Identification of Biologically Important Compounds In Neem Leaves Through GC-MS Analysis

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ABSTRACT

Objective: Neem (Azadirachta indica A. Juss.) is an important tree species that is known to posses anthelmintic, contraceptive, antimicrobial, antidiabetic and sedative properties. Therefore, the present study aimed to identify various compounds present in the leaf extract of neem and to enlist various biological activities of these compounds through literature survey. Methodology: Extract of neem was obtained by soaking 100 g dry powdered leaves in 1000 mL methanol followed by filtration. This was successively fractioned using n-hexane and chloroform. **Results**: GC-MS analysis of the chloroform fraction was performed and 7 compounds namely 2-pentanol, acetate (9.72%); decane (8.96%); 11-oxa-dispiro[4.0.4.1]undecan-1-ol (6.56%); nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)- (7.13%); quinoline-4-carboxamide, 2-phenyl-N-n-octyl- (9.79%); nonacosane (44.27%) and tetratriacontane (13.43%) were identified. Conclusion: Literature survey showed that the identified compounds possessed antibacterial, antifungal, anticancer, cytotoxic, anti-inflammatory, antioxidant, and antiviral properties.

Keywords: *Azadirachta indica*, Bioactive components, GC-MS analysis, Neem leaves.

INTRODUCTION

As a rich source of ingredients, medicinal plants can be used in the synthesis and development of drugs.[1] In addition, these plants also play a crucial role in human cultures as these are recommended for various therapeutic properties.[2] In the recent decade, neem (*Azadirachta indicia*) has attracted importance worldwide due to its diverse medicinal properties.[3] It is an evergreen plant native to Pakistan, India, Bangladesh, Burma, Thailand, Indonesia, Malaysia and Sri Lanka.[4] The tree has the adaptability to survive under a wide range of edaphic, topographic and climatic factors.[5] It naturally grows in areas where the rainfall is in range of 400 to 1150 mm, soil pH lies between 4.5 to 10, and temperature range from 0 to 50 °C.[6] It has been used in folk medicines for more than 4000 years ago with proven antipyretic, antiviral, antiseptic, antifungal, antibacterial, anti-inflammatory, antigastric, antimalarial and antiulcer properties.[4,7] It possesses a vast array of pharmaceutical and biologically active compounds which are structurally complex and diverse in nature.[8,9] Over 140 compounds have been identified in different parts of this plant including terpenes, limonoids, meliacin, margocin, margocilin, isoazadirolide, azadirachtin, salanin, gedunin, valssin, nimbin, nibonolone, gallic acid and others.[4,10,11] In agriculture, neem-based products are highly effective, easily biodegradable, cheap, easy in preparation with less or no hazardous effects. The registered neem formulations such as Neemark, Nimbicidine, Bioneem, Achook, Neemix, Margosan, Nim-76, Nim-20 and Azatin are in practice for the management of plant diseases.[12,13] On the other hand,

all parts of this plant are potentially beneficial for human health and are used traditionally for the treatment of jaundice, indolent ulcer, intestinal wounds, syphilitic sores, blood morbidity, itching, skin ulcer, rheumatism, leprosy, indigestion, pthysis, constipation, burning sensations, intestinal helminthiasis, pleuropneumonia, asthma, ringworm, urticaria, throat and respiratory disorders.[10,14,15] In West African counties, neem leaves and bark aqueous extracts are enriched with gedunin, an active anti-malarial component used particularly resistant to chloroquine strains.[16] Furthermore, neem bark possesses strong antiseptic properties that is used in the preparation of herbal tooth paste and dental gel to reduce the bacterial count and oral plaque index.[17] In a recent study, Khan et al.[18] reported promising antifungal activity of soil amendment with leaves of neem against *Sclerotium rolfsii*. Therefore, this study was carried out to analyze chloroform fraction of methanolic leaf extract through GC-MS for the identification of various antifungal and other biologically active compounds.

MATERIALS AND METHODS

Preparation of extract

Leaves of neem were collected from Lahore, Pakistan and dried under shade. The dried leaves were crushed thoroughly and 100 g material was dipped in 1000 mL methanol for 14 days and filtered. The solvent was evaporated on a rotary evaporator and the recovered material was mixed in 100 mL of distilled H_2O and mixed well. This mixture was partitioned with 100 mL of *n*-hexane using a separating funnel. After collecting the *n*-hexane soluble fraction, the process was repeated 5 times to separate all the *n*-hexane soluble compounds. Thereafter, the aqueous phase was partitioned with 100 mL of chloroform. The chloroform phase was collected, filtered through a Millipore filter paper and used for GC-MS analysis. In our previous studies, we found that generally chloroform fractions of methanolic extracts of different plant species were highly antimicrobial in nature and also contained other biologically important

compounds.[19,20] Therefore, this fraction was selected for identification of biologically important compounds in the present study.

GC-MS analysis

This study was performed to evaluate the possible biologically active phytochemical constituents in chloroform fraction. For this, samples were run on Agilant Technologies machine (Model GC-7890A) attached with a capillary column (0.25 μm × 0.25 μm × 30 m) and a mass spectrometer (MS 5975C). There was an operating system that operated the electron impact mode having 70 eV ionization energy. A carrier gas helium was used at a flow rate of 1 mL min⁻¹. The injector temperature was 260 °C, ion-source temperature was maintained at 200 °C and the oven was adjusted from 50 °C with an increase of 10 °C min⁻¹ ending with a 4 min isothermal at 310 °C.

Mass spectra were taken at source temperature of 250 °C, 70 eV with MS Quad temperature 150 °C. The total running time for GC-MS was 36 min with a solvent delay of 4 min. In order to identify the components of the extract, retention indices were used. In addition, these were also identified by comparing their mass spectral fragmentation patterns with those already reported and stored in the MS library (NIST database). The concentrations of these compounds were calculated from total area of GC peaks without applying any correction factor.

A thorough literature survey was carried out to collect the information regarding bioactivities of various identified compounds. Structures of the identified compounds were drawn using software ChemDraw.

RESULTS

The GC-MS investigation of chloroform fraction of methanolic leaf extract of neem indicated that there were eight phytochemicals as shown in Figure 1. The details of these constituents are shown in Table 1. Their bioactivities, as available in the literature, are given in Table 2 whereas their structures are illustrated in Figure 2. The identified compounds belonged to various classes of organic compounds. The most prevailing compound was

nonacosane with 44.27% peak area. The compounds namely, tetratriacontane; quinoline-4-carboxamide, 2-phenyl-N-n.-octyl-; 2-pentanol, acetate; decane; nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester, (Z,Z,Z)- and 11-oxa-dispiro[4.0.4.1]undecan-1-ol were moderately abundant with peak areas ranging from 6.56 to 13.43%.

DISCUSSION

Among the identified phytoconstituents, nonacosane was found as the most abundant one. It has also been identified from ethanolic extracts of *Cayratia trifolia* and *Moringa oleifera* leaves with strong anti-inflammatory, antioxidant and cytotoxic properties. It showed the highest antimicrobial potential against a wide range of bacterial strains including *Staphylococcus aureus, Klebsella pnemoniaea, Escherichia coli, Enterobacter faecalis* and *Salmonella typhi*.[21,22] Similarly, this compound was also found in ethanolic extract of *Ipomoea mauritiana* with strong antidiabetic potential and it can also be utilize on commercial basis.[23] Tetratriacontane was the second most abundant compound in this study and was previously identified from the methanolic extract of a medicinal plant *Plantago lanceolata* with excellent pharmaceutical, medicinal, antioxidant, anticancer and antibacterial activities against *Escherichia coli* and *B. cereus*.[24]

Likewise, decane was identified from methanolic extracts of *Camponotus fellah* and it is known for antifungal and antibacterial characteristics against a wide range of pathogens. Generally, it interacts with specific substances in the cell or either destabilizes the pathogenic cell wall or cell membrane permeability which eventually disrupts the signaling pathways leading to cell death.[25,26] Recently, nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)- was isolated from the methanolic leaf extracts of *Clinacanthus nutans* with potent antioxidant, cytotoxic, therapeutic and antiproliferative activities. It also exhibited excellent anticancer potential against aggressive HeLa cell line which is responsible for cervical cancer in humans.[27] Moreover, methanolic extracts of *Digitalis purpurea* revealed the presence of 11-oxa-dispiro[4.0.4.1]undecan-1-ol with promising antimicrobial potential.[28]

Conclusion

The present study concluded that neem leaf extract is a source of biologically important phytoconstituents such as nonacosane; tetratriacontane; decane and 11-oxa-dispiro[4.0.4.1]undecan-1-ol.
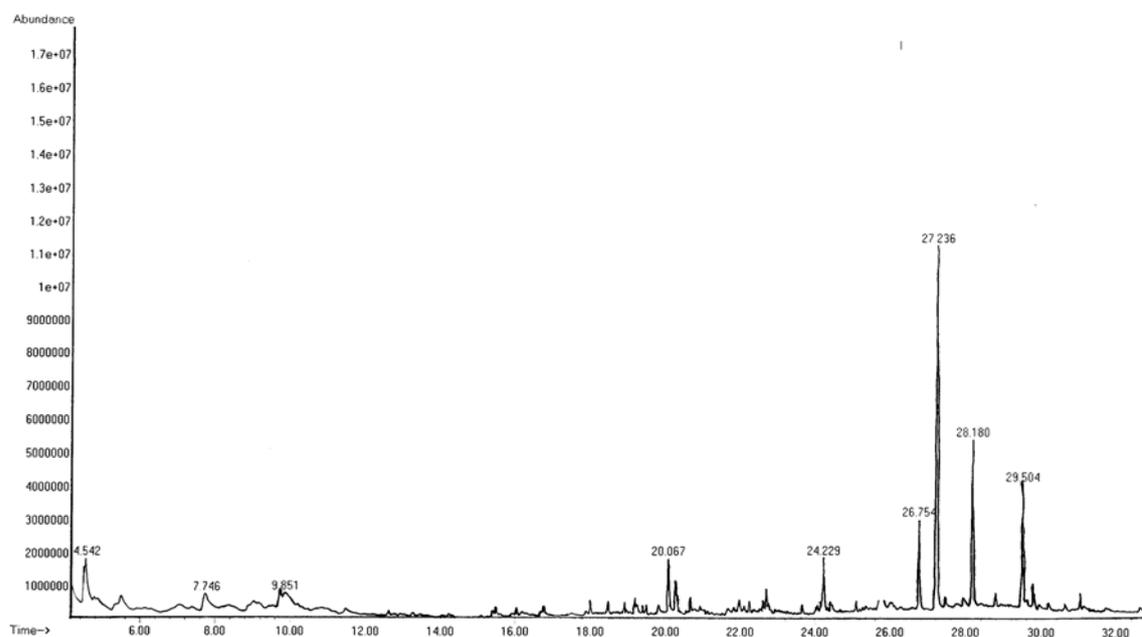
Author contributions: IH Khan conducted the study and wrote a part of paper. A. Javaid supervised the work and contributed in writing of manuscript.

Table 1. Compounds identified from chloroform leaf extract of *Azadirachta indica* through GC-MS analysis.

	Names of compounds	Formula	Weight $(g\ mol^{-1})$	Retention time (min)	Peak area (%)
1	2-Pentanol, acetate	$C_7H_{14}O_2$	130	4.542	9.72
2	Decane	$C_{10}H_{22}$	142	7.746	8.96
3	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	$C_{10}H_{16}O_2$	168	9.851	6.56
4	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-	$C_{21}H_{36}O_4$	352	24.229	7.13
5	Quinoline-4-carboxamide, 2-phenyl-N-*n*.-octyl-	$C_{24}H_{28}N_2O$	360	26.754	9.79
6	Nonacosane	$C_{29}H_{60}$	408	27.236	44.27
7	Tetratriacontane	$C_{34}H_{70}$	478	29.504	13.43

Table 2. Nature and properties of compounds identified from leaf extract of neem through GC-MS analysis.

Comp. No.	Names of compounds	Nature	Property	Reference
1	2-Pentanol, acetate	Ester	-	-
2	Decane	Alkane	Antimicrobial, antifungal, antibacterial	26
3	11-Oxa-dispiro[4.0.4.1]undecan-1-ol	Alcohol	Antimicrobial	28
4	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-	Ester	Antioxidant, antimicrobial, therapeutic agent, antiproliferative activity	27
5	Quinoline-4-carboxamide, 2-phenyl-N-*n*.-octyl-		-	-
6	Nonacosane	Aliphatic hydrocarbon	Antibacterial, antimicrobial, anti-inflammatory and antidiabetic	21,22
7	Tetratriacontane	Aliphatic hydrocarbon	Antimicrobial, antibacterial, anticancer, antioxidant, hypoglycaemic	24

Fig. 1. GC-MS chromatogram of chloroform leaf extract of *Azadirachta indica*.
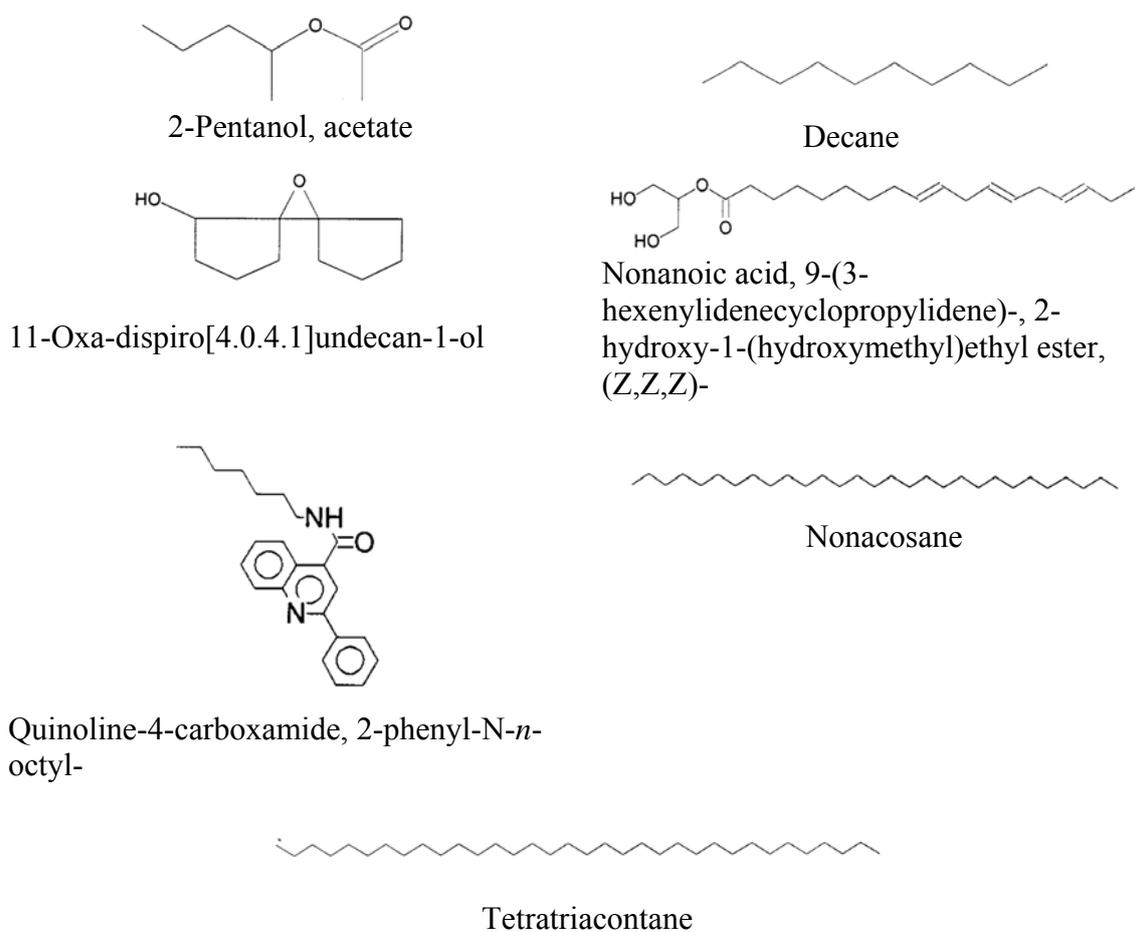
2-Pentanol, acetate

Decane

11-Oxa-dispiro[4.0.4.1]undecan-1-ol

Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester, (Z,Z,Z)-

Nonacosane

Quinoline-4-carboxamide, 2-phenyl-N-*n*-octyl-

Tetratriacontane

Fig. 2. Structures of compounds identified through GC-MS analysis of chloroform leaf extract of *Azadirachta indica.*

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تحديد المركبات المهمة بيولوجيًا في أوراق النيم من خلال تحليل GC-MS

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ملخص

خلفية: النيم (.Azadirachta indica A. Juss) هو أحد أنواع الأشجار الهامة المعروف بامتلاكه، خصائص طاردة للديدان، مانعة للحمل ، مضادة للميكروبات .مضادة لمرض السكر ومهدئة لذلك ، هدفت الدراسة الحالية إلى تحديد المركبات المختلفة الموجودة في مستخلص أوراق النيم وتسجيل الأنشطة البيولوجية المختلفة .لهذه المركبات من خلال مسح الأدبيات

الاساليب: تم الحصول على مستخلص النيم بنقع 100 .متبوعًا بالترشيح جم من أوراق البودرة الجافة في 1000 مل ميثانول. تم تجزئة هذا على التوالي باستخدام chloroform و n-hexane

النتائج: .الكلوروفورم وتم تحديد 7 مركبات تم إجراء تحليل GC-MS لكسر ديكان (8.96٪) ؛ بنتانول ، أسيتات (9.72٪) ؛ 2− وشملت هذه ؛ 11−6.56%) oxa-dispiro[4.0.4.1]undecan-1-ol) ، nonanoic acid, 9-(3- (7.13) -(Z,Z,Z) ,hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester٪); (9.79 quinoline-4-carboxamide, 2-phenyl-N-n-octyl٪)؛ (13.43٪) تتراتراكونتان و (نوناكوزان 44.27٪)

الاستنتاجات: أظهر المسح الأدبي أن المركبات المحددة لها خصائص مضادة للجراثيم، ومضادة للفطريات ، ومضادة للسرطان، وسامة للخلايا ، ومضادة للالتهابات ، ومضادة للأكسدة. ومضادة للفيروسات

الكلمات الدالة: أوراق النيم ، GC-MS تحليل ، المكونات النشطة بيولوجيا ، Azadirachta indica .