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Original Article

GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS IN LEAF EXTRACTS OF *NEOLAMARCKIA CADAMBA* (RUBIACEAE) FROM MALAYSIA

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ABSTRACT

Neolamarckia cadamba is one of the medicinal plants used in the treatment of various diseases traditionally. This study was conducted to identify the phytochemical constituents of *N. cadamba* leaf extracts using gas chromatography mass spectrometry (GC-MS). Solvents with increasing polarities viz. hexane, petroleum ether, chloroform, ethyl acetate and methanol were used in this study. The solvent extracts were analyzed using GC-MS and the mass spectra of the compounds found in the respective extract were matched with the National Institute of Standards and Technology (NIST) library. A total of 26 compounds were identified and the major chemical constituents were n-hexadecanoic acid (44.88%), hexadecanoic acid ethyl ester (17.96%) and octadecanoic acid ethyl ester (11.71%). Some of the identified compounds have been reported to possess various biological activities such as antioxidant, antimicrobial, anesthetic, antiseptic, antidiabetic, hypocholesterolemic and etc. The results thus concluded that *N. cadamba* leaves possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance.

Keywords: *Neolamarckia cadamba*, Gas chromatography mass spectrometry (GC-MS), Phytochemical, Secondary metabolite, Antioxidant

INTRODUCTION

Higher plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Plants are rich sources of secondary metabolites with interesting biological activities [1-2]. It is also the best sources for obtaining natural antioxidants for various medicinal uses such as aging and disease related to radical mechanism such as cancer [3]. *Neolamarckia cadamba* is one of the medicinal plants traditionally used by the Indian. It has been mentioned in many Indian medical literatures for the treatment of fever, anaemia, diabetes, uterine and liver complaints, menorrhagia, blood and skin diseases, diarrhoea, colitis, stomatitis, dysentery and in improvement of semen quality [4-8]. Various parts of this plant have been traditionally used to treat various diseases [9-10]. Bioactivity studies on this plant revealed its antimicrobial, antioxidant and wound healing properties, antimalarial, antihepatotoxic, hepatoprotective, analgesic, anti-inflammatory, antipyretic, anthelmintic, diuretic, laxative and antidiabetic activities [10-11]. In addition, the leaves and barks extracts of this plant showed antifungal activity against *Aspagillus fumigates* and *Candila albicans* [12]. The tribes of Ganjam district of Orissa drink the root paste duly suspended in water for antimicrobial and anthelmintic activities.

Various phytochemical compounds have been identified from *N. cadamba* using phytochemistry approaches to date. The leaf extracts of *N. cadamba* revealed the presence of various secondary metabolites and these include glycosides, alkaloids, tannins, phenolic, steroids, and flavonoids [13-14]. The bark contains alkaloids like cadambine and its derivatives, saponins, glycosides, triterpenoids, cadambagic acid, quinovic acid and β-sitosterol [7, 10]. Those alkaloids, steroids and flavonoids have potent antiepileptic effect in various seizure models [15]. In addition to this, saponins have also been able to modulate the neurotransmitter levels in the brain and to possess potent anti-convulsant activity [16]. The qualitative chemical tests revealed the presence of saponins, proteins, terpenes, carbohydrates and alkaloids in the bark powder of *N. cadamba* [12]. Phytochemical evaluation of methanolic extract of *N. cadamba* showed the presence of flavonoids, alkaloids, carbohydrate, proteins and glycoside compounds [17]. The flowers of *N. cadamba* yield essential oil and the main constituents of the essential oils were linalool, geraniol, geranylacetate, linalyl acetate, α-selinene, 2-nonanol, β-phellandrene, α-bergamottin, p-cymol,curcumene, terpinolene, camphene and myrcene [18]. The seeds of *N. cadamba* are composed of water-soluble polysaccharides D-xylose, D-mannose and D-glucose in the molar ratio 1:3:5 [19]. From literature survey it was found that almost every part of the *N. cadamba* is used in the treatment of various diseases traditionally. Unfortunately, thus far there is no phytochemical study on *N. cadamba*from Malaysia. In fact, it has been selected as one of the plantation tree species in forest rehabilitation projects in Malaysia due to its short rotation period which can give early commercial returns within 8-10 years [20-23]. Therefore ,the present study was carried out to determine the phytochemical constituents of *N. cadamba* leaves by using gas chromatography mass spectrometry (GC-MS). It is hoped that this study will provide another useful resource for future extraction of phytochemicals from this species which can be used as dietary supplements. To date, all published scientific findings are in agreement with the traditional use of the plant.

MATERIALS AND METHODS

Plant materials

N. cadamba seeds were obtained from the Seed Bank of Sarawak Forestry Corporation, Sarawak. The seeds were planted in trays of 50 holes and contained sand and compost (3:1) for one month and then planted in seed beds for the next 6 months. The leaves samples were dried in the shade in the open air condition for 6 – 12 days prior to extraction.

Sample extraction and column chromatography (CC)

About 300 g of dried leaves were ground into fine powder by using electric blender. The ground sample was percolated with methanol at room temperature for three days and filtered. The residue was extracted two more time to ensure complete extraction process. The residue was discarded and the filtrates were combined and evaporated to dryness using rotary evaporator. The crude dried methanol extract was partitioned with hexane, petroleum ether, chloroform, ethyl acetate and methanol. The entire steps were performed in three times in order to increase the effectiveness of the extraction process. All the hexane, petroleum ether, chloroform,

ethyl acetate and methanol partitions were evaporated to dryness using rotary evaporator and the weight of each fraction was determined. The selected partitions were separated by column chromatography (60 cm length and diameter of 3.2 cm) using silica gel 280-400 mesh as stationary phase. Initially, the column was rinsed with suitable solvent systems. The column was filled with the slurry of silica gel, which was prepared by adding 220 g of the silica gel into 350 ml suitable solvent systems. Glass rod was used to knock off the silica gel slowly into the column to ensure the silica gel was compacted and no air bubbles were present in the column. Then, the silica gel was rinsed with hexane. Combinations of solvents with increasing polarity were used. About 3 g of partitions were added into the column and then a number of fractions (25 ml each) were collected.

Table 1: Weight, percentage yield and colour of *N. Cadamba* leaf extracts with different solvents

Solvent Extracts	Colour	Weight (g)	Percentage (%)
Hexane	Yellowish	0.97	1.07
Petroleum ether	Yellowish brown	2.23	2.48
Chloroform	Blackish green	1.50	1.67
Ethyl acetate	Reddish	3.28	3.64
Methanol	Black bluish	4.62	5.13

Table 2: Phytochemicals screening of solvent extracts of *N. cadamba* leaves by GC-MS

No.	RT	Name of the compounds	Molecular Formula	MW	Peak Area (%)
Hexane extract					
1	26.27	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.84
2	27.25	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	17.96
3	28.68	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	298	0.41
4	29.17	Stearic acid, methyl ester	$C_{19}H_{38}O_2$	298	3.09
5	30.05	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	11.71
6	34.27	Docosanoic acid, methyl ester	$C_{23}H_{46}O_2$	354	0.74
7	34.50	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.44
8	35.43	Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	368	0.16
9	37.61	Pentacosanoic acid, methyl ester	$C_{26}H_{52}O_2$	396	0.15
10	38.16	Tetratetracontane	$C_{44}H_{90}$	618	0.43
Petroleum ether extract					
1	34.53	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.15
2	34.95	Heneicosane	$C_{21}H_{44}$	296	0.06
3	37.15	Tetratetracontane	$C_{44}H_{90}$	618	0.13
Chloroform extract					
1	26.34	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.49
2	28.87	Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	0.82
3	29.22	Stearic acid, methyl ester	$C_{19}H_{38}O_2$	298	0.14
4	33.79	Eicosane	$C_{20}H_{42}$	282	0.09
5	34.53	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.27
6	37.14	Tetratetracontane	$C_{44}H_{90}$	618	0.44
Ethyl acetate extract					
1	7.93	Benzaldehyde	C_7H_6O	106	6.08
2	9.82	Benzyl Alcohol	C_7H_8O	108	9.07
3	22.07	Pentanoic acid, 4-oxo-, phenylmethyl ester	$C_{12}H_{14}O_3$	206	0.82
4	22.49	Benzyl ether	$C_{14}H_{14}O$	198	0.77
5	24.13	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	0.52
6	27.48	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.35
7	30.04	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	0.31
8	34.51	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	1.32
9	39.34	Progesterone	$C_{21}H_{30}O_2$	314	1.87
10	40.13	Tetratetracontane	$C_{44}H_{90}$	618	0.23
Methanol extract					
1	20.88	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.32
2	24.24	Myristic acid	$C_{14}H_{28}O_2$	228	3.83
3	25.14	2-cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)	$C_{13}H_{18}O_3$	222	0.55
4	25.74	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.26
5	27.73	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	44.88
6	30.44	Hexadecanamide	$C_{16}H_{33}NO$	255	0.56
7	33.03	Octadecanamide	$C_{18}H_{37}NO$	283	0.10
8	33.76	Heneicosane	$C_{21}H_{44}$	296	0.10
9	34.50	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	2.15
10	37.11	Tetratetracontane	$C_{44}H_{90}$	618	0.20

Gas Chromatograph-Mass Spectroscopy (GC-MS)

GC-MS (Shimadzu QP 5000) was performed by using non-polar DB-5 cross linked column (30 m long x 0.25 mm ID x 0.25 µm film thickness composed of 5% phenyl methyl polysiloxane). The initial temperature was programmed at 50°C and held for two minutes, and then it was increased to 300°C with the rate of 6.5°C/min. The final temperature was held for ten minutes. The temperature of the injector and detector were set up to 280°C and 300°C, respectively. Helium gas was used as a carrier gas. 1 µl of the fractions was diluted in 200 µl dichloromethane and then injected into the GC-MS [24-25]. Interpretation of mass-spectrum was conducted using the

database of National Institute Standard and Technology (NIST). The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular mass and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

A total of 300 g of powdered dried leaves was percolated with methanol for three days and then filtered. The percolating procedure was repeated twice in order to complete the extraction process and increase the effectiveness of the extraction. A total of 180.34 g crude extract was obtained from the leaves of *N. cadamba*. 90 g of the crude extract were used for solvent partition using solvent with different polarities. Solvents with increasing polarities used in this study were hexane, petroleum ether, chloroform, ethyl acetate and methanol. The solvent partition was repeated until the solvent in the thimble becomes clear indicating the completion of extraction process. The weights and the percentage of yields of the solvent partitions are shown in Table 1. Methanol partition gave the highest yield compared to other partitions. This result showed the presence of polar compounds in the leaves of *N. cadamba*. Each partition was subjected to GC-MS analysis. The chemical components present in the leaf extracts of *N. cadamba* were identified by GC-MS analysis. The active principals with their retention time (RT), molecular formula, molecular mass (MW) and concentration (%) in the solvent extracts of *N. cadamba* leaves are presented in Table 2. Ten important compounds detected in the hexane extracts were hexadecanoic acid methyl ester, hexadecanoic acid ethyl ester**,** heptadecanoic acid ethyl ester**,** stearic acid methyl ester, octadecanoic acid ethyl ester, docosanoic acid methyl ester, 1,2-benzenedicarboxylic acid diisooctyl ester, tricosanoic acid methyl ester, pentacosanoic acid methyl ester and tetratetracontane (Fig.1). Meanwhile, three important compounds were detected in the petroleum ether extract of *N. cadamba* leaves. The compounds were 1, 2-benzenedicarboxylic acid, diisooctyl ester, heneicosane and tetratetracontane (Fig.2). Six compounds identified in the chloroform extract as hexadecanoic acid, methyl ester**,** octadecenoic acid, methyl ester**,** stearic acid, methyl ester**,** eicosane**,** 1,2-benzenedicarboxylic acid, diisooctyl esterand tetratetracontane (Fig.3)**.** Inethyl acetate extract, ten compounds were detected and these include benzaldehyde, benzyl Alcohol, pentanoic acid 4-oxo-phenylmethyl ester**,** benzyl ether**,** tetradecanoic acid**,** n-hexadecanoic acid**,** octadecanoic acid ethyl ester**,** 1,2-benzenedicarboxylic acid diisooctyl ester**,** progesterone and tetratetracontane (Fig.4)**.** Ten important compounds were also successfully identified in the methanol extract of *N. cadamba* leaves. The compounds were dodecanoic acid, myristic acid, 2-cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl), pentadecanoic acid, n-hexadecanoic acid, hexadecanamide, octadecanamide, heneicosane, 1,2-benzenedicarboxylic acid diisooctyl ester and tetratetracontane (Fig. 5).

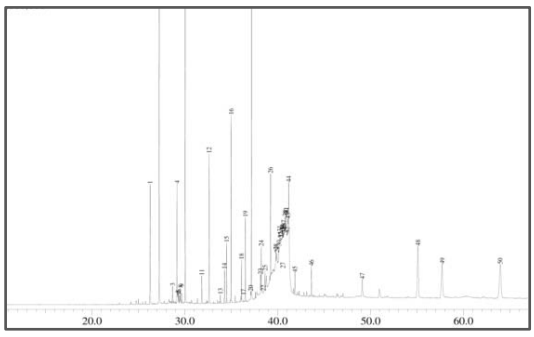

Fig. 1: Gas chromatogram of the hexane extract of the leaves of *N. cadamba.* Hexadecanoic acid, methyl ester (1), hexadecanoic acid, ethyl ester (2), heptadecanoic acid, ethyl ester (3), stearic acid, methyl ester (4), octadecanoic acid, ethyl ester (10), docosanoic acid, methyl ester (14), 1,2-benzenedicarboxylic acid, diisooctyl ester (15), tricosanoic acid, methyl ester (17), pentacosanoic acid, methyl ester (22), and tetratetracontane (23)

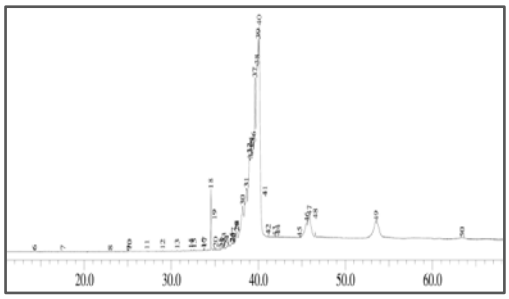

Fig. 2: Gas chromatogram of the petroleum ether extract of the leaves of *N. cadamba*. 1,2-benzenedicarboxylic acid, diisooctyl ester (18), heneicosane (19), and tetratetracontane (27)

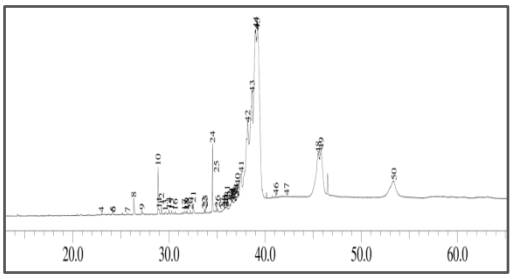

Fig. 3: Gas chromatogram of the chloroform extract of the leaves of *N. cadamba*. Hexadecanoic acid, methyl ester (8), octadecenoic acid, methyl ester (10), stearicacid, methyl ester (12), eicosane (23), 1,2-benzenedicarboxylic acid, diisooctyl ester (24), and tetratetracontane (40)

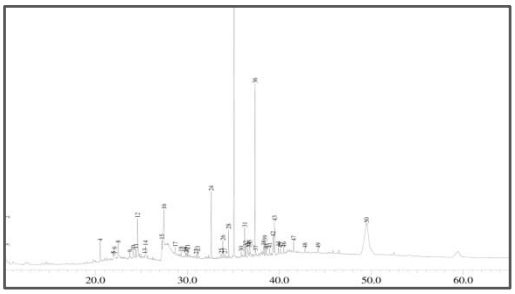

Fig. 4: Gas chromatogram of the ethyl acetate extract of the leaves of *N. cadamba*. Benzaldehyde (1), benzyl alcohol (2), pentanoic acid, 4-oxo-, phenylmethyl ester(6), benzyl ether (8), tetradecanoic acid (10), n-hexadecanoic acid (16),octadecanoic acid, ethyl ester (21), 1,2-benzenedicarboxylic acid, diisooctyl ester (28), progesterone (42), and tetratetracontane (45)

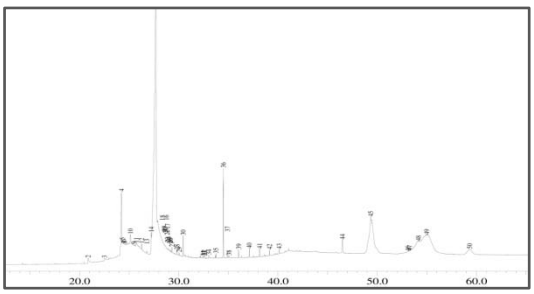

Fig. 5: Gas chromatogram of the methanol extract of the leaves of *N. cadamba*. Dodecanoic acid (2), myristic acid (4), 2-cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl) (10), pentadecanoic acid (11), n-hexadecanoic acid(15), hexadecanamide (30), octadecanamide (34), heneicosane (35), 1,2-benzenedicarboxylic acid, diisooctyl ester (36), and tetratetracontane (40)

Table 3: Summary of phytochemical compounds identified from the *N. cadamba* leaf extracts and their general biological activities (Modified from Dr. Duke's: Phytochemical and Ethnobotanical Databases [9])

S. No.	Compounds	Secondary metabolites	Biological activity
1	Progesterone	Steroid	Neuroactive, analgesic, anesthetic, antioxidant activity, anticancer
2	Hexadecanoic acid, ethyl ester (palmitic acid ethyl ester)	Fatty acid ester	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavor, hemolytic, 5-alpha reductase inhibitor
3	Hexadecanoic acid methyl ester	Fatty acid ester	Antioxidant, nematicide, pesticide, flavour, antiandrogenic
4	Stearic acid methyl ester	Fatty acid ester	5-Alpha reductase inhibitor, cosmetic, flavour, hypocholesterolemic, perfumery, suppository
5	n-hexadecanoic acid	Fatty acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic, flavour, hemolytic, 5-alpha reductase inhibitor
6	Tetradecanoic acid	Fatty acid	Antioxidant, hypercholesterolemic, cancer-preventive, cosmetic
7	Heptadecanoic acid	Fatty acid	Antioxidant
8	Dodecanoic acid	Fatty acid	Flavour
9	Myristic acid	Fatty acid	Antioxidant, hypercholesterolemic,cancer-preventive, cosmetic, nematicid.
10	Pentadecanoic acid	Fatty acid	Antioxidant
11	Benzaldehyde	Aromatic compounds	Allergenic, anesthetic, antibacterial, anticancer, antimutagenic, antipeptic, antiseptic, antispasmodic, antitumor, candidicide, flavour, insecticide, nematicide, pesticide, sedative, termiticide, tyrosinase inhibitor
12	Benzyl alcohol	Aromatic compounds	Allergenic, anesthetic, antiodontalgic, antipruritic, antiseptic, flavour, fungicide, pesticide, sedative
13	1,2-Benzenedicarboxylic acid, diisooctyl ester	Plasticizer compound	Antimicrobial, antifouling

In accordance with the previous findings, most of the identified compounds from this study have also been reported elsewhere in other species. For instance, n-hexadenoic acid was identified as the major compound (44.88%) of methanol extract of *N. cadamba* leaves and this compound was also reported as a major compound in the methanolic leaf extract of *Trichilia connaroides* [26]. 17 compounds were identified in the leaves of *Cleistanthus collinus* and n-hexadecanoic acid was identified as the major compound [27]. Eicosane, 1,2-benzenedicarboxylic acid, diisooctyl ester, n-hexadecanoic acid and ethyl ester were present in *Cassia italica* leaf methanol extract [28]. Heptadecanoic acid, ethyl ester, tricosanoic acid and stearic acid have been reported in the hexane extract of the leaves of *Desmodium elegans* [29]. The aromatic metabolites such as benzoic acid (BA) or benzyl alcohol constitute the backbone of numerous compounds in plants [30-31]. Benzaldehyde and benzyl alcohol were present in the dichloromethane extract of the leaves of *Drypetes gossweileri* and pentanoic acid-4-oxo- phenylmethyl ester has been reported in the ethyle acetate extract of the leaves of the same plant [32]. Palmitic acid, stearic acid, myristic acid and hexadecanamide were present in the methanolic extract of *Gigantochloa apus* leaf [33]. Octadecanamide was isolated from the aqueous extract of *Bacopa monnieri* [34].

The phytochemical screening has identified 26 compounds from all the solvent extracts from the *N. cadamba* leaves and some of the identified compounds have been reported to possess various biological activities such as anti-microbial, anti-cancer, antimutagenic, antipeptic, antiseptic, antispasmodic, anti-adrenogenic and hypocholesterolemic activities as summarized in Table 3. Palmitic acid is an intermediate in the biosynthesis of sexual pheromones of some insects [35]. It is used in the preparation of the ingredients of some drugs to decrease the hydrophobicity of virginiamycin, a drug used against *Mycobacterium avium* [36-37] and it is well known as insecticide and anti-microbial agents [38]. Myristic acid is one of saturated fatty acids in animal and vegetable fats that are commonly used in soaps, cosmetics, flavourings and perfumes. It has hypercholesterolemic activity as it increases low density lipoprotein cholesterol production [39].

CONCLUSION

The present phytochemical study of *N. cadamba* leaf extracts was studied for the first time using five different solvent extracts with increasing polarities. In fact, this is the first available information about the phytoconstituents of *N. cadamba* from Malaysia. *N. cadamba* possess various potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance. Its leaves can be used as antimicrobial, antiparasitic insecticide, nematicide, pesticide, antiandrogenic, hypocholesterolemic, antioxidant, cancer preventive, anticoronary, antiarthritic, hepatoprotective, neuroactive, analgestic and anesthetic, flavour and allergenic. The presence of various bioactive compounds has justified the use of *N. cadamba* leaf extracts for various ailments by traditional practitioners and therefore, further studies on isolation and identification of individual constituent are very much needed. It is also timely to explore its pharmacological values at the molecular level with the help of various biotechnological techniques in future.

CONFLICT OF INTERESTS

Declared None

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