Current Science International
ISSN: 2077-4435

Volume : 04 | Issue : 04 | Oct.- Dec. 2015
Pages: 677-683

Primary Metabolites and Saponin Constituents of *Taverniera lappacea* (Forssk.) DC.

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ABSTRACT

Investigations of the primary metabolites of *Taverniera lappacea* aerial parts revealed that, the total carbohydrates, total nitrogen, protein and total lipids, percentages were 7.13%, 1.46%, 9.14% and 2.42%, respectively. Meanwhile, investigation of free and combined sugars using HPLC instrument revealed the detections of 7 free sugars and 10 combined sugars. While the analysis of the amino acids using amino acid analyzer revealed the presence of 28 free amino acids and 17 protein amino acids. GLC analysis of the fatty acids, revealed the presence of 8 saturated fatty acids and 2 unsaturated fatty acids. Concerning Saponin contents, investigation of the plants using HPLC and LC-MS revealed the detections of 28- methyl serratagenate 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside, 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol, 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucuronopyranosyl-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol and one compound diterpne (Coronarin A).

Key words: *Taverniera lappacea*, *Fabaceae*, primary metabolites and saponins.

Introduction

Taverniera lappacea (Forssk.) Syn. *Hedsarum lappaceum* (Forssk.) belong to family *Fabaceae*. The genus of *Taverniera* in Egypt includes two species (*Taverniera lappacea* and *Taverniera aegyptiaca*) (Boulos, 1995). *Taverniera lappacea* is a perennial shrub with short branches, overall densely pubescent. Calyx densely thick-publescent, Corolla yellow. Vexillum 5-6 mm long or oven less. Fruit 1-2-seeded lomentum, l seeded part 6-7 mm wide, densely echinulate, fruit appendages profusely hairy (Tâckholm, 1974). Preliminary phytochemical screening of *Taverniera lappacea* revealed that it contained steroids, terpenoids, saponins, coumarins, flavonoids and phenolics and glycosides and/or carbohydrates, where ten flavonoid compounds, two phenolic acids and scopoletin as coumarin compound were separated and identified from the plant (Abd El-Moaty and Balah, 2009). Ayaz *et al*. (2007) reports the composition of carob pods (*Ceratonia siliqua* L. *Fabaceae*) sampled has sucrose (437.3 mg/g dry weight), glucose (395.8 mg/g dry weight) and fructose (42.3 mg/g dry weight) were the major sugars identified and quantified in the fruit. By tracing the UV triplet of heteroannular dienes.

Three novel saiko-saponins were isolated from the root bark of *Taverniera aegyptiaca.* They were identified as 22 β-hydroxyolean-l l, I 3 (18)-dien-3 β- yl- β –D- glucopyranoside. 1β, 22 β -dihydroxyolean- l 1, I3 (I8)-dien-3β- yl- β -D-glucopyranoside and 1β,22β-dihydroxyolcan-l 1,I3(18)-dien-3β-yl-D-xylopyranosyl(1→ 2) β- D-glucopyranoside on the basis of chemical and spectral evidences (Hassanean,1998). Ibraheim *et al.* (2003) isolated six new triterpenoidal saponins of oleanane type from *Taverniera aegyptiaca.* The primary metabolites and saponin constituents of *Taverniera lappacea* need more attentions for their essential values for the plant duration live, beside it maybe valuable for humans, hence our attentions turns toward investigations of this ingredients.

Materials and Methods

Plant material:

Taverniera lappacea aerial parts were collected from Ras Mohamed protected area, South Sinai, Egypt at spring season (2011). Samples were identified (Tâckholm, 1974) and deposited with the plant protection collection at the Desert Research Center at Cairo, Egypt. The aerial parts of *Taverniera lappacea* were cleaned, dried in an oven at 40°C, ground to fine powder and for different analysis.

Determination of certain pharmacopial constants:

Determination of percentages of inorganic (Ash) and organic matter (Brower and Zar,1984).

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Fig. 1: *Taverniera lappacea* at Ras Mohamed **(**South Sinai**)**

Metabolic products:

Carbohydrates content: Determination of total carbohydrates (Chaplin and Kennedy, 1994):

Identification of free sugars and combined sugars: by HPLC according to Zielinski *et al.* (2014).

Nitrogen content: Determination of total nitrogen:using Kjeldahl method (James, 1995)

Determination of total protein: (James, 1995)

Investigation of free and protein-amino acids: According to Pellet and Young (1980), using Amino Acid Analyzer.

Lipids content: Determination of total lipids: Farag *et al.* (1986).

Determination of saponifiable matter (fatty acids): Their components were determined using GLC according to Farag *et al.* (1986). .

Investigations of saponin constituents:

Sample preparation for analysis:

Two grams for each of the aerial parts of *Taverniera lappacea* and *Taverniera aegyptiaca* were used for separation of their saponin compounds according to Ibraheim *et al*. (2003). They were extracted in conical flask with 70 mL of ethanol 70% in static mode with a reciprocal water bath incubator for 7 hrs at 45°C, where during extraction, the contents of the flask were shaken gently for 1 min twice per an hour. After setting at room temperature for 17 hrs, the contents were filtered through filter paper, where the flask residue was washed with small portions of ethanol70% and again filtered. The final volume of the combined filtrate was adjusted to 100 mL. It was transferred into a round bottomed flask and concentrated under reduced pressure with a rotary evaporator. The concentrate was then dissolved in 10mL of methanol (HPLC-grade). One milliliter of the resulting solution was filtered through a 0.45-µm PVDF syringe filter (Gelman, Ann Arbor, MI) **(**Park *et al*., 2000) and used as the sample for HPLC and LC-MS analysis.

HPLC analysis of saponin compounds:

The analytical HPLC (Aglient 1100) diode array detector system (203 λ), the column used was an Inertsil ODS-3 C18 column (30 cm× 3.9 mm) with temperature set at 35ºC. The flow rate of 1.0 mL/min and detection wavelength of 203 nm with a gradient elution of acetonitrile–water from 40:60 (v/v) to 50:50 (v/v).

LC-MS analysis of saponin compounds: LC Quaternary pump,1290 infinity

*Auto sampler:*1290 infinity.

TCC (column compartment): 1290 TCC with temperature set at 35ºC.

Detector: 6420 Triple quad.

Column: Acquity UPLC BEH shield RP18 1.7 µm, 2.1 X 150 mm.

Mobil phase program: The flow rate of 0.35 ml/min with a gradient elution of acetonitrile–water from 40:60 (v/v) to 60:40 (v/v).

Detector parameters: ESI MS QQQ Mass spectrorometer with (+) mode.

*Gas parameters (ºC):*350.

Gas flow:(1/min): 9

*Nebulizer (Psi):*35

*Capillary (V):*5000

Results and Discussion

Determination of certain pharmacopial constants:

Inorganic and organic matter:

The percentage of total ash content of the aerial parts of *Taverniera lappacea* was 3.63%, while the percentage of organic matter was 96.37%.

Metabolic products:

Carbohydrates content: The percentage of total carbohydrates was 7.13%.

Investigation of free sugars:

The separation of the free sugars contents achieved using High Pressure Liquid Chromatography (HPLC), where the following sugars were obtained; inulin, glucose, maltose, glucuronic, xylose, galacturonic and stachyose as free sugars. The highest concentrations of the separated free sugars was inulin (40.31%) (Table 1).

Investigation of combined sugars:

The separation of the hydrolyzed combined sugars were achieved using HPLC, where the following sugars were obtained; xylose, maltose, fructose, inulin, sorbitol, ribose, glucose, glucuronic, glacturonic and stachyose as combined sugars. Where the concentration of xylose (3.01%) was the highest percentage of the separated sugars at Table (1).

Table 1: Relative percentage of free and combined sugars

Sugars	Free sugar (%)	Combined sugar (%)
Inulin	40.31	0.59
Glucuronic	1.09	0.27
Stachyose	0.06	0.08
Galacturonic	0.08	0.09
Maltose	1.34	1.73
Glucose	2.61	0.12
Xylose	0.68	3.01
Fructose	-	0.76
Sorbitol	-	0.38
Ribose	-	0.27

Nitrogen content

Total nitrogen and protein contents:

The percentage of total nitrogen was 1.46%, while the percentages of total protein was 9.14%.

Investigation of free amino acids:

The separation of free amino acids when achieved using amino acid analyzer, twenty nine free amino acids were detected, where the highest percentages of the separated free amino acids concentration were ornithine (22.5%), histidine (13. 6%) and lysine (12.6) (Table 2).

Table 2: Relative percentage of free and protein amino acids.

No.	Compound name	Free amino acids (%)	Protein amino acids (%)
1	Phospho-serine	2.0	-
2	Taurine	1.0	-
3	Phosphoethanolamine	0.8	-
4	Urea	0.7	-
5	Threonine	0.3	5.4
6	Serine	1.9	6.3
7	Asparagine	0.1	14.8
8	Glutamine	0.2	11.4
9	Glycine	0.4	6.3
10	Alanine	0.9	6.8
11	Valine	1.1	6.2
12	Methionene	-	0.1
13	Cystine	4.2	0.3
14	Isoleucine	0.3	4.9
15	Leucine	0.2	8.6
16	Tyrosine	1.2	1.9
17	Phenylalanine	0.2	4.5
18	b-Alanine	1.9	-
19	b-Aminobutyric acid	0.4	-
20	g-Aminobutyric acid	0.6	-
21	Ammonia	12.5	9.0
22	Ornithine	22.5	-
23	Lysine	12.6	5.6
24	3-Methylhistidine	5.3	-
25	Histidine	13.6	4.4
26	1-Methylhistidine	4.2	-
27	Tryptophan	4.5	-
28	Carnosine	1.5	-
29	Argenine	4.9	3.5

Investigation of protein amino acids:

The investigation of hydrolyzed protein-amino acids when achieved using amino acid analyzer, seventeen protein-amino acids of different types were detected. The concentration of Asparagine (14.8%) and glutamine (11.4%) were the highest percentages of the separated protein amino acids in the plant (Table 2).

Lipids content:

Total lipids content:

The percentages of total lipids of the aerial parts of *Taverniera lappacea* was 2.42%.

Investigation of saponifiable matter (fatty acids):

The fatty acid contents of the lipids were determined using GLC technique, where the obtained results revealed the presence of eight saturated fatty acids and two unsaturated fatty acids with different range of concentrations. Lauric acid (51.7 %) was the highest percentage of fatty acids, while Pentadecylic acid was the lowest percentage (0.1 %) in the plant (Table 3).

Investigations of saponin constituents:

HPLC analysis of saponin compounds:

HPLC analysis of the aerial parts of *Taverniera lappacea* when compared with HPLC analysis of the aerial parts of *Taverniera aegyptiaca* [(as reference) because the authentic sample wasn´t available] declared the appearance of three peaks in *Taverniera lappacea* at retention times (2.462, 3.020 and 4.725) (Fig. 2) similar to that presented peaks of *Taverniera aegyptiaca* at retention times (2.539, 2.960 and 4.999) (Fig. 3). Therefore the identification of these compounds was completed by using LC-MS.

Table 3: Relative percentage of fatty acids.

Compound name	No. of carbon atom	fatty acids (%)
Capric acid	C10:0	1.2
Undecylic acid	C11:0	0.2
Lauric acid	C12:0	51.7
Myristic acid	C14:0	1.1
Pentadecylic acid	C15:0	0.1
Palmitic acid	C16:0	38.3
Margaric acid	C17:0	1.3
Stearic acid	C18:0	3.8
Oleic acid	C18:1	1.4
Linoleic acid	C18:2	0.9

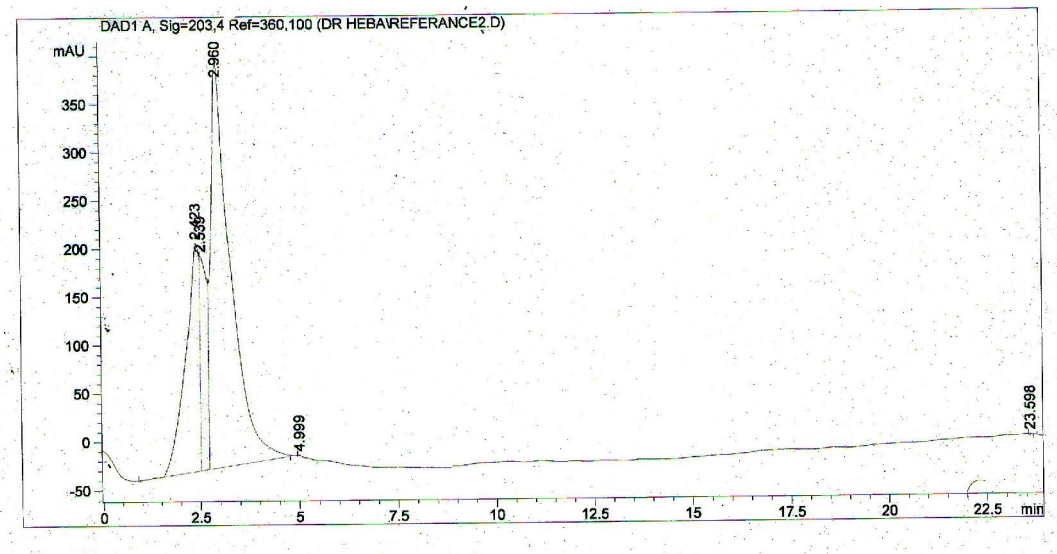


Fig. 2: HPLC of the saponin compounds of *Taverniera aegyptiaca*.

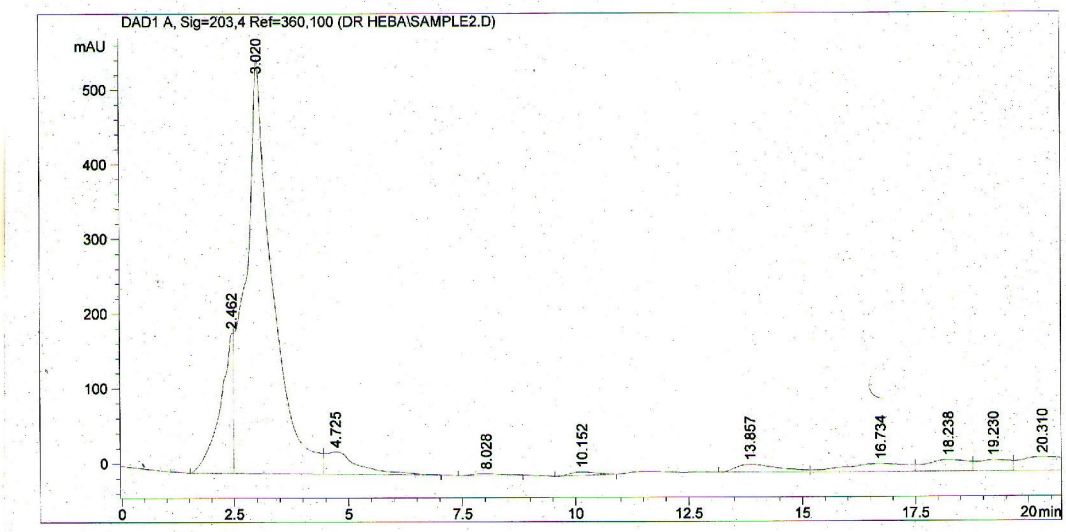


Fig. 3: HPLC of the saponin compounds of *Taverniera lappacea*.

LC-MS analysis of saponin compounds:

LC-MS analysis of the aerial parts of *Taverniera lappacea* declared the presence of four compounds

Compound No. (1):

LC-MS of the separated compound at (+ mode) indicated the molecular ion peak (M^+) of the compound was m/z 808 $(M+H)^+$ and other fragment 662 $[(M+H)$-methyl pentose$]^+$, 499 $[(M+H)$-methyl pentose hexose$]^+$ and 292 $[(M+H)$- 517$]^+$. This data indicted the compound was 28- methyl serratagenate 3-β-*O*-α-

rhmnopyranosyl (1→2)-β-glucopyranoside, where its spectral data are similar to those reported (Ibraheim *et al*., 2003 and Mangalorkar, 2013).

Compound No. (2):

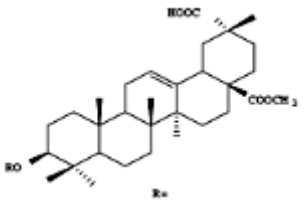
LC-MS of the separated compound at (+ mode) indicated the molecular ion peak (M^+) of the compound was m/z 764 $(M+H)^+$ and other fragment 618 [(M+H)-methyl pentose]$^+$ and 497 [(M+H)-methyl pentose hexose]$^+$.This data indicted the compound was 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol. where its spectral data are similar to those reported (Ibraheim *et al*., 2003 and Mangalorkar, 2013).

Compound No. (3):

LC-MS of the separated compound at (+ mode) indicated the molecular ion peak (M^+) of the compound was m/z 301 $(M+H)^+$. This data indicted the compound was diterpene compound (Coronarin A), where its spectral data are similar to those reported (Mangalorkar, 2013).

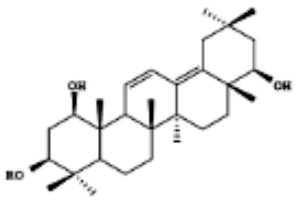
Compound No. (4):

LC-MS of the separated compound at (+ mode) indicated the molecular ion peak (M^+) of the compound was m/z 790 $(M+H)^+$ and other fragment 646 [(M+H)-methyl pentose]$^+$ and 443 [(M+H)-methyl pentose - GlcA]$^+$.This data indicted the compound was 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucuronopyranosyl-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol. where its spectral data are similar to those reported (Ibraheim *et al*., 2003 and Mangalorkar, 2013).



Compound No. (1):

28- methyl serratagenate 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside. [Rhamnose (1→2) glucose]

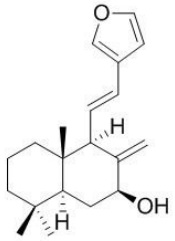


Compound No. (2):

3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol. [Rhamnose (1→2) glucose]

Compound No. (4):

3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucuronopyranosyl-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol. [Rhamnose (1→2) methylglucuronate]



Compound No. (3): Coronarin A

Conclusion

Taverniera lappacea aerial parts contained carbohydrates, nitrogen, protein and lipids with percentages of 7.13%, 1.46%, 9.14% and 2.42%, respectively. Meanwhile 7 free sugars and 10 combined sugars were detected, where 28 free amino acids and 17 protein amino acids were detected.

On other hand *Taverniera lappacea* aerial parts contained 8 saturated fatty acids and 2 unstaurated fatty acids. The plant contains the following saponins, 28- methyl serratagenate 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside, 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucopyranoside-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol, 3-β-*O*-α- rhmnopyranosyl (1→2)-β-glucuronopyranosyl-olean, 11, 13 (18)- dien-1β, 3β, 22β-triol and one compound diterpne (Coronarin A), which rate the economical values of *Taverniera lappacea* for humans.

References

Abd El-Moaty, H.I., H.I. and M.A. Balah, 2009. Phytochemical investigations of *Taverniera lappacea* forssk. and its activity as herbicides. Journal of Applied Sciences Research, 5(12): 2563-2573.

Ayaz, F.A., H. Torun, S. Ayaz, P.J. Correia, M. Alaiz, J. Gruz and M. Strnad, 2007. Determination of chemical composition of Anatolian carob pod (*Ceratonia siliqua* L.):sugars. Amino and organic acids, minerals and phenolic compounds. Journal of Food Quality, 30(6): 1040-1055.

Boulos, L., 1995. Flora of Egypt Checklist. Al Hadara publishing. Cairo, Egypt, p: 69.

Brower, J.E., J.H. Zar, 1984. Field and Laboratory Methods for General Ecology. Wm. C.Brown Publishers Dubuque, Iowa, pp: 226.

Chaplin, M.F. and J.F. Kennedy, 1994. Carbohydrate Analysis . a Practical Approach. Oxford University Press, Oxford, New York, Tokyo, 2nd Ed, pp: 324.

Farag, R.S., A.I. Ahmed, S.E. Rashad and M.W. Ewies, 1986. Unsaponifiable matter of six pollens collected by honey bees in Egypt. *J. Agric. Res*., 19(4): 52-58.

Hassanean, A.H., 1998. Salkosapnins from *Taverniera Egyptiaca.* Phytochemistry, 48 (4): 693-697**.**

Ibraheim, Z.Z., H.A. Hassanean and D.W. Bishay, 2003. Further saponins from *Taverniera aegyptiaca*. Phytochemistry, 62: 1201-1205.

James, C.S., 1995. Analytical Chemistry of Foods. Blackie Academic and Professional Publisher, an imprint of Chapman and Hall, pp: 178.

Mangalorkar, P.S., 2013. Bioprospecting the potential of *Taverniera cuneifolia* (Roth)Ali. Ph.D. Thesis, Fac. Sci. The Maharaja Sayajirao Univ., Baroda, pp: 244.

Park, I., E.M. Kang and N. Kim, 2000. High-Performance Liquid Chromatographic Analysis of Saponin Compounds in *Bupleurum falcatum*. Journal of Chromatographic Science, 38: 229-233.

Pellet, P.L. and V.R. Young, 1980. Nutritional Evaluation of Protein Foods. Published by the United Nations Univ., pp: 189.

Tâckholm, V., 1974. Students Flora of Egypt. 2nd Ed. Published by Cairo University, Printed by Cooperative printing compnay Beirat, pp: 888.

Zielinski, A.A.F., C.M. Braga, I.M. Demiate, F.L. Beltrame, A. Nogueria and G. Wosiacki, 2014. Development and optimization of a HPLC-RI method for the determination of major sugars in apple juice and evaluation of the effect of the ripening stage. Food Sci. Technology, 34(1): 38-43.