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Phytochemical insvestigation of the lipoidal fraction of *Passiflora caerulea* L. grown in Egypt

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Abstract

Phytochmemical investigation of the petroleum ether extract of the leaves of *Passiflora caerulea* L. grown in Egypt revealed the presence of fatty acids, esters of fatty acids, sterols and hydrocarbons. GLC analysis of lipid fraction led to the identification of phytosterols (19.97%), hydrocarbons (75.39%), saturated (54.42%) and unsaturated (36.62%) fatty acids. Moreover, three esters of fatty acids were isolated by column chromatographic fractionation, and their structures were established based on spectroscopic data including EI-MS, ^{1}H NMR and ^{13}C NMR; they were identified as myristic acid ethyl ester (T_1), ethyl linoleate (T_2) and ethyl oleate (T_3).

Keywords

Passiflora caerulea
GLC analysis
Fatty acids
Sterols

1. Introduction

Passiflora caerulea L. is a member of family Passifloraceae. This family contains about 580 species in 12 genera, many of which are tendril climbers and shrubs or trees that occur naturally in tropical and warm temperate regions (Mabberley, 1997). *Passiflora caerulea* L. (blue Passion flower), which synonyms includes: Abu sab'at alwan, Zahrat es saah is native to South America (Brazil, Argentina and Paraguay) and introduced into Britain in 17th century, it is the most vigorous and tender species having traditional use of its fruit as a sedative and anxiolytic (Rendle, 1959; Hickey and King, 1988). The authors previously studied the biological activities and chemical composition of the polar fractions of the leaves (El-Askary et al., 2017). From the reported data, *Passiflora* species are rich in the fatty acid either saturated or unsaturated; it was

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reported that the passion fruit (*Passiflora edulis*) seed oils established the presence of saturated and unsaturated fatty acids as linoleic, oleic, palmitic, stearic and myristic acids (Steven et al., 2004). Reviewing the current literature about the non-polar fraction of *Passiflora caerulea* L. growing in Brazil, few data was found revealing that oleic and linoleic and palmatic were the major constituents (Nolasco et al.,1999) but nothing was traced concerning the non-polar fraction of the plant cultivated in Egypt. Therefore, this study was carried out dealing with the isolation and identification of the major compounds in the non-polar fraction of *Passiflora caerulea* L. grown in Egypt, where environmental conditions are comparatively different from those of Brazil.

2. Materials and Methods

2.1. Plant material

Samples of *Passiflora caerulea* L. leaves used in the study were collected during 2008-2010 from El-Orman Botanical Garden, Giza, Egypt and the identification of the plant material was kindly verified by Eng. Therese Labib, consultant of plant taxonomy at Ministry of Agriculture and the former director of El-Orman Botanical Garden, Giza, Egypt. A herbarium (No.21.09.13) is deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

2.2. General experimental procedures

^{1}H-NMR (400 MHz), ^{13}C-NMR (100 MHz) were recorded on Varian Mercury NMR spectrometer and Bruker NMR spectrometer respectively, at 25^{o}C using TMS as an internal standard and chemical shifts were given in δ values. Molecular ion was recorded on mass spectrometer, Varian Mat 711, Finnigan SSQ 7000. Column chromatography was performed using silica gel 60 (70–230 Mesh, Merck), TLC analyses were conducted on pre-coated silica gel 60 (0.2 mm thickness, Merck), chromatographic spray reagents were p-anisaldehyde (Stahl, 1969).

2.3. Study of lipoidal content

Air-dried leaves of *Passiflora caerulea* L. were extracted by light petroleum till exhaustion, the solvent was then evaporated under vacuum. Two grams of the residue were used for preparation of unsaponifiable matter and the separated fatty acids were methylated prior to GLC analysis (Finar, 1973).

Unsaponifiable matter (USM) and fatty acids methyl esters (FAME) were separately subjected to GLC analyses according to the conditions previously described (El-Sakhawy et al., 2016). Peak identification was performed by comparing the retention time (R_t) of each compound with those of standard material.

2.4. Column chromatography of petroleum ether extract

Six grams of petroleum ether extract were purified with charcoal giving 4.8 g of purified fraction that was fractionated on silica gel column (50 cm L × 3 cm D). Gradient elution was performed using *n*-hexane-$CHCl_3$ mixtures. Fractions were collected and monitored by TLC. Fractions with similar chromatographic pattern were pooled, evaporated under reduced pressure. Three oily compounds were obtained (T_1-T_3) by elution with 7, 10 and 12% $CHCl_3$ in hexane, respectively.

3. Results and discussion

The yield of light petroleum extract constituted 0.7% of the plant material. Unsaponifiable matter represented 43.8% while fatty acids fraction constituted 33.7%.

The results of GLC analysis of USM (**Table 1**), revealed the presence of identified compounds representing (95.37%). The identified hydrocarbons and sterols were found to represent 75.39%, 19.97% of USM, respectively. *n-Pentacosane* (16.90%) was the major identified hydrocarbon. *Stigmasterol* (15.54%) was the major identified sterol. No data revealed the previous isolation of *n-pentacosane* and *stigmasterol* from the *Passiflora* species.

Table 1: GLC analysis of unsaponifiable matter

Identified Compounds	RRt*	Area %
n-Tetradecane	0.5594	1.684
n -Hexadecane	0.5963	0.234
n-Heptadecane	0.6814	0.898
n -Octadecane	0.6912	10.878
n -Nonadecane	0.7269	0.502
n -Eicosane	0.7702	2.982
n -Heneicosane	0.8123	0.400
n -Docasane	0.8522	3.747
n -Tricosane	0.9287	11.052
n -Tetracosane	0.9674	4.586
n-Pentacosane 25:0	1	16.906
n-Hexacnsane 26:0	1.007	1.723
n -Heptacosane27:0	1.0525	2.011
n-Octacosane 28:0	1.0661	4.574
n-Triacontane 30:0	1.1118	10.919
Stigmasterol	1.1391	15.541
B-sitosterol	1.1498	4.436
Hentriacontylic acid 31:0	1.1794	1.146
n-Doctriacontane 32:0	1.2048	1.151
Percentage of identified hydrocarbons	**75.39%**	
Percentage of identified sterols	**19.97%**	

*RRt: Retention time relative to *n*-Pentacosane (Rt= 38.817min)

While, the results of GLC analysis of FAME (**Table 2**) revealed the presence of at least 14 components, representing (91.042%). The percentage of identified saturated fatty acids (54.42%) was found to be higher than that of unsaturated ones (36.62%). Palmitic and stearic were the major identified saturated fatty acids (42.02% and 4.72%, respectively). Linoleic (21.85%) and oleic (7.72%) acids were the major identified unsaturated fatty acids. These findings were in accordance with a previous report revealing that oleic, linoleic and palmitic were the major in the *Passiflora* species (Nolasco et al., 1999; Sant'Anna et al., 2001).

Column chromatographic fractionation of the non-

Table 2: GLC analysis of fatty acids methyl esters

Identified fatty acids	RRt*	Area %
Lauric	0.126	5.326
Myristic	0.043	1.812
Palmitic	1	42.028
Palmitoleic	1.222	0.882
Stearic	1.506	4.723
Oleic	1.605	7.727
Linoleic	1.828	21.853
Linolenic	2.14	1.715
Gama-Linolenic	2.836	1.225
Arachidic	3.191	0.533
Arachidonic	3.548	0.695
Eicosapentaenoic acid (EPA)	4.234	0.585
Clupanodonic acid (DPA)	4.589	1.938
Saturated fatty acids %	54.42%	
Unsaturated fatty acids %	36.62%	

*RRt: Retention time relative to Palmitic acid (Rt =2.81 min).

polar extract led to the isolation of three compounds (T_1-T_3). Their structures (**Figure 1**) were elucidated by means of spectral data, *viz* EIMS, ^{1}H and ^{13}C NMR. Compound T_1 **(**19 mg, yellow oil) soluble in hexane and chloroform, its mass spectrum showed a molecular ion peak of 256 corresponding to a molecular formula $C_{16}H_{32}O_2$. The ^{1}H- NMR showed a triplet at 0.8 (J= 7Hz) that was assigned to CH_3 -14 representing vicinal coupling with an adjacent methylene group. The crowded multiplets at δ_H 1.11-1.20 were suggestive of a long carbon chain of methylene groups. A triplet at 2.28 (J =7 Hz) that is assigned to CH_2-2 indicated the attachment of this methylene group to a carbonyl function while the quartet at δ_H 4.11 (J =7 Hz) that was assigned to CH_2-1', was for a direct attachment to an oxygen atom. The ^{1}H- NMR is typical for a saturated acid ethyl ester and identified as myristic acid ethyl ester. The ^{13}C-NMR revealed the presence of 16 carbon including one methyl assigned at δ_c 14.3 ppm, methylene at δ_c 22.8-34.5 and one oxygenated carbon at δ_c 60.2 in addition to an ester carbonyl carbon at δ_c 174. T_1 was then identified as myristic acid ethyl ester (Richard et al., 2001).

Compound T_2 (18 mg, yellow oil) soluble in hexane and chloroform, its mass spectrum showed a molecular ion peak of 308, corresponding to a molecular formula $C_{20}H_{36}O_2$. The ^{1}H- NMR showed proton signals indicating unsaturated linear fatty acid: δ_H 0.87 ppm (3H, *t, J* = 6.9 Hz) for one terminal primary methyl proton, δ_H 0.89 (3H, *t, J* =7.6 Hz) for the methyl proton of the ethyl group, 1.22-1.29 (17H, *m*), 2.27 (2H, *t, J* =7 Hz) for straight hydrocarbon chain, δ_H 4.11 (2H, *q, J* =7.6) for ethoxy group and δ_H 5.3 (4H, *m*) for olefinic protons. The ^{13}C-NMR revealed the presence of 20 carbons including one methyl assigned at δ_c 14.3 ppm, methylene at δ_c 22.8-34.5 and one oxygenated carbon at δ_c 60.2 in addition to an ester carbonyl carbon at δ_c 174, and 4 olefinic carbons at 130.3, 130.3, 128.3 and 128.1. The data of T_2 was similar to those reported for ethyl linoleate (Adosraku et al., 1993; Richard et al., 2001).

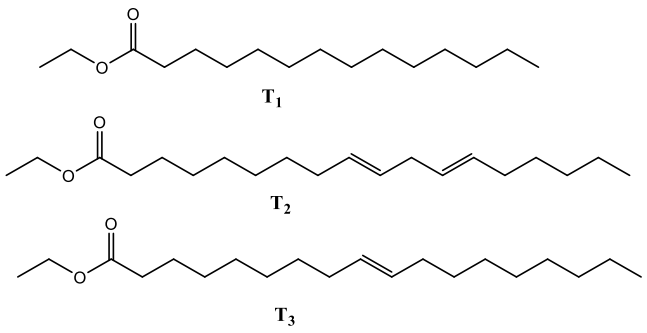

Figure 1: Structures of compounds T_1-T_3

Compound T_3 (18 mg, colorless oil) soluble in hexane and chloroform, its mass spectrum showed a molecular ion peak of 311, corresponding to a molecular formula $C_{20}H_{38}O_2$. The ^{1}H- NMR showed proton signals indicating unsaturated linear fatty acid: δ_H 0.87 ppm (3H, *t, J* =6.9 Hz) for one terminal primary methyl proton, δ_H 0.82 ppm (3H, *t, J* =7 Hz) for the terminal methyl group attached of the ethyl ester, δ 0.87-1.21ppm (30H, *m.*) for straight hydrocarbon chain, δ_H 4.11 ppm (2H, *q, J* =10), for ethoxy group and δ_H 5.4 ppm (2H, *m.*) for olefinic protons. The ^{13}C-NMR revealed the presence of 20 carbons including one methyl assigned at δ_c 14.3 ppm, methylene at δ_c 29.2-31 ppm, one oxygenated carbon at δ_c 60.2 ppm in addition to an ester carbonyl carbon at δ_c 174 ppm and 2 olefinic carbons at 140.49 ppm and 123.08 ppm. Then from spectral data results

and reported data (Choundhury et al., 1994; Siddiqui et al., 2004) the compound T_3 was identified as ethyl oleate.

4. Conclusion

Three fatty acid esters were isolated from the non-polar extract of the leaves of *Passiflora caerulea* L. Their corresponding fatty acids were previously reported to be present in the *Passiflora* species (Nolasco et al., 1999; Steven et al., 2005).

5. Conflict of interest

The authors report no declaration of conflict of interest.

6. Acknowledgements

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7. References

Adosraku, R. K., Anderson M. M., Anderson, G. J., Choi, C., Croft, S. L., Yardley, V., Phillipson, J. D., Gibbons, W. A., 1993. Proton NMR Lipid profile of leisshmania donovani promastigotes. Mol. Biochem. parasitol. 62, 251-262.

A.O.A.C., 2000. Official Methods of Analysis of Association of Official Analytical Chemist. Washington, D.C.

Choudhury, S. R., Traquair, J. A., Jarvis, W. R., 1994. New extracellular fatty acids in culture filtrates of Sporothrix flocculosa and S.rugulosa. J. Nat. Prod. 57, 700-704.

El-Askary, H. I, Haggag, M. Y., Abou-Hussein, D. R., Hussein, S. M., Sleem, A. A., 2017. Bioactivity-guided Study of Passiflora caerulea L. Leaf Extracts. IJPR 15, 46-57.

E-Sakhawy, F., Kassem, H., Abou-Hussein, D., El-Gayed, S., Mostafa, M., Ahmed, R., 2016. Phytochemical investigation of the bioactive extracts of the leaves of Ficus cyathistipula Warb. Z. Naturforsch. 71(5-6) c, 141–154.

Finar, I. L., 1973. Organic Chemistry, 6th ed., Longman group Ltd., England, 445.

Hickey, M., King, C., 1988. 100 Families of Flowering Plants. Cambridge University Press, Cambridge, 130–133.

Mabberley, D. J., 1997. The plant-book: A portable dictionary of the vascular plants. Second edition.Cambridge University Press.

Nolasco, S. M., Quriroga, O. E., Wiese, B., Vigo, M. S., 1999.Chemical study of medicinal plant seeds. Acta-Horticulturae. 501, 209-217.

Rendle, A. B., 1959. Classification of Flowering Plants. Cambridge University Press, Cambridge, 211–213.

Richard, C. L., Xiaoyang D., Steven, C., Kishan, R., Laurie, E. E., 2001. Preparation of Conjugated Soybean Oil and other Natural Oils and Fatty Acids by Homogeneous Transition Metal Catalysis. JAOCS. 78, 447–453.

Sant'Anna, E. S., Torres, R.C.O., Porto, A.C.S., 2001. Evaluation of different chromatographic methods to determine fatty acids in seeds of passion flower. Boletim-do-centro-de-Pesquisa-e-Processamento-de-Alimentos. 19(1), 85-94.

Siddiqui, B. S., Rasheed, M., Ilyas F., Gulzar, T., Tariq, R. M., Naqvi, S. N., 2004. Analysis of Insecticidal *Azadirachta indica* A. Juss. Fractions. Z. Naturforsch. 59 (1-2), 104-112.

Steven, A. N., Bernd, C., Wolfgang, S., 2005. Comparative Study of Fatty Acid Profiles of Passiflora Seed Oils from Uganda. JAOCS. 82, 41–44.

Yamamoto, H., Shimokawa, H., Haga, T., Fukui, Y., Iguchi, K., Unno, K., Hoshino, M., Takeda, A., 2014. The expression of relaxin-3 in adipose tissue and its effects on adipogenesis. Protein Pept. Lett. 21, 517-522.