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Investigation of the Unsaponifiable and Saponifiable Matters of *Pachypodium lamerei* Drake Leaves and Stems by GC/MS

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ABSTRACT
Pachypodium lamerei Drake (family Apocynaceae) is native to Madagascar and is known as Madagascar palm. The present study shows that, the unsaponifiable matter of *P. lamerei* contains various compounds identified as hydrocarbons (5.43%), pregnane (0.92%), steroids (11.54%), triterpenes (8.78%) as well as other oxygenated compounds (61.3%). While, the saponifiable matter contains twenty compounds from which ten were identified as methyl esters of saturated fatty acids (32.31%) in addition to hydroxylated fatty acids (9.96%) whereas ten fatty acids (57.73%) couldn't be identified. This is the first record of the GC/MS analysis for genus *Pachypodium*.

Keywords: *Pachypodium lamerei*, Apocynaceae, Leaves, Stems, Unsaponifiable Matter, Saponifiable Matter, Fatty Acids, GC/MS analysis, Gas Liquid chromatography (GLC), Electron Impact (EI).

1. Introduction
Pachypodium lamerei Drake (family Apocynaceae) is native to Madagascar and is known as Madagascar palm despite it is not being a palm at all. This name is attributed to the narrow leaves which grow only at the top of the trunk, like a palm tree. However *P. lamerei* Drake has been native to Madagascar, recently it is commonly produced as a commercial ornamental plant around the world. It needs to mention that there are no synonyms for *P. lamerei*. Generally speaking, Pachypodiums are succulents having very fat stems, capable of storing large volumes of water, so they can withstand long periods of drought [1-6].

2. Taxonomy
Pachypodium lamerei Drake [7, 8], Kingdom: Plantae, Subkingdom: Viridaeplantae, Infrakingdom: Steptophyta, Division: Tracheophyta, Subdivision: Spermatophytina, Infradivision: Angiospermae, Class: Magnoliopsida, Suborder: Asteranae, Order: Gentianales, Family: Apocynaceae, Subfamily: Apocynoidae, Tribe: Malouetieae, Genus: *Pachypodium* Lindl. and Species: *P. lamerei* Drake.

3. Materials, Equipments and Methods
3.1 Plant material:
The leaves and stems of *Pachypodium lamerei* Drake were collected in May 2010. It was identified by Agr. Eng. Tereez Labib, consultant of plant taxonomy at the Ministry of Agriculture and ex. director of El-Orman Botanical Garden. A voucher specimen has been deposited at the herbarium of pharmacognosy department, faculty of pharmacy, Minia University, Minia, Egypt under registration (Mn-Ph-Cog-006).

3.2 Equipments
Trace GC 2000 produced by THERMO provided by FID (Flame Ionization Detector), connected to the Mass Spectrometer Model: DSQII produced by THERMO, using DB-5 capillary column (30 m x 0.25 mm) (U.S.A) for analysis, Rotary evaporator (BÜCHI R-144, Switzerland), (HEIDOLPH 4000, Germany), Water distiller (BHANU Basic/PH4 MK-I, India) and Water bath (BÜCHI B466, Switzerland).

3.3 Methods:
3.3.1 GLC of unsaponifiable matter:
The column used was a capillary column (50 m x 0.25 mm) and packed with DB-5 (5% Phenyl,

95% Methyl polysiloxane). The injected volume was 1 µl. The analysis was carried out at a programmed temperature. The initial temperature was 50 °C then increasing at a rate of 5 °C/min and final temperature 300 °C (kept for three min.). Injector temperature was 250 °C and detector temperature at 300 °C. Helium was used as the carrier gas at a flow rate of one ml/min. The total run time was 58 min.

3.3.2 GLC of fatty acid methyl esters:
The analysis was done on the same column used for unsaponifiable matter with the same injection volume, flow rate, injector temperature and carrier gas. However, the initial temperature was 150 °C then increased at a rate of 5 °C/min and final temperature 280 °C (kept for five min). The detector temperature was 280 °C. The total run time was 34 min.

3.3.3 Preparation of the samples:
3.3.3.1 Preparation of the Unsaponifiable Matter:
About 1 gm of the dried petroleum ether fraction of the air-dried powder of the leaves and stems of *P. lamerei* was subjected to alkaline hydrolysis for saponification. The petroleum ether fraction was refluxed with 50 ml of N/2 alcoholic potassium hydroxide for about 8 hrs. on a boiling water bath. The major part of the alcohol present was distilled off and the liquid left was diluted with twice its volume of water, then extracted with several portions of chloroform until exhaustion. The combined chloroformic extracts were washed with alcoholic sodium hydroxide (10%) then with distilled water until the wash were free from any alkalinity. The chloroformic extracts were dehydrated over anhydrous sodium sulphate and then the chloroform was distilled off. The residue obtained (represent the unsaponifiable matter) was dark orange red in color and semisolid at room temperature [9-10].

3.3.3.2 Preparation of saponifiable matter (fatty acids):
The alkaline aqueous solution (soap) remained after removal of the unsaponifiable matter was acidified with sulphuric acid (10%). The liberated fatty acids were extracted with successive small portions of chloroform. The combined chloroformic extracts were washed with distilled water, till the wash was neutral to litmus paper. The chloroform was distilled off and the residue of total fatty acids was dried over anhydrous calcium chloride overnight. It was semisolid at room temperature and brown in color [9-10].

3.3.3.3 Preparation of fatty acid methyl esters:
The fatty acids were converted to their methyl esters by refluxing with 50 ml. absolute methanol and 1.5 ml. conc. sulphuric acid for two hrs. The major part of alcohol was distilled off and the residue was solubilized with distilled water and then extracted with several portions of chloroform. The combined chloroformic extracts were washed with distilled water, till the wash was free from any acidity. The chloroformic extract was concentrated and the residue was dried over anhydrous calcium chloride overnight and then kept for further investigation [9, 11].

3.3.3.4 MS Analysis of the lipid fraction:
Electron Impact (EI) mode of ionization, with mass range of 50-700 *m/z*, was applied in the GC/MS analysis of the unsaponifiable matter, while the GC/MS analysis of the fatty acid methyl esters was with mass range 50-500 *m/z*.

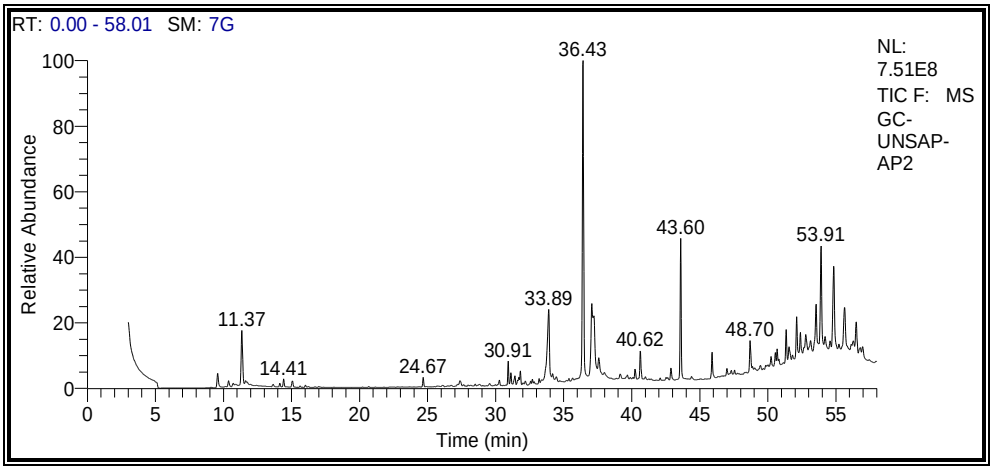

Fig 1: GLC chromatogram of the unsaponifiable matter of *P. lamerei* leaves and stems.

4. Results and Discussion
4.1 The Unsaponifiable Matter
The results of the GC/MS analysis of the unsaponifiable matter of *P. lamerei* leaves and stems shown in Figure 1 and Table 1 revealed the presence of 31 compounds from which 23 (87.97%) were identified whereas eight compounds (12.03%) couldn't be identified.
The identified compounds were classified as hydrocarbons (acyclic and cyclic) (5.43%), pregnane (0.92%), steroids (11.54%), triterpenes (8.78%) and other oxygenated compounds (61.3%).
Phytol was the major identified compound from the unsaponifiable matter (18.82%) followed by tetradecyl oxirane (14.16%), 2-acetyl-3-hydroxy-1,4-naphthoquinone (10.13%) and stigmasterol (9.69%). Phytol, which is an acyclic diterpene alcohol functions as a precursor for vitamin E and K1 and it has antioxidant and anticancer activities [12].
Identification of the compounds was carried out by matching their retention times and fragmentation patterns with those of reference compounds analyzed under the same conditions [13-18].

Table 1: Identification of the components of the unsaponifiable matter of *P. lamerei* leaves and stems.

Peak No.	Compounds	Molecular formula	Molecular weight	Rt (min)	Relative Area%
1	*P*- Cresol	C_7H_8O	108	11.37	8.44
2	Unknown	---	---	13.64	0.11
3	Unknown	---	---	14.15	0.16
4	N-Decane	$C_{10}H_{22}$	142	14.41	0.39
5	Unknown	---	---	15.07	0.31
6	Unknown	---	---	15.62	0.05
7	α- Citronellol	$C_{10}H_{20}O$	156	16.02	0.09
8	Cis- Geraniol	$C_{10}H_{18}O$	154	16.75	0.03
9	Unknown	---	---	17.01	0.06
10	Bibenzene	$C_{12}H_{10}$	154	20.67	0.04
11	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	$C_{11}H_{16}O_2$	180	24.67	0.46
12	4-(2,2,6-Trimethylcyclohexyl)-2-butanone	$C_{13}H_{24}O$	196	27.36	0.68
13	4-(2,6,6-Trimethyl-cyclohex-1-enyl)-butan-2-ol	$C_{13}H_{24}O$	196	30.27	0.32
14	Unknown	---	---	30.91	3.43
15	2-Acetyl-3-hydroxy-1,4-naphthoquinone	$C_{12}H_8O_4$	216	33.89	10.13
16	Phytol	$C_{20}H_{40}O$	296	36.43	18.82
17	Tetradecyl oxirane	$C_{16}H_{32}O$	240	37.07	14.16
18	3,7,11-Trimethyl-1-dodecanol	$C_{15}H_{32}O$	228	39.15	0.28
19	(*Z*)-9-Hexadecenal	$C_{16}H_{30}O$	238	40.24	0.57
20	Unknown	---	---	40.62	1.91
21	Octadecane	$C_{18}H_{38}$	254	42.89	0.67
22	Diisoctyl phthalate	$C_{24}H_{38}O_4$	390	43.60	7.32
23	Heptacosane	$C_{27}H_{56}$	380	45.90	1.27
24	3-Hydroxy-pregn-5-en-20-one	$C_{21}H_{32}O_2$	316	47.00	0.92
25	Nonacosane	$C_{29}H_{60}$	408	48.70	1.70
26	Octacosane	$C_{28}H_{58}$	394	51.35	1.36
27	Cholesterol	$C_{27}H_{46}O$	386	52.12	1.85
28	Stigmasterol	$C_{29}H_{48}O$	412	53.91	9.69
29	Unknown	---	---	54.84	6.00
30	β-Amyrin	$C_{30}H_{50}O$	426	55.65	3.64
31	Lupeol	$C_{30}H_{50}O$	426	56.49	5.14
Total identified hydrocarbons (acyclic and cyclic)					5.43
Total identified pregnane					0.92
Total identified steroids					11.54
Total identified triterpenes					8.78
Other total identified oxygenated compounds					61.3
Total unidentified compounds					12.03

4.2 Investigation of the saponifiable matter (Fatty acids)

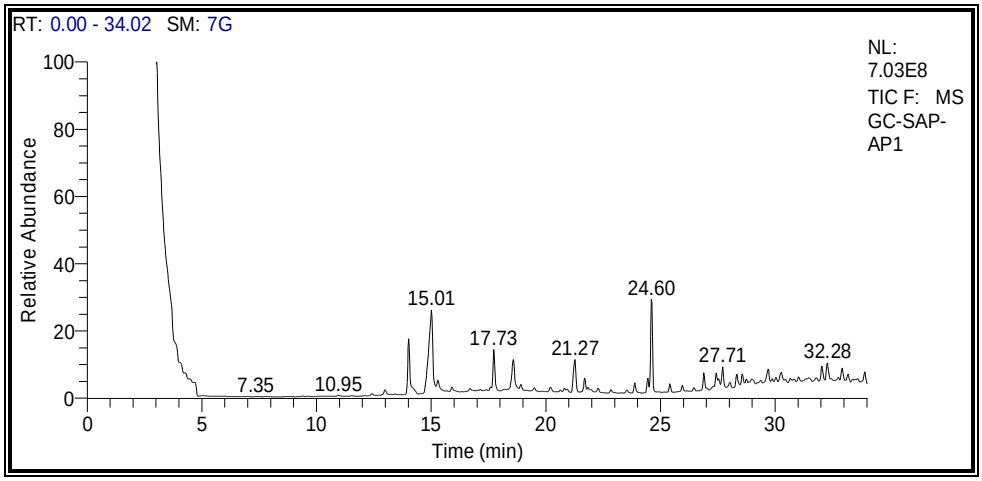

Fig 2: GLC chromatogram of the saponifiable matter of *P. lamerei* leaves and stems.

Table 2: Identification of the components of the saponifiable matter of *P. lamerei* leaves and stems.

Peak No.	Compounds	Molecular formula	Molecular weight	Rt (min)	Relative Area%
1	Unknown	---	---	12.98	1.03
2	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270	14.02	8.90
3	Unknown	---	---	15.01	28.17
4	Stearic acid methyl ester	$C_{19}H_{38}O_2$	298	17.73	5.86
5	Nonadecanoic acid methyl ester	$C_{20}H_{40}O_2$	312	18.59	9.77
6	Unknown	---	---	20.20	0.59
7	Unknown	---	---	20.79	0.73
8	4-Hydroxyoctadecanoic acid methyl ester	$C_{19}H_{38}O_3$	314	21.27	9.96
9	Arachidic acid methyl ester	$C_{21}H_{42}O_2$	326	23.88	1.25
10	Unknown	---	---	24.60	13.62
11	Behenic acid methyl ester	$C_{23}H_{46}O_2$	354	25.41	0.86
12	Tricosanoic acid methyl ester	$C_{24}H_{48}O_2$	368	25.96	0.79
13	Heptacosanoic acid methyl ester	$C_{28}H_{56}O_2$	424	26.89	1.69
14	Unknown	---	---	27.71	5.25
15	Montanic acid methyl ester	$C_{29}H_{58}O_2$	438	28.33	1.31
16	Unknown	---	---	28.57	2.80
17	Nonacosanoic acid methyl ester	$C_{30}H_{60}O_2$	452	29.69	1.88
18	Unknown	---	---	32.04	1.62
19	Unknown	---	---	32.28	2.38
20	Unknown	---	---	32.92	1.54
Total identified saturated long chain fatty acids					32.31%
Total identified hydroxylated fatty acids					9.96%
Total unidentified compounds					57.73%

The results of GC/MS analysis of the saponifiable matter of *P. lamerei* leaves and stems shown in Figure 2 and Table 2 revealed the presence of 20 compounds from which ten were identified as methyl esters of saturated fatty acids (32.31%) and hydroxylated fatty acids (9.96%) whereas ten fatty acids (57.73%) couldn't be identified.

4-Hydroxyoctadecanoic acid methyl ester was the major identified fatty acid methyl ester (9.96%) followed by nonadecanoic acid

methyl ester (9.77%), palmitic acid methyl ester (8.90%), stearic acid methyl ester (5.86%), nonacosanoic acid methyl ester (1.88%) and heptacosanoic acid methyl ester (1.69%) while the other identified fatty acids methyl esters were in minor amounts.

Identification of the fatty acid methyl esters was done by comparison of their retention times and pattern of fragmentation with those of reference compounds analyzed under the same conditions [14-19].

It is worthy mentioned that all the identified compounds in both unsaponifiable and saponifiable matters are first to be reported in genus *Pachypodium.*

5. Conclusion

The present study that includes the investigation of the unsaponifiable and saponifiable matters of *Pachypodium lamerei* Drake leaves and stems By GC/MS, could be helpful in authentication of plant. This is the first record for the GC/MS analysis of any of *Pachypodium* species. Other techniques could be employed to identify the unknown peaks in order to obtain authentic peaks of all the obtained compounds. Having a record of the authentic peaks could be helpful, especially for the constituents that are medically important.

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