DE GRUYTER
OPEN

herba polonica
From Botanical to Medical Research
Vol. 62 No. 1 2016
DOI: 10.1515/hepo-2016-0002

EXPERIMENTAL PAPER

Seed oil composition of *Acacia nilotica* (L.) Delile from Iran

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S u m m a r y

Introduction: *Acacia nilotica* (L.) Delile belongs to the *Fabaceae* family and the subfamily Mimosoideae; and commonly called Gum arabic tree. As the young pods and seeds are eaten roasted by the people in South Iran. **Objective:** The oil composition of the mature seeds of *A. nilotica* collected from natural habitats of the south of Iran were analyzed in order to determine their potential for human or animal consumption. **Methods:** The extracted oil was analyzed by gas-chromatography/mass spectroscopy method (GC/MS). **Results:** The oil content in these edible seeds was found to be 3.4% (*v*/*w*) fresh weight. A rare phytosterol, six fatty acids, nine hydrocarbons and one diterpenoid were identified which constituted about 83.5% of the oil. The phytosterol, 26-ethylcholesta-5,25(Z)-dien-3.*β*-ol (20.8%) as well as essential saturated and unsaturated fatty acids were the main components. Other components were present

in amounts lower than 5%. **Conclusion:** It is concluded that the seed oil could be a new natural source for human nutrition.

Key words: *Acacia nilotica*, seed oil, phytosterol, fatty acid

INTRODUCTION

Acacia is a genus of *Fabaceae* family and comprises about 135 species of trees or shrubs which are widely spread throughout the arid and semi-arid tropics [1]. *A. nilotica* belongs to the sub-family Mimosoideae; and commonly called Gum arabic tree. It is a 5–20 m high tree with a dense spheric crown. Leaves of *A. nilotica* locally called "Chesh" are used for feeding sheep and goats in south of Iran. Bark and leaves are used in Iranian traditional medicine to treat haemorrhage, colds, diarrhea, scurvy, dysentery and ophtalmia. Antibacterial, antifungal, antidiarrhea, antioxidant, antimutagenic, anthelmintic, analgesic, anti-inflammatory, antihypertensive and vasoconstrictional activities of different parts of the plant were reported [2].

As evident from the literature, the roasted young pods and seeds have been eaten by humans. The seeds of some *Acacia* species were used as food and have been assessed for nutritional compositions and were shown to contain considerable amount of oil [3]. Since there was no phytochemical investigation on *A. nilotica* seed oil growing wild in South of Iran, we were prompted to extract and characterize the *A. nilotica* seed oil to evaluate its oil potential and explore a new source of fatty acid for nutritional purposes.

MATERIAL AND METHODS

Plant material

A. nilotica seeds were collected in August 2014 from Sarkhun village, Bandar Abbas, Hormozgan Province, Iran (27°23'34" N 56°23'59" E, 100 m.a.s.l). Specimen was identified by R. Asadpour and voucher was deposited in the Herbarium of Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran under the code number 409-PMP.

Oil extraction

Oil extraction was performed with a Soxhlet apparatus using *n*-hexane as the solvent. 100 g of powdered seeds was extracted for 6 h and then the solvent was evaporated by using a rotary evaporator at 40°C. The pure oil was transferred into a small glass vial, flushed with nitrogen and maintained at –18°C until analyzed.

Preparation of fatty acid methyl esters

Fatty acid methyl esters of the extracted oil were prepared according to the method previously reported by Metcalfe *et al*. [4]. 1 g of the oil was weighed into a volumetric flask. Then, 25 ml of 0.5 N methanolic potassium hydroxide was added and placed in the boiling water for 20 min. Then 12 ml boron trifluoride (BF_3) was added and boiled again for 3 min. After that, the flask was cooled and 5 ml *n*-hexane and adequate saturated NaCl solution were added. The flask was shaken vigorously and left to stand for 5 min. The fatty acid methyl esters were prepared and dissolved in *n*-hexane (the upper phase). 2 ml of phase was collected, dried with anhydrous Na_2SO_4 and transferred to clean glass vial and kept at 0˚C until analyzed by GC/MS.

Oil analysis

Oil sample analysis was performed on a Hp-6890 gas chromatograph (GC) equipped with a FID and a DB-5 capillary column, 30 m × 0.25 mm, 0.25 μm film thickness, temperature programmed as follows: 60°−240°C at 4°C/min. The carrier gas was N2 at a flow of 2.0 ml/min; injector port and detector temperature were 250°C and 300°C, respectively. Sample was injected by splitting and the split ratio was 1:10.

The GC/MS analysis was performed on a Hewlett-Packard 6890/5972 system with a DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The operating conditions were the same conditions as described above but the carrier gas was He. Mass spectra were taken at 70 eV. Scan mass range was from 40 to 400 m/z at a sampling rate of 1.0 scan/s. Quantitative data were obtained from the electronic integration of the FID peak areas. The components of the oil was identified by their retention time, retention indices, relative to C_9-C_{30} *n*-alkanes, computer matching with the WILEY275 library as well as by comparison of their mass spectra with those of authentic samples or with data already available in the literature [5, 6]. The percentage of composition of the identified compounds was computed from the GC peak areas without any correction factors and was calculated relatively.

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

In this study, the oil composition of *A. nilotica* seeds native to south of Iran was determined. The oil extracted was viscous and yellow-green in color with the total oil content of 3.4% (*v*/*w*). The quality of the oil is mainly governed by the

fatty acid composition hence the standardization of the oil on the basis of fatty acid composition is mandatory. The general chemical profiles of the tested oil, the percentage content of the individual components and the retention indices are summarized in Table 1.

From the table 1, it is evident that the major constituents of the seed oil consists mainly of a rare phytosterol, 26-ethylcholesta-5,25(Z)-dien-3.β-ol (20.8%) and essential saturated and unsaturated fatty acids. Other components were presented in amount less than 5% (tab. 1).

Table 1.

GC-MS analysis of *A. nilotica* seed oil

Compound[a]	KI[b]	KI[c]	Percentage
n-Decane	998	1000	3.5
Undecane	1096	1100	3.3
n-Dodecane	1195	1200	6.7
Naphthalene, decahydro-1,6-dimethyl	1214	1211	2.1
Naphthalene, decahydro-2,3-dimethyl	1221	1219	2.5
Naphthalene, decahydro-1,5-dimethyl	1295	1299	7.4
n-Tridecane	1306	1300	3.9
n-Tetradecane	1398	1400	1.6
n-Hexadecane	1592	1600	0.6
Palmitic acid methyl ester	1930	1927	3.2
Linoleic acid methyl ester	2088	2092	8.6
Oleic acid methyl ester	2120	2116	5.0
Phytol acetate	2214	2218	2.4
Stearic acid methyl ester	2125	2130	2.0
Eicosanoic acid methyl ester	2333	2329	0.8
n-Pentacosane	2494	2500	0.8
Docosanoic acid methyl ester	2520	2513	0.7
n-Heptacosane	2692	2700	2.4
n-Nonacosane	2912	2904	4.8
α-Tocopherol	3140	3149	0.4
26-ethylcholesta-5,25(Z)-dien-3.β-ol	3430	3432	20.8
Total			83.5

[a]Compounds listed in order of elution.
[b]KI (Kovats index) measured relative to *n*-alkanes (C_9-C_{28}) on the non-polar DB-5 column under condition listed in the Material and Methods section.
[c]KI (Kovats index) from literature [4, 5].

Phytosterols are steroid compounds similar to cholesterol which occur in plants. Phytosterol enriched foods possess well documented LDL cholesterol lowering effects [7]. Phytosterols may inhibit lung, stomach, ovarian and breast cancers [8]. Phytosterols have a long history of safe use and have generally recognized as safe status in the US [9]. Figure 1 shows the 26-ethylcholesta-5,25(Z)-dien-3.β-ol molecular structure. The mass spectrum for this major component which had [M]+ at 412 m/z suggested the molecular formula $C_{29}H_{48}O$. The other fragment peaks at m/z were 379, 299, 271, 213, 145, 105 and 55. The structure was elucidated just by mass spectra. This rare phytosterol ($C_{29}H_{48}O$) has been not reported from any natural source by now. Future biological studies are suggested to investigate the possible valuable pharmacological properties of 26-ethylcholesta-5,25(Z)-dien-3.β-ol.

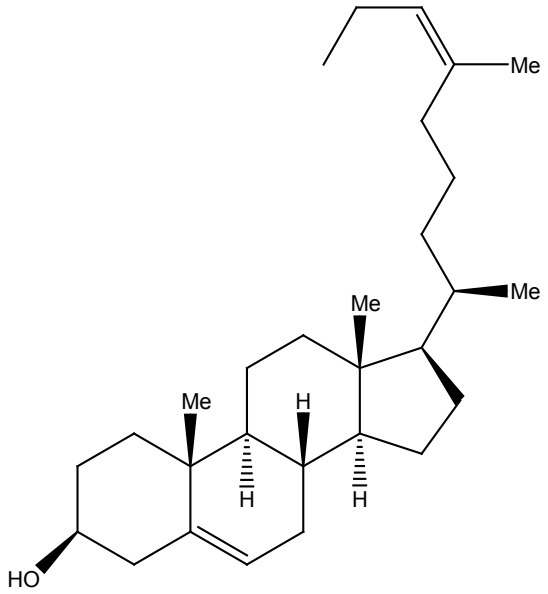

Figure 1.

Molecular structure of 26-ethylcholesta-5,25(Z)-dien-3.β-ol

Linoleic acid (8.6%) was found to be in maximum in *A. nilotica* seed oil, followed by oleic acid (5.0%), palmitic acid (3.2%) and stearic acid (2.0%). Higher content of linoleic acid in analyzed oil is noteworthy. Phytol acetate was also identified with a considerable amount of 2.4% in the oil. It is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E [10].

The literature survey revealed that fatty acid composition of *A. nilotica* seed oil growing in India has been investigated previously. The oil content was reported to be little lower (3.1%) than that of *A. nilotica* seeds. The coronaric acid was reported as the major fatty acid [11].

CONCLUSION

In conclusion, the present study revealed that the seed oil of *A. nilotica* growing in south of Iran could be a new source of high phytosterol-rich edible oil and its full potential should be exploited. The use of oil from the seeds is of potential economic benefit to the poor native population of the areas where it is cultivated. Despite that previous studies reported that the different parts of the plant were not toxic to human [12], the seed oil of *A. nilotica* could be a new source of edible vegetable oil after the future toxicological studies. The 26-ethylcholesta-5,25(Z)-dien-3.β-ol structure was elucidated just by mass spectra and the most possible and inexhaustible structure was determined for this phytosterol. Further phytochemical investigations are suggested to elucidate the exact molecular structure.

ACKNOWLEDEGMENTS

Supports from the Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS) are gratefully acknowledged.

Conflict of interest: Authors declare no conflict of interest.

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SKŁAD OLEJU Z NASION *ACACIA NILOTICA* (L.) DELILE ROSNĄCEJ W IRANIE

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S t r e s z c z e n i e

Wstęp: *Acacia nilotica* (L.) Delile należy do rodziny *Fabaceae,* podrodziny Mimosoideae; otrzymuje się z niej gumę arabską. W południowym Iranie są spożywane pieczone młode strąki i nasiona tej rośliny **Cel:** Badano skład oleju z dojrzałych nasion *A. nilotica* zebranych z naturalnych stanowisk na południu Iranu w celu określenia jego przydatności do spożycia przez ludzi i zwierzęta. **Metody:** Wyekstrahowany olej analizowano metodą chromatografii gazowej sprzężonej ze spektrometrią mas (GC/MS). **Wyniki:** Zawartość oleju w jadalnych nasionach wynosiła 3.4% (*v/w*) świeżej masy. Olej zawierał rzadko spotykany fitosterol, sześć kwasów tłuszczowych, dziewięć węgolwodorów i jeden diterpenoid; związki te stanowiły łącznie około 83.5% oleju. Głównymi składnikami oleju były: fitosterol, 26-ethylcholesta-5,25(Z)-dien-3.*β*-ol (20.8%) oraz nasycone i nienasycone kwasy tłuszczowe. Zawartość pozostałych składników nie przekroczyła 5%. **Wniosek:** Olej z nasion omawianego gatunku może być nowym naturalnym środkiem odżywczym dla ludzi.

Słowa kluczowe: *Acacia nilotica, olej z nasion, fitosterol, kwas tłuszczowy*