Research Article

GC-MS profile and phytochemical analysis of methanol extract of *Atalantia racemosa* Wight ex Hook leaves

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Abstract

Objective: *Atalantia racemosa* belongs to the family Rutaceae, comprises of 11 species which are closely-related. The present study was mainly done to evaluate and identify the phyto-compounds present in the leaves of *Atalantia racemosa*. **Materials and Methods:** Phytochemical analysis and Thin layer chromatography proved to be a potent method for the presence of phyto-constituents. Also, the GC-MS analysis was performed in order to find out the active compounds in the extract which might be responsible for antioxidant, antibacterial activity. **Results:** Phytochemical investigation revealed the presence of phenols, flavonoids, steroids, terpenoids, etc. Toluene: Ethyl acetate: Methanol was preferred as the solvent system for the separation of compounds by chromatographic technique. The study revealed that *Atalantia racemosa* explored seven to eight bioactive compounds such as Beta-Asarone, Cis-Lanceol, Catechol from GC-MS analysis. **Conclusion:** The leaves of *Atalantia racemosa* could be considered as a potent antioxidant source against many free radicals, antibacterial activity. Presence of phenolic compounds, flavonoids might be responsible for antioxidant activity. Tannin-rich compounds would be definitely responsible for antibacterial activity.

***Keywords*:** Catechol, Flavonoids, GC-MS, Phenols, Steroids, Retention time, Thin layer chromatography

Introduction

The term "Pharmaceutical" means food product/by-product that provides good health benefits (Dhan Prakash et al., 2012). These products include micro and macro nutrients, dietary supplements, specific diets, herbal-based products, processed foods (Biesalski, 2001; Kalra, 2003). The good healthy benefits are mainly due to active ingredients (or) phytochemicals in case of medicinal plants. Phytochemicals are bioactive compounds and they are natural components that may have either defensive or disease protective property. When these phytochemicals are supplemented as intake, they provide healthier life. Phytochemicals when combined as polyherbal formulation have significant therapeutic potential against several diseases such as cancer, diabetes, high blood pressure, microbial infections, osteoporosis, etc. Various types of phytochemicals include phenols, flavonoids, saponins, tannins, alkaloids, terpenoids, steroids, glycosides, pigment producers, etc. Each phyto-constituent possess their own specific biological application.

Phenolic compounds have one or more aromatic rings with one hydroxyl group (Prakash et al., 2004). They are found to be rich in apple, pears, red wine, tea, coffee, etc. Examples of phenolic compounds include Catechin, Quercetin, Gallic acid, Chlorogenic acid, etc. Also, citrus-rich fruits are major source of flavones (Kris-Etherton et al., 2002; Nyamai et al., 2016; Piero et al., 2015). Polyphenols are soluble in water, intermolecular complexation and have antioxidant activities.Ferulic acid, have wide range of beneficial effects by preventing lipid peroxidation. They preserve cells integrity when exposed to alcoholic stress and thereby scavenge free radicals. Low molecular weight compounds are flavonoids with anti-hyperglycemic effect (Piero et al., 2015; Scalbert et al.,

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2005; Prakash and Kumar, 2011). Genistein, an isoflavone, when combined with cisplatin reduce proliferation of cancer cells. Epicatechin, an flavonoid compound facilitate invitro release of insulin through change of pro-insulin to insulin (Packer and Weber, 2001; Iwase et al., 2000; Muriithi et al., 2015). Tannin-rich compounds exhibit anti-diabetic activity, Ellagic acid and Resveratrol are known to inhibit skin tumorgenesis mice (Zhang et al., 2008; Cassidy et al., 2000).

The genus *Atalantia* (Family: Rutaceae) comprises nearly 11 species which are very closely related one. Decoction of leaves of *A. racemosa* is used in the treatment of bronchitis, asthma and cough. Leaf powder is used as blood purifier agent (Devanand L. Luthria et al., 1989). Leaves of *Atalantia racemosa* along with other herbal formulation (*Callicarpa lanata* L. and *Clerodendrum infortunatum* L.) is boiled in water and this water is used for bath to prevent repeating fever. The root paste with salt is given to treat allergy (Pullaiah, 2006). The Kurichia tribes are using the leaf juice of *Atalantia racemosa* Wight var. internally to treat acidity (Harsha et al., 2002). The aerial parts of *A. racemosa* were found to possess insect antifeedant activity (Shyma and Devi Prasad, 2013). The petroleum ether extract of the *A. racemosa* was found to contain terpene compound, friedlin and four coumarins identified as xanthyletin, luvangetin, rasemosin and xanthotoxin (Joshi et al., 1978). Therefore, in this study the methanolic extract of *A. racemosa* leaves were tested for phytochemical analysis, separation of compounds and also to identify the bioactive compounds present in the extract through GC-MS analysis.

Materials and methods

Preparation and extraction of *Atalantia racemosa* leaves

The leaves of *Atalantia racemosa* were carefully washed with tap water followed by rinsing in distilled water and air-dried at room temperature for few hours. Then leaves were separated and taken to separate clean place and dried at room temperature for one week. Then they were ground into fine powder and sieved through fine mesh, finally stored in cool and dry place in a clean air-tight container. In the extraction process, finely ground plant material was extracted with methanol in 1:10 ratio. The extract was filtered through the Whatmann No.1 filter paper in a separate container. The above process was repeated 3 times with the same plant material but using fresh solvent and condensation in a rotary evaporator was carried out at 40^0C-45^0C resulting in smooth, semi-viscous green coloured extract (Harborne, 1998; Trease and Evans, 1989).

Qualitative phytochemical analysis

Preliminary screening of secondary metabolites such as alkaloids, flavonoids, saponins, coumarins, anthraquinones, terpenoids, steroids and sterols were carried out according to the standardized phytochemical methods (Harborne, 1998). The different qualitative chemical tests were performed for establishing the profile of given extract for its chemical composition.

Determination of total Phenols content

Folin-Ciocalteau reagent method was used to determine the total phenolic compounds with slight modifications (Spanos and Wrosltad, 1990). One hundred µL of methanol extract of leaves of *Atalantia racemosa* (1 mg/mL) was mixed with 900 µL of distilled water and 1 mL of Folin-Ciocalteau reagent (1:10 diluted with distilled water). After 5 mins, 1 mL of sodium carbonate (20% w/v) solution was added. The mixture was then allowed to stand for 30 mins incubation in dark at room temperature. The absorbance was measured by UV-vis spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (GAE/mg of extract), which is a common reference compound.

Determination of total flavonoids

The total flavonoid content of methanol extract of leaves of *Atalantia racemosa* was determined using aluminium chloride reagent method with slight modifications (Liu et al., 2007). Five hundred µL of extract (1 mg/mL) was mixed with 0.5 mL of methanol and 0.5 mL of (5% w/v) sodium nitrite solution. Then, 0.5 mL (10% w/v) aluminium chloride solution was added followed by 1 mL of 1M sodium hydroxide. The mixture was incubated for 30 minutes at room temperature and the absorbance was measured by UV-vis spectrophotometer at 510 nm. The result was expressed as (QE/mg of extract) quercetin equivalent.

Separation of bioactive compounds by Thin Layer Chromatography

Thin layer chromatrography technique for separation of active compounds extracted from *Atalantia racemosa* was achieved (Stahl, 2005). Separation of phyto-constituents is based on the exact solvent system that is optimized clearly. The elution mainly takes based on solvent polarity system-from higher to lower. The sample was spotted on the silica gel plates and was run in the selected solvent system and then the dried plate was placed under UV-light for visualization of bands. The same dried plate was placed in a chamber containing a few crystals of iodine. The iodine vapour in the chamber oxidizes the substances in the various spots.

Identification of bioactive compounds by Gas chromatography-Mass spectrometry analysis

The presence of active compounds were been confirmed by thin layer chromatography and the compounds were identified using gas chromatography and mass spectrometry (GC-MS) method, (TSQ QUANTUM XLS).

The name of the instrument is Gas Chromatography-Mass Spectrometry and the instrument made is of Thermo scientific. The software required for analytical studies is XCALIBUR (ver-2.2) (Saraswathi et al., 2019). The column size is of TG-5MS (30mX0.25mmX0.25um). The injector temperature and interface temperature (°C) was at 280°C.

Results and discussion

Determination of phytochemicals

The phytochemical analysis of methanol extract of leaves of *Atalantia racemosa* showed the presence of tannins, glycosides, terpenoids, flavonoids and phenols in major amounts (Table 1) and was quantified. Quantitative analysis showed that the total phenolic content in the methanol extract of leaves of *Atalantia racemosa* is 114.80±0.34 GAE/mg, total flavonoids content is 50.03±0.19 QE/mg.

Separation of bioactive compounds by Thin Layer Chromatography

Methanolic extract of *Atalantia racemosa* was subjected to TLC in order to identify the bioactive compounds. The most appropriate TLC system for analysis was shown to be (Toluene: Ethyl acetate: Methanol (v/v)) in the ratio 2.5:1:0.5, in which the separation of compounds was most distinct and clear with Six bands 0.91, 0.71, 0.51, 0.48, 0.37 and 0.22 (Figure 1) under Ultra Violet light. The preliminary phytochemical screening of *A.racemosa* revealed the presence of phenolics and alkaloids in high amounts followed by saponins in trace. The chromatogram developed with methanol, ethyl acetate and chloroform in the ratio of 0.5:0.5:9 revealed the presence of seven major compounds at R_f value of 0.97, 0.89, 0.82, 0.79, 0.56, 0.35 and 0.13 as visualized under iodine vapour and UV illumination. The phytochemical profile revealed the presence of terpenoids, glycosides, phenols, flavonoids, etc showing identical results with Dhanalakshmi et al., 2013. Similar results were obtained for the methanol extract of leaves of *Atalantia racemosa*.

Table 1. Qualitative analysis of methanol extract of leaves of *Atalantia racemosa*

Phytochemicals	Results
Saponins-Foam test	---
Terpenoids-Salkowski test	+++
Glycosides-Legal's test	+++
Steroids-Libermann-Burchard test	+++
Flavonoids-Sodium hydroxide test	+++
Reducing Sugars-Fehling's test	+++
Alkaloids: (a) Mayer's test	
(b) Hager's test	---
Phenols-Ferric chloride test	+++
Tannins-Lead acetate test	+++
Proteins-Xanthoproteic test	---

Identification of bioactive compounds by Gas chromatography-Mass spectrometry analysis

Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST-011 library. The names of the components of the test materials were ascertained and mentioned in table 2 and figure 2. The GC-MS analysis for the tested plant extract indicated the presence of phyto-active compounds and the analysis study is used to identify volatile compounds, alcohols, hydrocarbons, etc.

Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid, n-Hexadecanoic acid was the major compounds identified from the methanolic extract of *A. racemosa* fruit by

Table 2. GC-MS profile of methanol extract of leaves of *Atalantia racemosa*

RT	Compounds Name	Peak area%	CAS#
8.617	Dihydroxyacetone	18.24	000096-26-4
12.055	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	17.37	028564-83-2
14.478	4(1H)-Quinolinone, octahydro-1-methyl-	3.51	1000320-16-7
15.962	Beta-Asarone	44.49	005273-86-9
16.193	Diethyl Phthalate	5.20	000084-66-2
16.249	Cis-Lanceol	3.65	010067-28-4
16.823	Beta-Asarone	2.74	005273-86-9
17.531	Catechol	4.80	000120-80-9

*RT-Retention Time

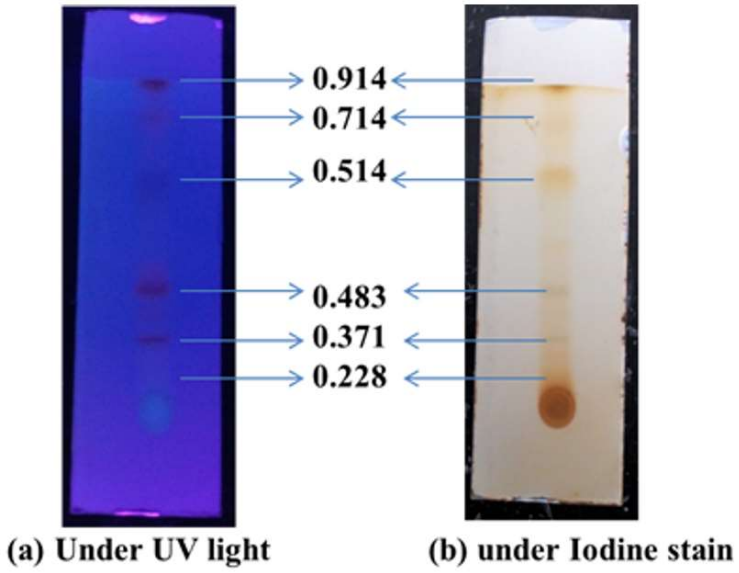

Figure 1. Visualization of bands of methanol extract of leaves of *Atalantia racemosa*

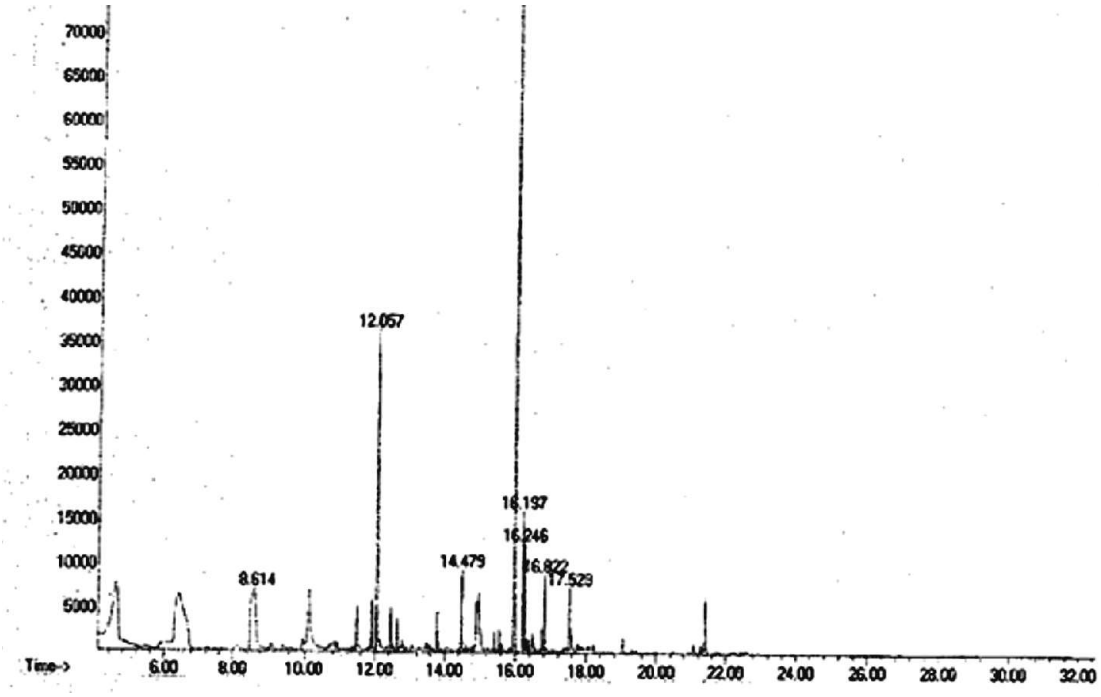

Figure 2. GC-MS Chromatogram of methanol extract of leaves of *Atalantia racemosa*

GC-MS analysis. Significant results were obtained for methanolic extract of *Atalantia racemosa* leaves in which seven to eight bioactive compounds such as Beta-Asarone, Cis-Lanceol, Catechol, etc were identified from GC-MS analysis. From the GC-MS profile of *Atalantia racemosa*, the compound of interest is Catechol (Molecular weight: 110.112 g/mol; Molecular formula: $C_6H_6O_2$), Cis-lanceol (Molecular weight: 220.356 g/mol; Molecular formula: $C_{15}H_{24}O$) having high phenolic and terpenoid compounds. Literature survey proves that these phyto-compounds are responsible for antibacterial, antioxidant activity against free radicals, anti-proliferative activity against Breast and Colon cancer (Das and Swamy, 2016; Holst and Williamson, 2008; Arika et al., 2015; Middleton et al., 2000).

Conclusion

From the results obtained in this study, it is evident that the leaves of *Atalantia racemosa* are effective with active compounds. Also, the chromatogram developed, suggests that six-seven major compounds are present in the leaf extract of *Atalantia racemosa* which could contribute to its antioxidant, antibacterial activity. These results reveal that the leaves of *Atalantia racemosa* could be a potential source of traditional medicine for several infections and diseases.

Further the research work shall be done to purify the exact compound by chromatographical methods and also the structure could be predicted using bioinformatics tools.

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References

Arika WM, Abdirahman YA, Mawia MM, Wambua KF, Nyamai DM. 2015. Hypoglycemic Effect of *Lippia javanica* in Alloxan Induced Diabetic Mice. Journal of Diabetes & Metabolism 6: 2.

Das AK, Swamy S. 2016. Antioxidant activity and determination of bioactive compounds by GC-MS in fruit methanol extracts-a comparative analysis of three *Atalantia* species from south India. Journal of Applied Pharmaceutical Science 6(02): 130-134.

Biesalski HK. 2001. Nutraceuticals: the link between nutrition and medicine. In: K. Kramer, PP. Hoppe and L. Packer eds. Nutraceuticals in health and disease prevention. New York: Marcel Deckker Inc. pp. 1-26.

Cassidy A, Hanley B, Lamuela-Raventos RM. 2000. Isoflavones, lignans and stilbenes-origins, metabolism and potential importance to human health. Journal of the Science of Food and Agriculture 80: 1044-1062.

Luthria DL, Ramakrishnan V, Verma GS, Prabhu BR, Banerji A. 1989. Insect Antifeedants from *Atalantia racemosa*. Journal of Agricultural and Food Chemistry 37: 1435-1437.

Prakash D, Gupta C, Sharma G. 2012. Importance of Phytochemicals in Nutraceuticals. Journal of Chinese Medicine Research and Development 1(3): 70-78.

Dhanalakshmi P, Jaya Prakash Priya A, Harini R, Sindhu S, Sagadevan E, Aroumougame S, Arumugam P. 2013. Investigation of Antibacterial Activity of *Atalantia racemosa* using Different Dye Assays. African Journal of Basic & Applied Sciences 5(4): 174-178.

Harborne JB. 1998. Phytochemical Methods, A guide to Modern Techniques of Plant analysis, second ed. Chapman and Hall, London, pp 54-84.

Harsha VH, Hebbar SS, Hedge GR, Shripathy V. 2002. Ethnomedical knowledge of the plants used by kunabi tribe of Karnataka, India. Fetoterapia 73: 281-287.

Holst B, Williamson G. 2008. Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. Current opinion in Biotechnology 19: 73-82.

Iwase Y, Takemura Y, Ju-ichi M, Ito C, Furukawa H. 2000. Inhibitory effect of flavonoids from Citrus plants on Epstein-Barr virus activation and two-stage carcinogenesis of skin tumors. Cancer Letters 154: 101-105.

Joshi BS, Gawad DH, Ravindranath KR. 1978. Chemical constituents of *Atalantia racemosa* Wt. and Arn. Structure and synthesis of racemosin, a novel pyranocoumarin. Proceedings of the Indian Academy of Sciences 87A: 173-179.

Kalra EK. Nutraceutical - Definition and Introduction. APS PharmSciTech 2003; 5, 1-2.

Kris-Etherton P, Hecker K, Bonanome A, Coval S, Binkoski A, Hilpert K. 2002. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. American Journal of Medicine 113: 71S–88S.

Liu X, Dong M, Chen X, Jiang M, Lv X, Yan G. 2007. Antioxidant activity and phenolics of endophytic *Xylaria* sp. from *Ginkgo biloba*. Food Chemistry 105: 548-554.

Middleton E, Kandaswami C, Theoharides TC. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological reviews 52: 673-751.

Muriithi NJ, Maina GS, Maina MB, Kiambi MJ, Juma KK. 2015. Determination of Hematological Effects of Methanolic Leaf Extract of *Vernonia lasiopus* in Normal Mice. Journal of Blood &Lymph 5: 139.

Nyamai DW, Arika W, Ogola PE, Njagi ENM, Ngugi MP. 2016. Medicinally Important Phytochemicals: An Untapped Research Avenue; Research and Reviews. Journal of Pharmacognosy and Phytochemistry 2321-6182.

Packer L, Weber SU. 2001. The role of vitamin E in the emerging field of nutraceuticals. Marcel Dekker. New York, 27-43.

Piero NM, Kimuni NS, Ngeranwa JJN, Orinda GO, Njagi JM. 2015. Antidiabetic and Safety of *Lantana rhodesiensis* in Alloxan Induced Diabetic Rats. Journal of Developing Drugs 4: 129.

Prakash D, Dhakarey R, Mishra A. 2004. Carotenoids: the phytochemicals of nutraceutical importance. Indian Journal of Agricultural Biochemistry 17: 1-8.

Prakash D, Kumar N. 2011. Cost Effective Natural Antioxidants. In: RR Watson, JK Gerald and VR Preedy eds. Nutrients, Dietary Supplements and Nutriceuticals. Humana Press, Springer. USA., pp 163-188.

Pullaiah T. Encyclopaedia of World Medicinal Plants. Regency Publisher., 2006; New Delhi. p.2442.

Saraswathi K, Sivaraj C, Arumugam P. 2019. Antioxidant Activities, Thin Layer Chromatographic Analysis and

GCMS Analysis of *Capsicum annuum* L.: A Comparison of Green and Red Chilli. Journal of Biological and Chemical Research 36(1): 184-197.

Scalbert A, Manach C, Morand C, Remesy C. 2005. Dietary polyphenols and the prevention of diseases. Critical Reviews in Food Science and Nutrition 45: 287-306.

Shyma TB, Devi Prasad AG. 2013. Traditional use of medicinal plants and its status among the tribes in Mananthavady of Wayanad district, Kerala. World Research Journal of Medicinal and Aromatic Plants 1(2): 22-26.

Spanos GA, Wrosltad RE. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural & Food Chemistry 38, 1565-1571.

Stahl E. Thin Layer Chromatography, 2nd ed., Springer Pvt. Ltd., New Delhi, 2005; 85.

Trease GE, Evans WC. Pharmacognosy. 13th (ed), 1989; ELBS/Bailliere Tindall, London, 345-6, 535-6, 772-3.

Zhang Y, Seeram NP, Lee R, Feng L, Heber D. 2008. Isolation and identification of strawberry phenolics with antioxidant and human cancer cell anti-proliferative properties. Journal of Agricultural and Food Chemistry 56, 670-675.