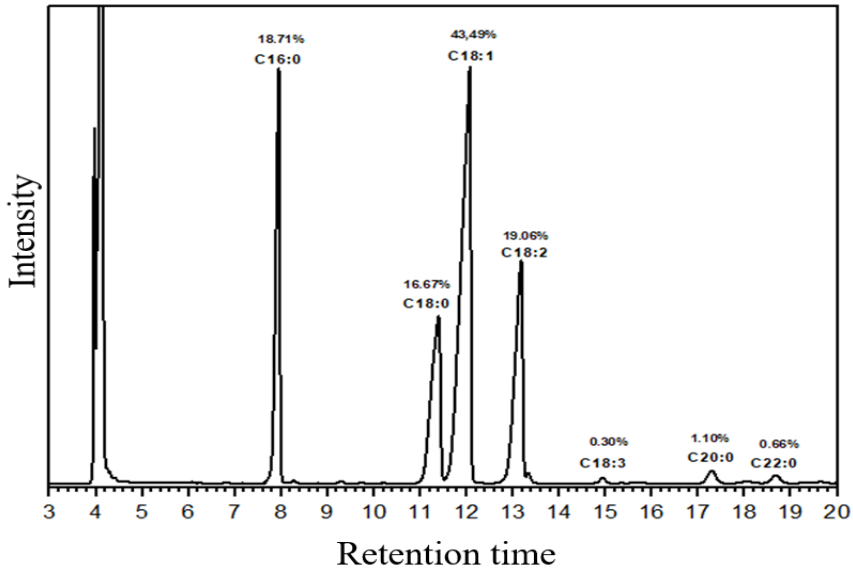

Figure 5 - GC-FID chromatogram of fixed neem oil extracted from seed. Palmitic acid C16:0 (18.71%); stearic acid C18:0 (16.67%); C18:1 oleic acid (43.49%); C18:2 linoleic acid (19.06%); linolenic acid C18:3 (0.30%); 20:0 arachidonic acid (1.10%); behenic acid C22:0 (0.66%).

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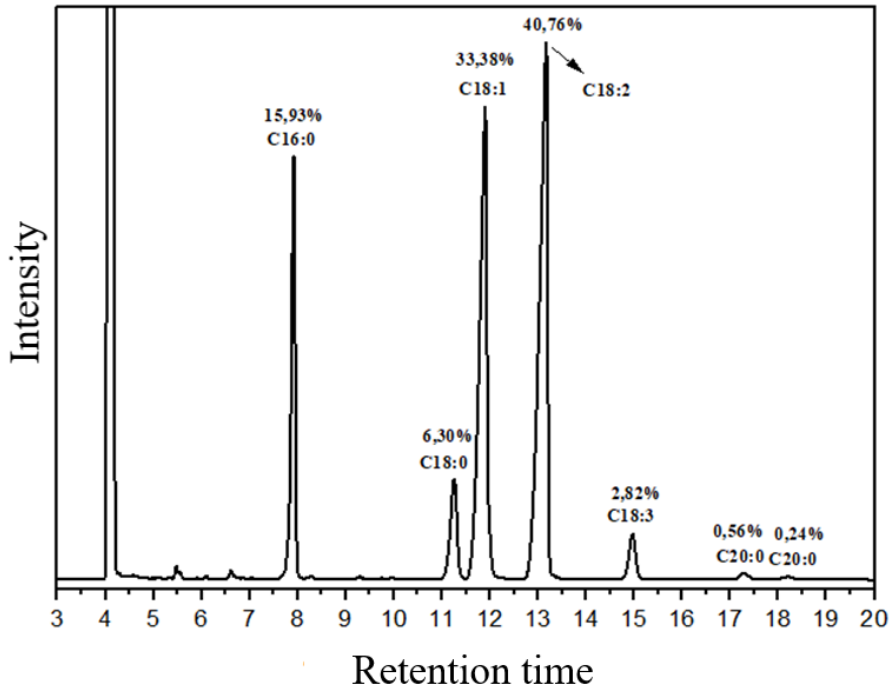

Figure 6 - GC-FID chromatogram of commercial neem oil. Palmitic acid C16:0 (15.93%); stearic acid C18:0 (6.30%); C18:1 oleic acid (33.38%); C18:2 linoleic acid (40.76%); linolenic acid C18:3 (2.82%); arachidic acid C20:0 (0.56%).

The major fatty acids with their respective values of percentage areas found in seed oil were oleic acid 43.49 %; linoleic acid 19.06 %; palmitic acid 18.71 % and stearic acid 16.67 %. While in commercial oil, the data for the same compounds were linoleic acid 40.76 %; oleic acid 33.38 %; palmitic acid 15.93 % and stearic acid 6.30 %. In the literature, all the compounds mentioned are among the most abundant in the neem oil analyzed in the GC-FID, with the highest concentration of oleic acid and linoleic acid (Mulla et al., 2020; Saha tchinda et al., 2021; Ismaila et al., 2022).

In other oils, it has been detected the presence of most of the fatty acids mentioned above, for example, in the oil of Moringa oleifera seeds, oleic acid is more abundant, with a percentage area of 70.2%; followed by palmitic acid 7.8%; stearic acid 7.5% and linoleic acid 3.1% (Zhao et al., 2019). In soybean oil fatty acid analysis studies, the major compounds are linoleic acid, oleic acid, palmitic acid, linolenic acid and stearic acid (Kostik et al., 2013; Zambiazi et al., 2007). As presented, soybean oils and Moringa Oleifera contain a strong presence of oleic acid and linoleic acid, as well as in our work with neem oil extracted from seeds and commercial oil.

Some fatty acids found in our work have pesticide activity. The mixture of oleic, linoleic, palmitic and stearic and oleic effects with stearic acids showed ovicidal effects of egg positioning and death of larvae of C. maculatus (Lienard et al., 1993). In another study the fight against C. maculatus was also observed an insecticide property, with the use of oleic and linoleic acid that had greater efficiency and with the mixture with the four fatty acids mentioned above (Aider et al., 2016). Other properties of oleic acidic fatty acids, linoleic, palmitic and stearic are antibacterial activity (Casillas-Vargas et al., 2021), linoleic is related to decreased risks of cardiovascular diseases (Marangoni et al., 2020) and the high intake of oleic acid can help improve cases of hypertension, obesity, insulin resistance and inflammations (Granado-Casas e Mauricio, 2019).

CONCLUSIONS

The efficient extraction of neem seeds oil was performed by the soxhlet system using the hexane solvent, obtaining a good output equal to 23.73% ± 0.07. It was possible to detect 35 volatile compounds in the neem seeds oil, belonging to several chemical classes, which are responsible for the characteristic aroma of the oil. These same compounds are known for their antioxidant, biological, antibactericidal, antifungal, antimicrobial, insecticide, and anticancer activities.

Seed and commercial oils are rich in fatty acids and have practically the same compounds identified by GC-FID. However, the concentration of fatty acids is higher in the oil extracted from the seeds, and the oleic and linoleic acids are the

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compounds with the highest concentration. Fatty acids as well as volatile acids have biological activities essential to the functioning of the human organism and tree development. Thus, it's considered that all these chemical constituents present in the neem oil contribute synergistically to the various known benefits in using neem.

Given the presence of these chemical compounds in neem oil, it is suggested, for future research, to isolate and apply organosulfur compounds as insecticides. The extraction of oil from neem flowers and seeds, with solvents that are less harmful to the environment, makes it possible to assess its pesticide potential. Evaluating the impact of neem on bees, analyzing mortality and seeking solutions against environmental damage, is crucial. Additionally, the potential of neem oil as an industrial and hospital cleaning product is highlighted, exploring its antimicrobial, antibacterial and biodegradable properties.

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BIBLIOGRAPHIC REFERENCES

Alba SL, García-Gonzáles C, Coronado OMA, Ayla BJR, Alpírez GM, Montes DGLNN. extraction methods and applications of bioactive compounds from neem (*Azadirachta indica*): A mini-review. Mini-Reviews in Organic Chemistry, v.20, n.7, p.644-654, 2023. https://doi.org/10.2174/1570193X19666220707125726

Aider FA, Kellouche A, Fellag H, Debras JF. Evaluation of the bio-insecticidal effects of the main fatty acids of olive oil on *Callosobruchus maculatus* F. (*Coleoptera-Bruchidae*) in cowpea (*Vigna unguiculata* (L.). Journal of Plant Diseases and Protection, v.123, p.235-245, 2016. https://doi.org/10.1007/s41348-016-0034-z

Ayalew AA. Chromatographic and spectroscopic determination of solvent-extracted Lantana camara leaf oil. Journal of International Medical Research, v.48, n.10, p.1-12, 2020. https://doi.org/10.1177/0300060520962344

Ayinde AA, Morakinyo OM, Sridhar MKC. Repellency and larvicidal activities of *Azadirachta indica* seed oil on *Anopheles gambiae* in Nigeria. Heliyon, v.6, n.5, p. 1-7, 2020. https://doi.org/10.1016/j.heliyon.2020.e03920

Baby AR, Freire TB, Marques GA, Rijo P, Lima FV, Carvalho JCM, Rojas P, Magalhães WV, Velasco MVR, Morocho-Jácome AL. *Azadirachta indica* (Neem) as a potential natural active for dermocosmetic and topical products: a narrative review. Cosmetics, v. 9, n. 3, p.1-17, 2022. https://doi.org/10.3390 /cosmetics9030058

Balandrin MF, Lee SM, Klocke JA. Biologically active volatile organosulfur compounds from seeds of the neem tree, *Azadirachta indica* (Meliaceae). Journal of Agricultural and Food Chemistry, v.36, n.5, p.1048–1054, 1988. https://doi.org/10.1021/jf00083a035

Barbosa LMP, Santos JO, Sousa RCM, Furtado JLB, Vidinha P, Garcia MAS, Vitorino HA, Dall'Oglio DF. Bioherbicide from *Azadirachta indica* seed waste: exploitation, efficient extraction of neem oil and allelopathic effect on senna occidentalis. Recycling, v. 8, n. 3, p.1-15, 2023. https://doi.org/10.3390/recycling 8030050

Beyecha Hundie K, Abdissa D, Bekele Bayu A. Extraction, optimization, and characterization of neem seed oil via box-behnken design approach. Journal of the Turkish Chemical Society Section A: Chemistry, v.9, n.2, p.513-526, 2022. https:// doi.org/10.18596/jotcsa.1039997

Breu W. Allium cepa L. (Onion) Part 1: Chemistry and analysis. Phytomedicine, v.3, n.3, p.293–306, 1996. https://doi.org/10.1016/S0944-7113(96)80069-9

Bukvicki DR, Tyagi AK, Gottardi DG, Veljic MM, Jankovic SM, Guerzoni ME et al. Assessment of the chemical composition and in vitro antimicrobial potential of extracts of the liverwort scapania aspera. Natural Product Communications, v.8, n.9, p.1-4, 2013. https://doi.org/10.1177/1934578X1300800932

Cantrell MS, Seale JT, Arispe SA, McDougal OM. Determination of Organosulfides from Onion Oil. Foods, v.9, n.7, p.884, 2020. https://doi.org/10.3390/foods9070884

Carvalho AMS, Heimfarth L, Pereira EWM, Oliveira FS, Menezes IRA, Coutinho HDM et al. Phytol, a chlorophyll component, produces antihyperalgesic, anti-inflammatory, and antiarthritic effects: possible NFκB pathway involvement and reduced levels of the proinflammatory Cytokines TNF-α and IL-6. Journal of Natural Products, v.83, n.4, p.1107-1117, 2020. https://doi.org/10.1021/acs.jnatprod.9b01116

Casillas-vargas G, Ocasio-Malavé C, Medina S, Morales-Guzman C, Valle RG Del, Carballeira NM, Carballeira NM, Sanabria-Ríos DJ. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next-generation of antibacterial agents. Progress in Lipid Research, v.82, p.1-10, 2021. https://doi.org/10.1016/j.plipres.2021.101093

Chaudhary MF, Ashraf A, Waseem M, Hayat S, Nadeem HU, Siddique MH et al. Neem oil. In: Inamuddin, Boddula R, Asiri AM (org.). Green Sustainable Process Chemical and Environmental. Elsevier, 2021. p.57-73.

Chinnasamy G, Chandrasekharan S, Koh TW, Bhatnagar S. Synthesis, characterization, antibacterial and wound healing efficacy of silver nanoparticles from *Azadirachta indica*. Frontiers in Microbiology, v.12, p.1-14, 2021. https://doi.org/10.3389/fmicb.2021.611560

Choi SM, Lee D-J, Kim J-Y, Lim S-T. Volatile composition and sensory characteristics of onion powders prepared by convective drying. Food Chemistry, v.231, p.386-392,

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v11n4.16258

2017.
https://doi.org/10.1016/j.foodchem.2017.03.129

Desai SN, Jadhav AJ, Holkar CR, Pawar BG, Pinjari DV. Extraction and microencapsulation of Buchanania lanzan Spreng seed oil. Chemical Papers, v.76, n.6, p.3521-3530, 2022.
https://doi.org/10.1007/s11696-022-02116-0

Ebrahimi M, Safaralizade MH, Valizadegan O. Contact toxicity of *Azadirachta indica* (Adr. Juss.), *Eucalyptus camaldulensis* (Dehn.) and *Laurus nobilis* (L.) essential oils on mortality cotton aphids, *Aphis gossypii* Glover (*Hem.: Aphididae*). Archives of Phytopathology and Plant Protection, v.46, n.18, p.2153–2162, 2013.
https://doi.org/10.1080/03235408.2013 .774526

Fagbemi KO, Aina DA, Olajuyigbe OO. Soxhlet extraction versus hydrodistillation using the clevenger apparatus: A comparative study on the extraction of a volatile compound from *Tamarindus indica* seeds. The Scientific World Journal, v.2021, p.1-8, 2021.
https://doi.org/10.1155/2021/5961586

Fernandes SR, Barreiros L, Oliveira RF, Cruz A, Prudêncio C, Oliveira AI, Pinho C, Santos N, Morgado J. Chemistry, bioactivities, extraction and analysis of azadirachtin: State-of-the-art. Fitoterapia, v.134, p.141-150, 2019.
https://doi.org/10.1016/j.fitote.2019.02.006

Ghosh S, Mali SN, Bhowmick DN, Pratap AP. Neem oil as natural pesticide: Pseudo ternary diagram and computational study. Journal of the Indian Chemical Society, v.98, n.7, p.1-7, 2021.
https://doi.org/10.1016/j.jics.2021.100088

Granado-Casas M, Mauricio D. Oleic acid in the diet and what it does: implications for diabetes and its complications. In: Watson RR, Preedy VR (ed.). Bioactive food as dietary interventions for diabetes. Academic Press. 2019. p.211-229.
https://doi.org/ 10.1016/ B978-0-12-813822-9.00014-X

Guchhait KC, Manna T, Barai M, Karmakar M, Nandi SK, Jana D, Dey A, Panda S, Raul P, Patra A, Bhattacharya R, Chatterjee S, Panda Ak, Ghosh C. Antibiofilm and anticancer activities of unripe and ripe *Azadirachta indica* (neem) seed extracts. BMC Complementary Medicine and Therapies, v.22, n.1, p.1-18, 2022.
https://doi.org/10.1186/s12906-022-03513-4

Gutbrod K, Romer J, Dörmann P. Phytol metabolism in plants. Progress in Lipid Research, v.74, p.1-17, 2019.
https://doi.org/10.1016/j.plipres.2019.01.002

Hirondart M, Rombaut N, Fabiano-Tixier AS, Bily A, Chemat F. Comparison between pressurized liquid extraction and conventional soxhlet extraction for rosemary antioxidants, yield, Composition, and Environmental Footprint. Foods, v.9, n.5, p.1-14, 2020.
https://doi.org/10.3390/foods9050584

Hossain MA, Al-Toubi WAS, Weli AM, Al-Riyami QA, Al-Sabahi JN. Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. JTUSCI, v.7, n.4, p.181-188, 2013.
https://doi.org/10.1016/j.jtusci .2013.05.003

Islam MD T, Alencar MVOB de, Machado KC, Machado KC, Melo-Cavalcante A A de C, Sousa DP de et al. Phytol in a pharma-medico-stance. Chemico-Biological Interactions, v.240, p.60–73, 2015.
https://doi.org/10.1016/j.cbi.2015.07.010

Islam MT, Ali ES, Uddin SJ, Shaw S, Islam Md A, Ahmed Md I et al. Phytol: A review of biomedical activities. Food and Chemical Toxicology, v.121, p.82-94, 2018.
https://doi.org/ 10.1016/j.fct.2018.08.032

Islas JF, Acosta E, G-Buentello Z, Delgado-Gallegos JL, Moreno-Treviño MG, Escalante B et al. An overview of neem (*Azadirachta indica*) and its potential impact on health. Journal of Functional Foods, v.74, p.1-13, 2020.
https://doi.org/10.1016/j.jff.2020.104171

Ismaila SS, Sani Y, Sani AA, Yakasai SM, Momoh H, Mohammed Sa'a. E. Determination of fatty acids and physicochemical properties of neem (Azadrachta Indica L) seed oil extracts. AJOL, v.8, n.1, p.149-160, 2022.
https://doi.org/10.4314/dujo pas.v8i1a.16

Jeelani I et al. Pharmaceutical potential of constituents from *Azadirachta indica* and their specific role as anti-cancer agents. Current Bioactive Compounds, v.19, n.3, p.94-103, 2023.
http://dx.doi.org/10.2174/1573407218666220818110059

Jishma P, Hussain N, Chellappan R, Rajendran R, Mathew J, Radhakrishnan EK. Strain-specific variation in plant growth promoting volatile organic compounds production by five different *Pseudomonas* spp. as confirmed by response of *Vigna radiata* seedlings. Journal of Applied Microbiology, v.123, n.1, p.204–216, 2017.
https://doi.org/10.1111/jam.13474

Kaura SK, Gupta SK, Chowdhury JB. Morphological and oil content variation in seeds of *Azadirachta indica*tes A. Juss. (Neem) from northern and western provenances of India. Plant Foods for Human Nutrition, v.52, p.293-298, 1998.
https://doi.org/10.1023/A: 1008013424150

Keneni YG, Marchetti JM. Oil extraction from plant seeds for biodiesel production. AIMS Energy. v. 5, n. 2, p. 316-340, 2017.
https://doi.org/10.3934/energy.2017.2.316

Kilani-Morakchi S, Morakchi-Goudjil H, Sifi K. Azadirachtin-Based Insecticide: Overview, Risk Assessments, and Future Directions. Frontiers in Agronomy, v.3, p.1-13, 2021.
https://doi.org/10.3389/fagro.2021.676208

Kostik V, Memeti S, Bauer B. Fatty acid composition of edible oils and fats. Journal of Hygienic Engineering and Design. v.4, p.112-116, 2013.

Koul O. Biological activity of volatile di-n-propyl disulfide from seeds of neem, *Azadirachta indica* (Meliaceae), to two species of stored grain pests, Sitophilus oryzae (L.) and Tribolium castaneum (Herbst). Journal of Economic Entomology, v.97, n.3, p.1142-1147, 2004.
https://doi.org/10.1093/jee/97.3.1142

Kumar S, Singh N, Devi LS, Kumar S, Kamle M, Kumar P et al. Neem oil and its nanoemulsion in sustainable food preservation and packaging: Current status and future prospects. Journal of Agriculture and Food Research, v.7, p.1-9.
https://doi.org/10.1016/j.jafr.2021.100254

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v11n4.16258

Kumar Tyagi A, Bukvicki D, Gottardi D, Veljic M, Guerzoni ME et al. Antimicrobial potential and chemical characterization of serbian liverwort (*Porella arboris-vitae*): SEM and TEM Observations. Evidence-Based Complementary and Alternative Medicine, v.2013, p.1-7, 2013. https://doi.org/10.1155/2013/382927

Lienard V, Seck D, Lognay G, Gaspar C, Severin M. Biological activity of Cassia occidentalis L. against *Callosobruchus maculatus* (F.) (*Coleoptera: Bruchidae*). Journal of Stored Products Research, v.29, n.4, p.311-318, 1993. https://doi.org/10.1016/0022-474X(93)90046-7

Loganathan T, Barathinivas A, Soorya C, Balamurugan S, Nagajothi TG, Ramya S, et al. Physicochemical, druggable, ADMET pharmacoinformatics and therapeutic potentials of azadirachtin - a prenol lipid (triterpenoid) from seed oil extracts of *Azadirachta indica* A. Juss. Journal of Drug Delivery and Therapeutics, v.11, n.5, p.33-46, 2021. https://doi.org/10.22270/jddt.v1 1i5.4981

López-Bascón MA, Luque de Castro MD. Soxhlet Extraction. In: Poole CF (org.). Liquid-Phase Extraction. Elsevier. 2020. p.27 https://doi.org/10.10 16/B978-0-12-816911-7.00011-6

Marangoni F, Agostoni C, Borghi C, Catapano AL, Cena H, Ghiselli A, Vecchia CL, Lercker G, Manzato E, Pirillo A, Riccardi G, Risé P, Visioli F, Poli A. Dietary linoleic acid and human health: Focus on cardiovascular and cardiometabolic effects. Atherosclerosis, v.292, p.90–98, 2020. https://doi.org/10.1016/j.atherosclerosis.2019 .11.018

Mohamadpoor M, Amini J, Ashengroph M, Azizi A. Evaluation of biocontrol potential of Achromobacter xylosoxidans strain CTA8689 against common bean root rot. Physiological and Molecular Plant Pathology, v.117, p.101769, 2022. https://doi.org/10.1016/j.pmpp.2021.101769

Mubarak AM, Kulatilleke CP. Sulphur constituents of neem seed volatiles: A revision. Phytochemistry, v.29, n.10, p.3351-3352, 1990. https://doi.org/10.1016/0031-9422(90)80213-Z

Mulla M, Ahmed J, Alagarsamy S, K Habeebullah SF. Utilization of novel and rapid techniques for characterization of neem *Azadirachta indica* seed oil and palm oil blends. International Journal of Food Engineering, v.16, n.10, p.20200047, 2020. https://doi.org/10.1515/ijfe-2020-0047

Neves EJM, Carpanezzi AA. The cultivation of neem (*Azadirachta indica*) for fruit production in Brazil. Colombo: Embrapa Forests, 8p. 2008.

Olofsson P, Hultqvist M, Hellgren LI, Holmdahl R. Phytol: A chlorophyll component with anti-inflammatory and metabolic properties. In: Jacob C, Kirsch G, Slusarenko A, Winyard P, Burkholz T, (org.). Recent advances in redox active plant and microbial products: From basic chemistry to widespread applications in medicine and agriculture. Dordrecht: Springer Netherlands. 2014. p.14.

Perera AGWU, Karunaratne MMSC, Chinthaka SDM. Bioactivity and volatile profiling of *Azadirachta indica* leaves for the management of maize weevil, *Sitophilus zeamais* (motsch.) infestations. JTFE, v.8, n.1, p.10-24, 2018. https://doi.org/10.31357/jtfe.v8i1 .3479

Perera WPRT, Liyanage JA, Dissanayake KGC, Gunathilaka H, Weerakoon WMTDN, Wanigasekara DN et al. Antiviral potential of selected medicinal herbs and their isolated natural products. BioMed Research International, v.2021, p.1-18, 2021. https://doi.org/10.1155/2021/7872406

Ragunath C, Kumar YAS, Kanivalan I, Radhakrishnan S. Phytochemical screening and GC-MS analysis of bioactive constituents in the methanolic extract of *Caulerpa racemosa* (Forssk.) J. Agardh and Padina boergesenii Allender & Kraft. Current Applied Science And Technology, v.20, n.3, p.380-393, 2020.

Rana S, Saxena A. Medicinal chemistry of neem: A state of art. Kochi, Kerala, India: 1, 2023. https://doi.org/10.1063/5.0114567

Raza W, Ling N, Liu D, Wei Z, Huang Q, Shen Q. Volatile organic compounds produced by Pseudomonas fluorescens WR-1 restrict the growth and virulence traits of Ralstonia solanacearum. Microbiological Research, v.192, p.103-113, 2016. https://doi.org/10.1016/j.mi cres.2016.05.014

Saha tchinda J-B, Tchebe TMF, Tchoukoua A, Yona AMC, Fauconnier ML, Kor M N, et al. Fatty acid profiles, antioxidant, and phenolic contents of oils extracted from Acacia polyacantha and *Azadirachta indica* (Neem) seeds using green solvents. Journal of Food Processing and Preservation, v.45, n.2, p.1-13, 2021. https://doi.org/10.1111/jfpp.15115

Sarkar S, Singh RP, Bhattacharya G. Exploring the role of *Azadirachta indica* (neem) and its active compounds in the regulation of biological pathways: an update on molecular approach. 3 Biotech, v.11, n.4, p.178, 2021. https://doi.org/10.1007/s13205-021-02745-4

Satyal P, Craft JD, Dosoky NS, Setzer WN. The chemical compositions of the volatile oils of garlic (*Allium sativum)* and wild garlic (*Allium vineale*). Foods, v.6, n.8, p.63, 2017. https://doi.org/10.3390/foods6080063

Shariare MH, Noor HB, Khan JH, Uddin J, Ahamad SR, Altamimi MA, Alanazi FK, Kazi M. Liposomal drug delivery of Corchorus olitorius leaf extract containing phytol using design of experiment (DoE): In-vitro anticancer and in-vivo anti-inflammatory studies. Colloids and Surfaces B: Biointerfaces, v.199, p,1-11, 2021. https://doi.org/10.1016/j.colsurfb.2020.111543

Suganthy M, Gajendra CV. Chemical characterization of *Strychnos nux-vomica* L. leaves for biopesticide properties using GC-MS. International Journal of Chemical Studies, v.8, n.1, p.1112-1116, 2020. https://doi.org/10.22271/chemi.2020.v8.i1o.8398

Tesfaye B, Tefera T, Misikir O, Tsegaye G. Extraction and comparison of essential oil from neem seed by using soxhlet extraction and simple distillation methods. International Journal of Engineering Technologies and Management Research, v.5, n.9, p.74-81, 2018. https://doi.org/10.29121/ijetmr.v5.i9.2018 .291

Tesfaye B, Tefera T. Extraction of essential oil from neem seed by using soxhlet extraction methods. IJAEMS, v.3, n.6, p.646-650, 2017. https://dx.doi.org/10.24001/ij aems.3.6.5

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v11n4.16258

Tulashie SK, Adjei F, Abraham J, Addo E. Potential of neem extracts as natural insecticide against fall armyworm (*Spodoptera frugiperda* (J. E. Smith) (*Lepidoptera: Noctuidae*). Case Studies in Chemical and Environmental Engineering, v.4, p.1-7, 2021. https://doi.org/10.1016 /j.cscee.2021.100130

Ungo-Kore HY, Ibrahim YKE, Tytler BA. Anti-dermatophytic activity of hexane extracts of *Azadirachta indica*tes A. Juss. African Journal of Microbiology Research, v.13, n.26, p.421-429, 2019. https://doi.org/10.5897/AJMR2018.8819

Winnett V, Sirdaarta J, White A, Clarke FM, Cock IE. Inhibition of Klebsiella pneumoniae growth by selected australian plants: natural approaches for the prevention and management of ankylosing spondylitis. Inflammopharmacology, v.25, p.223-235, 2017. https://doi.org/10.1007/s10787-017-0328-1

Yami AM, Ibrahim ME, Raji A. Extraction and characterization of oil from neem and yellow oleander seeds for biodiesel production. European Journal of Materials Science and Engineering, v.5, n.4, p.212-221, 2020. https://doi.org/10.36868/ejmse.2020.05.04.212

Zambiazi RC, Przybylski R, Zambiazi MW, Mendonça CB. Fatty acid composition of vegetable oils and fats. Bulletin of the Center for Food Research and Processing, v.25, n.1, p.111-120, 2007. http://dx.doi.org/10.5380/cep.v25i1.8399

Zatelli A, Fondati A, Maroli M. The knowns and unknowns of the efficacy of neem oil (*Azadirachta indica*) used as a preventative measure against *Leishmania* sand fly vectors (*Phlebotomus genus).* Preventive Veterinary Medicine, v.202, p.1-5, 2022. https://doi.org/10.1016/j.prevetmed.2022.105618

Zhang H, Mallik A, Zeng RS. Control of panama disease of banana by rotating and intercropping with chinese chive (*Allium Tuberosum* Rottler): role of plant volatiles. Journal of Chemical Ecology, v.39, n.2, p.243-252, 2013. https://doi.org/10.1007/s10886-013-0243-x

Zhao B, Li H, Lan T, Wu D, Chen Z. Characterization of the chemical composition of chinese Moringa oleifera seed oil. Journal of the American Oil Chemists' Society, v.96, n.5, p.523-533, 2019. https://doi.org/10.1002/aocs.12203

Zhao Y, Smyth HE, Tao K, Henry RJ, Gilbert RG. Starch molecular structural features and volatile compounds affecting the sensory properties of polished australian wild rice. Foods, v.11, n.4, p.1-22, 2022. https://doi.org/10.3390/foods11040511

Zheng L, Shen X, Wang Y, Liang J, Xu M, Fang X, Gang C, Guilliang C. Content and composition of phospholipids, fatty acids and sterols in commercial natural phospholipid excipients. Current Pharmaceutical Analysis, v.17, n.7, p.892-902, 2021. https://doi.org/10.2174 /1573412916999200605162707

ISSN: 2179-4804
DOI: https://doi.org/10.20873/jbb.uft.cemaf.v11n4.16258