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THE *AEGLE MARMELLOS* LEAVES CATIONIC SALTS COMPOSITION AND PHYTOCHEMICAL SCREENING

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ABSTRACT

Haravey reported the *Aegle marmelos* leaves to possess cardiotoxic property, but not much is known about the chemical constituent responsible nor the mechanism involved; in this regard the present study is a preliminary attempt to identify some of the cationic salt constituents (sodium, potassium, calcium and magnesium) of the Aqueous and Alcoholic extracts involved in the regulation of cardiotoxic activity using Atomic Absorption Spectrophotometer, determination of phytochemicals and the extracts chemical analysis using HPTLC. Our study results showed variation in cationic salts composition in both the extracts but in the same order Ca>Mg>K>Na. The alcohol extract was positive for flavanoids, glycosides, tannins and saponins; while the aqueous extract was negative for flavanoids but positive for glycosides, alkaloids, tannins and saponins.

KEYWORDS

Aegle marmelos, Cardiotoxic, Cationic salts, Atomic Absorption Spectrophotometer and HPTLC.

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INTRODUCTION

Aegle marmelos a deciduous tree native to India belonging to family Rutaceae has its mention in writings dating back to 800 BC. Many medicinal properties are claimed to be possessed by the plant but the available scientific literature on the leaves of this plant is mostly on the isolation of chemical constituents like γ -sitosterol¹, Aegeline², Aegelenine³, Lupeol, sitosterol, Aegeline⁴, Rutin, marmesinin, β – sitosterol – B – D - glucoside⁵, Cinnamides⁶, and on anti-diabetic activities^{7,8}. Haravey⁹ reported the leaves to possess cardiotoxic property. The reported cardiotoxic activity in the

leaf extracts could not be due to the presence of a particular alkaloid or glycoside because the plant extract contains multifarious compounds and salts within them; in this regard the Aqueous and Alcoholic extracts cationic salts, which would have intimately involved in the regulation of cardiotoxic activity were estimated using Atomic absorption spectrophotometry, phytochemical screening and the chemical analysis of the extracts using HPTLC were carried out.

MATERIALS AND METHODS

MATERIALS

Collection and identification of the plant

Fresh leaves of *Aegle marmelos* collected locally from single source during the month of July were used for the study. Identification and certification of the leaves of *Aegle marmelos* was done by the taxonomist, Prof. R. Viswanathan at the Centre for Advanced Studies in botany, University of Madras, Chennai, India. A specimen sample was deposited for future reference. The allotted voucher number was CASB H-3. The leaves were washed, kept under shade and air dried.

METHODS

Preparation of the Aqueous and Alcohol extracts

Aqueous extraction was done using 500 g of leaf material soaked in deionized water in a 5 liters round bottom flask. After 24 h, this was decanted and filtered. The filtrate obtained was subjected to tannin removal procedure and concentrated on a water bath at 60°C. Yield of the extract obtained for 500 g of dry leaf powder was 12.5 g. Similar procedure using 95% was carried out for alcohol extraction were the yield of the extract obtained for 500 g of dry leaf powder was 8.3 g.

The concentrated crude extracts thus obtained were stored in refrigerator and they were used during the qualitative studies.

Phytochemical screening of the Aqueous and Alcohol extracts

The Extracts were subjected to qualitative phytochemical analysis as described by Harborne¹⁰.

Chemical Analysis - HPTLC

This was carried out to validate the authenticity of the plant material (leaves) for future studies. The alcohol extract and aqueous extract of the *Aegle marmelos* were subjected to get the individual chemo-profiles. For this precoated TLC plate of silica gel 60 F254 (E. Merck, India) of 0.2mm was used. On this plate 20 µL of both extracts (Alcohol and Aqueous) were spotted in the form of a band using Linomat IV Automatic Spotter (Camag, Switzerland). The spotted plates were kept in TLC chamber. The separated constituents were visualized using appropriate spraying reagents. Then the plates were scanned in CAMAG-TLC Scanner and the peaks were recorded at a wavelength of 550 nm. The R_f values and % of separated compounds were determined.

Elemental analysis in the Aqueous and Alcohol extracts – by Atomic Absorption Spectrophotometry (AAS)

The extracts were subjected to acid digestion prescribed for elemental analysis according to the procedure of Cid and Yebra¹¹. The digested material was analysed by atomic absorption spectrophotometry (AAS: GBC 932 plus Australia) at Indian Institute of Technology (IIT), Chennai.

RESULTS

Phytochemical analysis of the Aqueous and Alcohol extracts

The alcohol extract was found to contain flavonoids, glycosides, tannins and saponins while the aqueous extract contained glycosides, alkaloids, tannins and saponins. Thus flavonoids have been found to be preferentially extracted in the aqueous extract whereas alkaloids have to been found to be present only in the alcohol extract. Glycosides, tannins and saponins have been found to be present in both the extracts (Table No.1).

Chemical Analysis - HPTLC

The alcohol extract was positive for rutin and lupeol (Figure No.1 (a and b)).

Elemental analysis in aqueous extract and Alcohol extract by Atomic absorption spectrophotometry (AAS)

Elemental analysis of the extracts has shown that the Calcium content of aqueous extract is 23.4mg/gm and Alcohol extract is 6.23 mg/gm (Table No. 2).

DISCUSSION

The aqueous and alcohol leaf extracts of *Aegle marmelos* were used for the studies. An array of chemical constituents has been reported to be present in *Aegle marmelos* leaves. The chemical entities identified in the present investigation by qualitative chemical testing of the alcohol extract was found to contain flavonoids, glycosides, tannins and saponins; while the aqueous extract contained glycosides, alkaloids, tannins and saponins. Lupeol and rutin have been identified in alcohol extract by

HPTLC. The leaf extracts of *Aegle marmelos* claimed to possess cardiotoxic properties by haravey⁹ was estimated for their cationic salts using atomic absorption spectrophotometry (AAS) as they play a key role in electrical action potentials across cellular membranes. Cardiac cells rely on them for cardiac contraction (depolarization and repolarization), rhythm regulation and strength of heart contractions. The extracts were analyzed and the calcium content of aqueous extract was found to be approximately four times the quantity present in alcohol extract. Since the cardiotoxic effect largely depends on the cytosolic calcium, further studies are in line to find out whether the increased extracts calcium could have either augmented the phytoconstituents cardiotoxic activity or would have supplemented with calcium in producing the activity as seen in CaCl₂ supplementation by Ringer in 1889.

Table No.1: Phytochemical analysis of the Aqueous and Alcoholic extracts

S.No	Test	Test Methods	Aqueous extract test Results	Alcohol extract test Results
1	Test for flavonoids	1. Sodium hydroxide test 2. Ferric chloride test 3. Lead acetate test	--- --- ---	++ + ++
2	Tests for glycosides	1. Molisch test 2. Benedict's test 3. Fehling's test	+++ +++ ++	+ ++ +
3	Tests for alkaloids	1. Dragendorff's test 2. Wagner's test 3. Mayer's test	++ + ++	--- --- ---
4	Tests for tannins	1. Lead acetate test 2. Ferric chloride test	++ ++	+ +
5	Tests for saponins	1. Foam test 2. Libermann Test	++ +++	+ +

(+) Positive for qualitative tests (-) Negative for qualitative tests

Table No.2: Elements in the extracts estimated by Atomic Absorption Spectrophotometer

S.No	Elements	AqE /gm	AIE /gm
1	Calcium(µg)	23.4	6.23
2	Sodium(µg)	1.899	1.657
3	Potassium(µg)	23.93	25.22
4	Magnesium(µg)	0.280	0.127

AqE - Aqueous leaf extract, AIE – Alcohol leaf extract

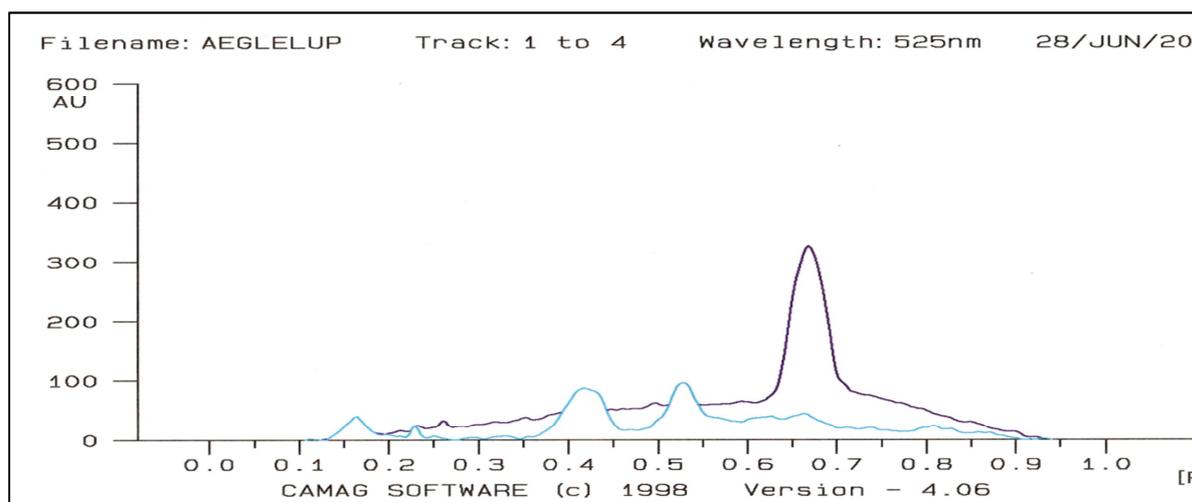


Figure No.1 (a): Comparative analysis of HPTLC spectrum of Alcohol extract of *Aegle marmelos* with Lupeol standard

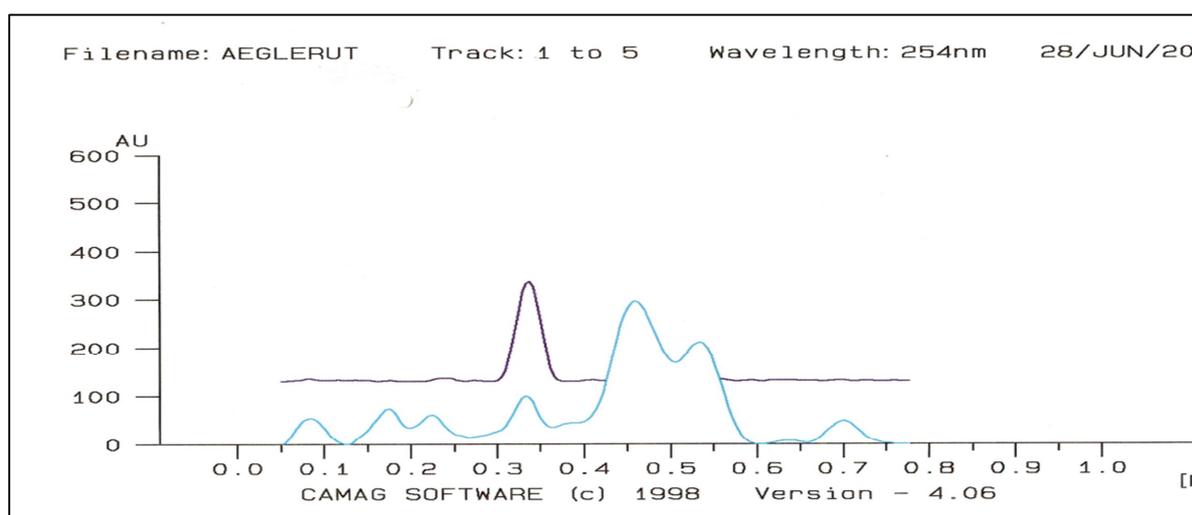


Figure No.1 (b): Comparative analysis of HPTLC spectrum of Alcohol extract of *Aegle marmelos* with Rutin standard

CONCLUSION

The evaluations conducted for the cationic constituents points to the presence of more calcium in both the extracts. Further studies are in line to understand the underlying mechanisms of the reported cardiotoxic activity in relation to the calcium.

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CONFLICT OF INTEREST

None declared.

BIBLIOGRAPHY

1. Chakravarti R N, Dasgupta B. β -sitosterol from the leaves of *Aegle marmelos* Correa, *J Indian Chem Soc*, 35(3), 1958, 194-6.
2. Chatterjee A, Bose S, Srimany S K. Studies on the constitution, stereochemistry, and synthesis of Aegeline, an Alkaloidal-Amide of *Aegle marmelos* Correa, *J Org Chem*, (24), 1959, 687-90.
3. Chatterjee A, Majumder R. Structure of Aegelenine, the minor alkaloid of *Aegle marmelos* Correa, *Indian J Chem*, (9), 1971, 763-66.
4. Patra A, Mukhopadhyay A K, Ghosh A, Mitra A K. Constituents of *Aegle marmelos*: Carbon-13 NMR spectra of Aurapten and Marmin, *Indian J Chem*, 17B, 1979, 385-87.
5. Sharma B R, Rattan R K, Perveen sharma. Constituents of leaves and fruits of *Aegle marmelos*, *Indian J Chem*, 19B, 1980, 162.
6. Govindachari T R, Premila M S. Some Alkaloids from *Aegle marmelos*, *Phytochem*, 22(3), 1983, 755-57.
7. Ponnachan P T, Paulose C S, Panikkar K R. Effect of leaf extract of *Aegle marmelose* in diabetic rats, *Indian J Exp Biol*, 31(4), 1993, 345-7.
8. Rao V V, Dwivedi S K, Swarup D, Sharma S R. Hypoglycaemic and antihyperglycaemic effects of *Aegle marmelos* leaves in rabbits, *Curr Sci*, 69(11), 1995, 932-33.
9. Haravey S K. A preliminary communication of the action of *Aegle marmelos* (Bael) on heart, *Indian J Med Res*, 56(3), 1968, 327-31.
10. Harbone J. In: *Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis*, Chapman Hall, London, 2nd edition, 1984, 288.
11. Moreno-Cid A, Yebra M C. Continuous ultrasound-assisted extraction coupled to a flow injection-flame atomic absorption spectrometric system for calcium determination in seafood samples, *Anal Bioanal Chem*, 379(1), 2004, 77-82.

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