ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Research Article / JAPR
Ahmed et al., 2019, 3 (2), 83-89

Journal of Advanced Pharmacy Research

# Phytochemical Screening and Chemical Investigation of Lipoidal Matter of *Arenga engleri* Leaves

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***Submitted on: 13-02-2019; Revised on: 19-03-2019; Accepted on: 26-03-2019***

**To cite this article:** Ahmed, W. A.; Kamal, A. M.; Ibrahim, R. R. Phytochemical Screening and Chemical Investigation of Lipoidal Matter of *Arenga engleri* Leaves. *J. Adv. Pharm. Res.* **2019**, *3* (2), 83-89 DOI:10.00000/APRH.2019.0000.0000

## ABSTRACT

**Objectives**: In this study, a preliminary phytochemical screening and lipoidal matter of *Arenga engleri* Becc. leaves (family Arecaceae) were studied for the first time. **Methods:** Gas chromatography coupled with mass spectroscopy (GC/MS) was used for the identification of compounds of saponifiable and unsaponifiable content. **Results:** The preliminary phytochemical screening showed the presence of saponins, tannins, flavonoids, cardiac glycosides, carbohydrate and/or glycosides, unsaturated sterols and/or triterpenes and absence of anthraquinones, coumarins, volatiles and alkaloids or compound containing nitrogenous bases. GC/MS analysis revealed the higher percentage of unsaturated fatty acids (51.39%) than that of saturated ones (31.47%). The major unsaturated fatty acids present were linoleic acid (31.55%) and 7,10-hexadecadienoic acid (11.42%) while the major saturated one was palmitic acid (17.27%). The unsaponifiable matter was represented as hydrocarbons (41.19%), fatty alcohols (28.31%), terpenes (9.68%) and sterol (0.14%). 1-Octadecene (17.65%) and 1-hexadecene (12.41%) represented the major hydrocarbons while behenic alcohol (14.71%) was the major fatty alcohol, phytol (4.89%) was the major terpene and ethylisoallocholate (0.14%) was the only sterol identified.

***Keywords:*** *Arenga engleri*; *GC/MS; Lipoidal matter; Phytochemical screening.*

## INTRODUCTION

Arecaceae, previously called the Palmae family, comprises about 200 genera and 2600 species which are distributed throughout tropical and subtropical regions[1,2]. Palms are called the "Trees of Life" as they have a potential role in people's life supplying them with foods, fibers, shelter, fuels, oils, gums, waxes, poisons and medicines[2]. Genus *Arenga* contains 22 species, distributed in South China, the Ryukyu Islands and Taiwan in the North to Christmas Island in the South and from India in the West to Queensland, Australia in the East[3]. The genus is economically important as it is useful for sugar, starch, thatch production and potentially has an ornamental value[4]. Previous reports showed the identification of squalene, lutein, *β*-sitosterol and stigmasterol from *Arenga tremula*[5] and RP-HPLC analysis of *Arenga wightii* revealed the presence of caffeine and major phenolic compounds: gallic acid, ascorbic acid and chlorogenic acid[6]. Different species were reported to have hypocholesterolaemic[7], antioxidant, anti-microbial[6], anti-hypertensive, anti-inflammatory and analgesic activities[8] and treating skin allergies[9], headache , malaria and tuberculosis[10].

*Arenga engleri* Becc., commonly called the Formosa palm, Taiwan sugar palm or dwarf sugar palm, is an attractive medium-sized ornamental clustering

palm which is native to Taiwan (Formosa) and Ryukyu Islands (South of Japan)[11]. The stems are covered with delicate black fibers which have been used for a long time to prepare coir raincoat, shoes, rope, fishing nets, brushes and pads[12]. The leaves are known to be useful in thatching and wickerwork[4]. The young ones and apical buds are edible, the stems pith used to produce starch while the sweet sap obtained from the inflorescence stem used to make sugar[13] and the flowers have a very pleasant fragrance. The mesocarp of the fruits are rich in irritant calcium oxalate raphides like other most species of this genus and this make them inedible and poisonous[4]. Since there are no reported studies about phytochemical constituents of *Arenga engleri* Becc. leaves and its lipoidal profile, it was found essential to carry out preliminary phytochemical screening and investigation of lipoidal matter of this palm.

## MATERIALS AND METHODS

### Plant material

Fresh leaves of *Arenga engleri* Becc. were collected on October 2014 from Al Zohriya Garden, Cairo, Egypt and kindly identified by Dr. Terase Labib, Head of the Taxonomists at Orman Botanical Garden, Giza, Egypt. A Voucher specimen (03Aen/2019) was kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Egypt.

### Chemicals

For phytochemical screening: 1 % Hydrochloric acid, 5 % alcoholic potassium hydroxide, concentrated ammonium hydroxide solution, 10 % alcoholic solution of α- naphthol, sulfuric acid, 1% aluminum chloride solution, magnesium turning, concentrated hydrochloric acid, 0.1% ferric chloride solution, 1 % lead acetate solution, picric acid, sodium hydroxide, acetic anhydride, glacial acetic acid, chloroform, Fehling's solution, Barfoed's reagent, Mayer's reagent, Dragendorff's reagent.

For lipoidal matter: *n*-Hexane, 10 % alcoholic potassium hydroxide, ether, 10 % 2N hydrochloric acid, sulphuric acid, methanol.

### Apparatus

TRACE™ 1310 Gas Chromatograph produced by Thermo Scientific™ provided by FID (Flame Ionization Detector), attached with ISQ LT single quadrupole Mass Spectrometer (Regional center for Mycology and Biotechnology, Al-Azhar University).

### Methods

### Preliminary phytochemical screening

Air dried powdered leaves of *A. engleri* were screened for its constituents using standard protocols in the mentioned references [14,15,16].

### Preparation of lipoidal matter

The air-dried powder of *A. engleri* leaves (100 g) were extracted by *n*-hexane. The solvent was evaporated at 40°C under reduced pressure to give 7 g residue of lipoidal matter[17].

### Fractionation of lipoidal matter

Two gm of lipoidal matter were saponified by refluxing with 50 ml of 10% alcoholic potassium hydroxide solution for 2 hr followed by evaporating the alcohol, diluting with distilled water and extracting with ether exhaustively. The collected ethereal extract was washed with distilled water till being free from alkalinity, dried over anhydrous sodium sulphate, and then evaporated to give 1.16 g (58%) unsaponifiable matter (USM) residue.

The remaining saponifiable aqueous layer left after extraction with ether was acidified with 10% 2 N hydrochloric acid and the liberated fatty acids were extracted exhaustively with ether. The collected ethereal extract was washed with distilled water until neutralization, dried over anhydrous sodium sulfate, and then evaporated to give 0.54 g (27%) total fatty acids (TFA) residue[18].

### Preparation of fatty acid methyl esters

The preparation of methyl esters of free fatty acids (0.54 g) was carried out by refluxing with 100 ml of absolute methanol and 5 ml sulphuric acid for 2 hr, extracting with ether and drying the ethereal layer over anhydrous sodium sulfate followed by evaporation of ether to give residue of the fatty acid methyl esters (FAME), kept for GC-MS analysis[19].

### GC-MS analysis of the FAME

Fatty acid methyl esters were analyzed according to the following conditions: TR-FAME, Thermo 260 M 142 P (30 m, 0.25 mm ID, 0.25 µm film), 70% cyanopropyl- polysilphenylene siloxane capillary column. The used gas: Helium (1.5 ml/ min). Injector temperature: 200°C; temperature transfer line: 250°C; initial column temperature 80°C, programmed by 3°C/ min up to the final temperature 230°C within 50 min and the ionization energy was 70 ev.

### GC-MS analysis of the unsaponifiable matter

The analysis was carried out under the following conditions: DB-17 P/N 122-1751 (30 m, 0.25 mm ID, 1 µm film), 50% phenyl-methylpolysiloxane capillary column. The used gases: $H_2$, $N_2$, air. Injector temperature: 280°C; temperature transfer line: 300 °C; initial column temperature: 100°C, programmed by

**Table 1. GC-MS analysis of USM of *A. engleri* leaves**

| Identified compounds | | RRT* (min) | Area % |
|---|---|---|---|
| 3-Phenyl decane | $C_{16}H_{26}$ | 0.809 | 0.07 |
| 1-Hexadecene | $C_{16}H_{32}$ | 0.837 | 12.41 |
| 6-Phenyl undecane | $C_{17}H_{28}$ | 0.866 | 0.57 |
| 5-Phenyl undecane | $C_{17}H_{28}$ | 0.869 | 1.10 |
| 4-Phenyl undecane | $C_{17}H_{28}$ | 0.878 | 0.74 |
| 3-Phenyl undecane | $C_{17}H_{28}$ | 0.896 | 0.49 |
| *n*-Dotriacontane | $C_{32}H_{6}$ | 0.923 | 0.33 |
| 2-Phenyl undecane | $C_{17}H_{28}$ | 0.928 | 0.40 |
| 6-Phenyl dodecane | $C_{18}H_{30}$ | 0.945 | 1.37 |
| 5-Phenyl dodecane | $C_{18}H_{30}$ | 0.948 | 1.26 |
| 4-Phenyl dodecane | $C_{18}H_{30}$ | 0.959 | 0.81 |
| 3-Phenyl dodecane | $C_{18}H_{30}$ | 0.977 | 0.77 |
| 1-Octadecene | $C_{18}H_{36}$ | 1 | 17.65 |
| 2-Phenyl dodecane | $C_{18}H_{30}$ | 1.01 | 0.24 |
| 6-Phenyl tridecane | $C_{19}H_{32}$ | 1.02 | 1.05 |
| 5-Phenyl tridecane | $C_{19}H_{32}$ | 1.024 | 0.83 |
| 3-Phenyl tridecane | $C_{19}H_{32}$ | 1.05 | 0.27 |
| 2-Phenyl tridecane | $C_{19}H_{32}$ | 1.08 | 0.34 |
| 17-Pentatriacontene | $C_{35}H_{70}$ | 1.17 | 0.49 |
| 1-Hexadecanol | $C_{16}H_{34}O$ | 0.66 | 5.52 |
| Behenic alcohol | $C_{22}H_{46}O$ | 1.14 | 14.71 |
| *n*-Tetracosanol-1 | $C_{24}H5_{0}O$ | 1.26 | 8.08 |
| Phytol | $C_{20}H_{40}O$ | 1.22 | 4.89 |
| Squalene | $C_{30}H_{50}$ | 1.67 | 4.79 |
| Ethylisoallocholate | $C_{26}H_{44}O_{5}$ | 1.09 | 0.14 |
| **Total hydrocarbons** | | | **41.19** |
| **Fatty alcohols** | | | **28.31** |
| **Total terpenes** | | | **9.68** |
| **Total sterols** | | | **0.14** |
| **Total identified compound** | | | **79.32** |
| **Unidentified compounds** | | | **20.68** |

*RRT*: Relative retention time of 1-Octadecene with RT = 29.83 min.*

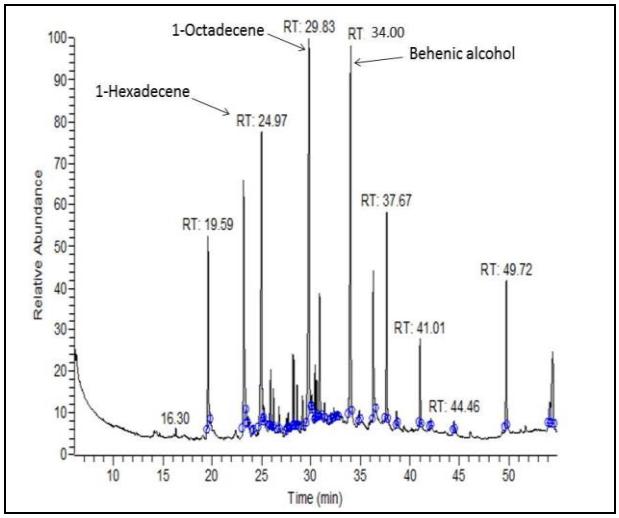


**Figure 1. GC-chromatogram of unsaponifiable matter of *A. engleri* leaves.**

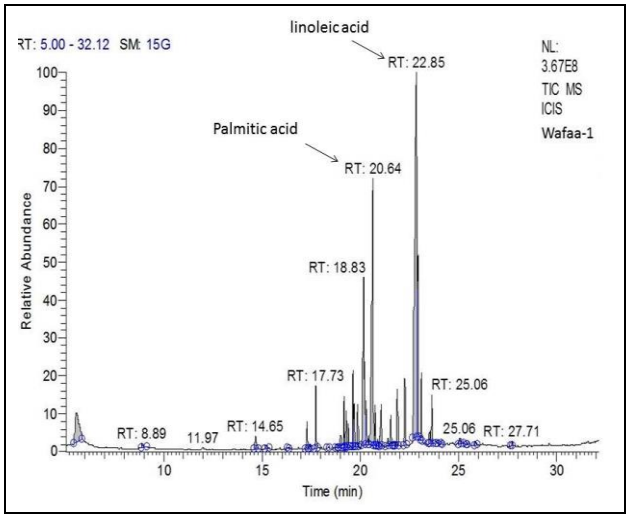


**Figure 2. GC- chromatogram of fatty acids of *A. engleri* leaves.**

10 ºC/ min up to the final temperature 270 ºC within within 30 min and the ionization energy was 70 ev.

**Identification of USM and TFA**

After GC-MS analysis of USM and TFA, the compounds were identified by comparing their retention times and mass fragmentation patterns with those of the reference standard data of WILEY and NIST libraries[20]. Quantitative determination was based on peak area integration[21].

## RESULTS

**Preliminary phytochemical screening**

Preliminary phytochemical screening of *A. engleri* leaves showed the presence of saponins, tannins, flavonoids, cardiac glycosides, carbohydrate and/or glycosides, unsaturated sterols and/or triterpenes while anthraquinones, coumarins, volatiles and alkaloids or compound containing nitrogenous bases were absent.

**Investigation of lipoidal matter of *A. engleri* leaves**

Fractionation of lipoidal matter of *n*-hexane extract of *A. engleri* leaves yielded 58% unsaponifiable matter and 27% fatty acids. As shown in **Table 1** and **Figure 1**, GC-MS results of unsaponifiable matter revealed that leaves of *A. engleri* contain 41.19% hydrocarbons, 28.31% fatty alcohols, 9.68% terpenes and 0.14% sterols. The most abundant compounds identified in hydrocarbons content were 1-octadecene (17.65%) and 1-hexadecene (12.41%). Behenic alcohol (14.71%) was the major fatty alcohol. Total terpenes identified as 4.89% phytol and 4.79% squalene. Ethylisoallocholate was the only sterol identified. As shown in **Table 2** and **Figure 2**, the percentage of unsaturated fatty acids (51.39 %) was higher than saturated ones (31.47 %). The major unsaturated fatty acids were linoleic acid (31.55 %) and 7,10-hexadecadienoic acid (11.42%) while the major saturated one was palmitic acid (17.27 %).

## DISCUSSION

The results of preliminary phytochemical screening of *Arenga engleri* leaves showed the presence of sterols and triterpene that was confirmed by the GC-MS analysis of its lipoidal matter. As revealed in GC-MS analysis, the percentage of unsaponifiable matter was higher than saponifiable one and unsaturated fatty acids were more than saturated ones. Linoleic acid and palmitic acid represented the major identified unsaturated and saturated fatty acids, respectively. For unsaponifiable matter, hydrocarbons represented the major component then fatty alcohols and then terpenes while the only sterol identified was ethylisoallocholate. The most abundant compounds identified in hydrocarbons content were 1-octadecene and 1-hexadecene. Behenic alcohol was the major fatty alcohols and total terpenes identified were phytol and squalene.

Many biological activities have been reported for linoleic acid as antioxidant, anti-inflammatory by cyclooxygenase-I (COX-I) and cyclooxygenase-II (COX-II) inhibition[22]and hypocholesterolemic activities[23]. Studies revealed that elevated levels of linoleic acid in the plasma prevented and controlled hypertension[24,25]. It helped in glycemic control and reduced the risk of Type 2 diabetes[26] and also had anti-cancer activity[27]. Palmitic acid showed antibacterial,

**Table 2. GC-MS analysis of total fatty acids of *A. engleri* leaves**

| Identified compounds | RRT* (min) | Area% |
|---|---|---|
| 4- oxo, Pentanoic acid (levulinic acid) C5:0 | 0.243 | 5.07 |
| 4-Hydroxy-4-methylhex-5-enoic acid C7:1 | 0.389 | 0.27 |
| 10-methyl Undecanoic acid (Isolauric acid) C12:0 | 0.641 | 0.97 |
| Nonanedioic acid (Azelaic acid) C9:0 | 0.664 | 0.35 |
| Octadec-6,9-dien-12-ynoic acid C18:2 | 0.715 | 0.04 |
| cis-5,8,11,14,17-Eicosapentaenoic acid (Timnodonic acid) C20:5 | 0.764 | 0.19 |
| Tetradecanoic acid (Myristic acid) C14:0 | 0.776 | 2.31 |
| 10,13-Octadecadiynoic acid C18:0 | 0.804 | 0.09 |
| Pentadecanoic acid C15:0 | 0.839 | 1.68 |
| 7,10-Hexadecadienoic acid C16:2 | 0.882 | 11.42 |
| 9-Hexadecenoic acid (Z) (Palmitoleic acid) C16:1 | 0.888 | 1.95 |
| Hexadecanoic acid (Palmitic acid) C16:0 | 0.903 | 17.27 |
| 9,12-Octadecadienoic acid (Z,Z) (linoleic acid) C18:2 | 1 | 31.55 |
| 9,12,15-Octadecatrienoic acid (linolenic acid) C18:3 | 1.003 | 5.52 |
| Octadecanoic acid (Stearic acid) C18:0 | 1.011 | 2.00 |
| Hexanoic acid C6:0 | 1.034 | 1.71 |
| 2-Octyl-cyclopropaneoctanoic acid C19:0 | 1.056 | 0.06 |
| 6,9,12-Octadecatrienoic acid C18:3 | 1.097 | 0.36 |
| Docosanoic acid (Behenic acid) C22:0 | 1.112 | 0.05 |
| **Saturated fatty acids** | | **31.47** |
| **Unsaturated fatty acids** | | **51.39** |
| **Un identified compounds** | | **17.14** |

*RRT*: Relative retention time of linoleic acid with RT = 22.85 min*

antifungal, antioxidant, anti-inflammatory and hypocholesterolemic activities[28].

1-Octadecene showed anticancer, antioxidant and antimicrobial activities[20]. Behenic alcohol was a fatty alcohol of antiviral activity[29] while 1-hexadecene had antibacterial, antifungal and antioxidant activities[20]. Comparably to other species, fatty acids like 3-nonenoic acid, 13-docosenoic acid, butanoic acid, and 2-hydroxyisobutyric acid were identified in leaf and fruit of *Arenga wightii* Griff. by GC-MS analysis[6].

## CONCLUSION

From preliminary phytochemical screening, it is revealed that *Arenga engleri* leaves are rich in saponins, tannins, flavonoids, cardiac glycosides, carbohydrate and/or glycosides, unsaturated sterols and/or triterpenes and laking anthraquinones, coumarins, volatiles and alkaloids or compound containing nitrogenous bases. Lipoidal matter investigation by GC/MS analysis showed that leaves contain valuable compounds that suggest the probability of using this palm medicinally and it is the first report for lipoidal investigation and phytochemical screening of *Arenga engleri* leaves growing in Egypt.

### Conflict of Interest

The authors declare that they don't have any kind of conflict of interest.

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