J. Indian bot. Soc. (2025) Vol. 105 (1): 50-55
e-ISSN:2455-7218, ISSN:0019 - 4468
https://doi: 10.61289/jibs2024.08.30.103
www.indianbotsoc.org

RESEARCH ARTICLE

HR-LCMS Based Metabolites Profiling of *Careya arborea* Roxb.

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Abstract

Novel chemicals and bioactive compounds found in medicinal plants can be used in the discovery and development of new drugs. *Careya arborea* Roxb. (Family: Lecythidaceae) is known to be one of the important Ayurvedic medicinal plants which has an illustrious history of use in traditional medicine. This study aims to establish the phytochemical profile of Hydroalcoholic stem bark and leaf extracts of *C. arborea* Roxb. using High Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS). The results showed the detection of 9 major phytochemical compounds in bark and 28 major phytochemical compounds in leaves. This HR-LCMS study manifested the presence of several compounds of pharmaceutical importance in the bark and leaf extracts. Furthermore, this study confirmed that *C. arborea* Roxb. is a rich source of biologically active phytochemical constituents which can be the reason for its bioactive potential.

Keywords: *Careya arborea*, High resolution liquid chromatography-mass spectrometry (HR-LCMS), Leaves, Phytochemicals, Stem bark.

Introduction

Medicinal plants are a vital source for improving health and overcoming the side effects of modern medicine. Several scientific studies have shown that medicinal plants have many benefits and have biochemical and molecular effects (Gratus *et al*. 2009). Plants are acknowledged as a biosynthetic laboratory due to the presence of numerous chemical compounds that includes primary and secondary metabolites (Kokate *et al*. 2008). It is of vital importance to acquire knowledge about these phytochemical components in order to discover new therapeutic agents and to synthesize new complex chemicals (Mojab *et al*., 2003). The development of various analytical methods has enabled the isolation, identification and structure elucidation of these phytochemicals (Feng *et al*., 2019).

Liquid Chromatography-Mass Spectrometry (LC-MS) or High-Resolution Liquid Chromatography-Mass Spectrometry (HR-LCMS) is an analytical technique that combined the outstanding separation efficacy of liquid chromatography with sensitive qualitative analysis and specific detection of mass spectroscopy (Ardrey, 2003). The development of hyphenated analytical approaches such as HR-LCMS, GC-MS, LC-NMR, GC- IR, etc. has advanced the identification of new molecules in plants (Singh *et al*., 2018).

HR-LCMS is a much more widely applicable method and facilitates the analysis of large, polar, ionic, thermally unstable and non-volatile compounds that traditionally have been difficult to analyse. HR-LCMS system is helpful in the correct determination and structural elucidation of known and unknown components. It also offers excellent sensitivity within minimum acquisition time and attain high-quality data (Primer, 1998).

HR- LCMS has a wide range of applications in the field of biomedical sciences for structural interpretation, pharmacokinetic analysis, genetic analysis, etc. (Parasuraman *et al*., 2014). In particular, this hyphenated analytical technique is greatly used in the identification and very sensitive quantification of natural products which are

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How to cite this article: Gupta, P., Patil, D., Patil, A. (2025). HR-LCMS Based Metabolites Profiling of *Careya arborea* Roxb. *J. Indian bot. Soc.*, 105(1):50-55 Doi: 10.61289/jibs2024.08.30.103

Source of support: Nil

Conflict of interest: None.

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Received: 15/07/2024 **Accepted:** 30/08/2024 **Published online**: 10/10/2024

present at minute concentrations in complex matrices. It also makes easy to find and identify suspected impurities present at trace levels in the complex sample (Sauvage *et al*., 2006; Pitt, 2009 and Pawar *et al*, 2019). Also, it allows the detection of molecular weight to the nearest 0.001 a.m.u (atomic mass units) (Wu *et al*., 2012).

Careya arborea Roxb. belongs to family Lecythidaceae, is a medium sized deciduous tree of about 8–20-meter height with a spreading crown. It is commonly called Padmaka in Ayurveda. It is distributed throughout India in deciduous forests and grasslands (Anonymous, 1993). It is globally found in India, Ceylon, Malay Penninsula, Cambodia and Australia up to an altitude of 1500 meters (Kirtikar and Basu, 1975 and Anonymous, 2006). In the present study, *Careya arborea* Roxb. bark and leaf extracts were subjected to High Resolution - Liquid Chromatography and Mass Spectrometry (HR-LCMS) analysis for the separation, identification and characterization of the phytoconstituents based on their retention time.

Materials And Methods

Plant Materials- Collection and Authentication

Stem bark and leaves were collected from the forest area of Badlapur, Mumbai (Maharashtra) and authenticated from Agharkar Research Institute, Pune, India. Leaves were washed with running tap water to remove soil particles and air dried. Bark was also air dried. After drying, both the plant parts were ground into fine powder and stored in an airtight container at room temperature for further studies.

Preparation of Plant extracts

Extracts of bark and leaf were prepared by refluxing 10 grams of air-dried powdered materials in 100 ml of Hydro alcohol (50 % Ethanol) for 6 hours at 70 °C. The extracts were filtered through Whatmann filter paper no. 1. They were evaporated to dryness using a rotary vacuum evaporator at 70 °C and 629 mm of Hg. Plant extracts were reconstituted in HPLC grade ethanol at a concentration of 100 ppm and submitted for HR-LCMS to Sophisticated Analytical Instrument Facility (SAIF), Indian Institute of Technology (IIT), Powai, Mumbai.

High resolution liquid chromatography and mass spectrometry (HR-LCMS) analysis

HR-LCMS studies and accurate mass measurements were carried out by 6550i funnel Q-TOF LCMS (Agilent Technologies, USA) equipped with a dual AIS ESI ion source. The stationary phase (Columm) used was Zorbax SB-C18, 2.1×50mm, 1.8 microns (Agilent Technologies, USA) and the mobile phase used were

A. 0.1% Formic acid in Water

B. 90% Acetonitrile+ 10% Water+ 0.1% Formic acid

The data acquisition and processing were performed using Mass Hunter software.

The HR-LCMS mass spectrum was interpreted by comparing the spectrum of unknown components with the spectrum of known components. For comparison, we have utilized the SAIF -IIT Bombay database, which contains over 62000 patterns of the spectrum. The components of the trial materials were identified by their names, molecular weights, and structures.

Results And Discussion

The phytochemical profile of *Careya arborea* Roxb. bark and leaf were characterized by using HRLC-MS spectra. In a chromatogram, the relative concentrations of various compounds get eluted as a function of retention time. Based on the height of the peak, we could determine the relative concentration of bioactive compounds present. The mass spectrometer analyses the compounds eluted at different times in order to identify the nature and structure of the compounds. In the data library, these mass spectra can be identified as fingerprints of that compound (Figs 1 and 2).

Phytochemical screening of Hydroalcoholic (50 % Ethanolic) extract of *Careya arborea* Roxb. bark and leaf through High Resolution (HR)-LCMS detected several

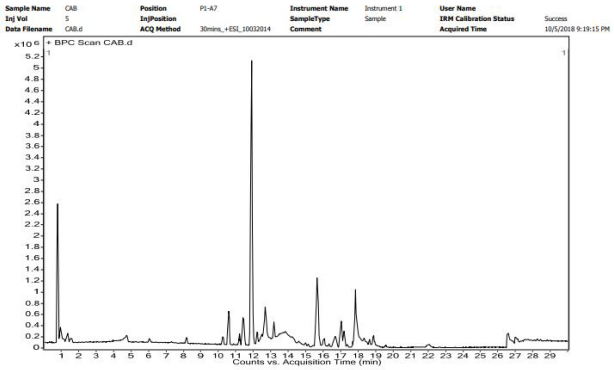

Plate 1: HR-LCMS chromatogram of Hydroalcoholic (50 % Ethanolic) extract of *Careya arborea* Roxb. bark

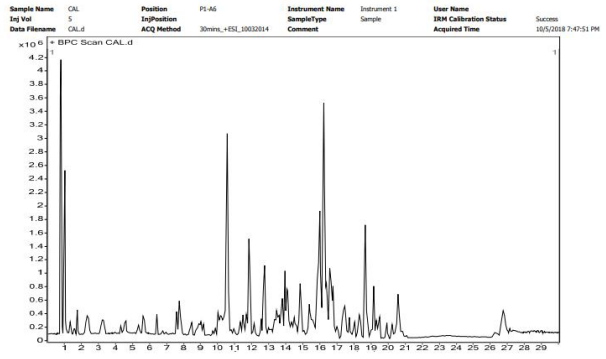

Plate 2: HR-LCMS chromatogram of Hydroalcoholic (50 % Ethanolic) extract of *Careya arborea* Roxb. leaves

phytocompounds but in the present study, compounds having Hits (DB) 5 or less than 5 are selected as the most probable compounds present in bark and leaf.

Major known compounds found to be present in *Careya arborea* Roxb. bark are Mebeverine metabolite (Veratric acid glucuronide), Esculetin, Phe His, Convallatoxin, Dihydrodeoxystreptomycin, 11alpha-acetoxykhivorin, Oleamide, Dodecylbenzene and 2-Oxo-4-methylthiobutanoic acid (Table 1).

Esculetin, Oleamide, Dodecylbenzene, Convallatoxin, Dihydrodeoxy streptomycin and 2-Oxo-4-methyl thio butanoic acid found in bark extract possess various biological activities (Table 2). Oleamide shows hypnotic and antidepressant properties (Basile *et al*., 1999 and Akanmu *et al*., 2007) which is commercially used in the form of capsules and powder (Nootropics Depot) for promoting relaxation and supporting healthy sleeping habits. Dodecylbenzene is used as a surfactant cleansing agent in its sulphonic acid form (Becker *et al*., 2010) and commercially available by the name Sulfonax (Kao chemicals, Europe) for laundry and cleaning purposes.

Careya arborea Roxb. leaf showed the presence of 28 major phytocompounds i.e. Triparanol, Anabasamine, m-Salicylic acid, Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-, Demeclocycline, Tuberonic acid, Vanillylmandelic acid, Betaxolol, Ubiquinone, Convallatoxin, Dihydrodeoxystreptomycin, Nostoxanthin sulfate, Undecanedioic acid, Cucurbitacin P, Madecassic acid, Neamine (Neomycin A), Betamethasone dipropionate, 2-methyl-tridecanedioic acid, Hydrocortisone-17- butyrate, ZK 168281, Atocalcitol, Digitoxigenin, Taxa-4(20),11(12)-dien-5alpha-acetoxy-10beta-ol, (22E, 24E)-1alpha, 25 dihydroxy-26, 27-diethyl-22, 23, 24, 24a-tetradehydro-24a homovitamin D3/(22E, 24E), 1-Hexadecanoyl-sn-glycerol, 2 alpha-(3- Hydroxypropyl)-1 alpha, 25- dihydroxy-19-norvitamin D3, Spheroidene and 1, 2-di-(13Z- docosenoyl)-sn-glycerol (Table 3).

Betaxolol, Triparanol, Anabasamine, Neamine (Neomycin A), m-Salicylic acid, Demeclocycline, Tuberonic acid, Madecassic acid, Digitoxigenin, Betamethasone dipropionate, Convallatoxin and Dihydrodeoxy streptomycin found in leaf extract show various biological activities (Table 3).

Table 1: List of compounds identified in the Hydroalcoholic extract of *C. arborea* Roxb. bark

No.	*Compound Name*	*RT*	*Mass*	*Formula*	*Hits (DB)*
1	Mebeverine metabolite (Veratric acid glucuronide)	1.02	358.093	$C_{15}H_{18}O_{10}$	3
2	Esculetin	8.175	178.0273	$C_9H_6O_4$	3
3	Phe His	10.603	302.1393	$C_{15}H_{18}N_4O_3$	4
4	Convallatoxin	11.894	550.2712	$C_{29}H_{42}O_{10}$	1
5	Dihydrodeoxy streptomycin	11.895	567.2961	$C_{21}H_{41}N_7O_{11}$	1
6	11alpha-Acetoxykhivorin	12.238	644.2737	$C_{34}H_{44}O_{12}$	1
7	Oleamide	17.856	281.2743	$C_{18}H_{35}NO$	2
8	Dodecylbenzene	17.86	246.2359	$C_{18}H_{30}$	1
9	2-Oxo-4-methylthiobutanoic acid	18.888	148.0168	$C_5H_8O_3S$	1

Table 2: Biological activities of some important bioactive compounds found in 50 % Hydroalcoholic extract of *C. arborea* Roxb. bark and leaf

No.	*Compound name*	*Plant part*	*Biological activities*	*References*
1	Esculetin	Bark	Antioxidant, Liver protecting, Antidiabetic, Antibacterial and Antitumor activity	Kaneko *et al*., 2003; Gilani *et al*., 1998; Prabakaran and Natarajan, 2012; Lee *et al*., 2014 and Jay *et al*., 2013
2	Oleamide	Bark	Hypnotic properties, Antidepressant property and vasodilation activity	Basile *et al*., 1999; Akanmu *et al*., 2007 and Hiley and Hoi, 2007
3	Dodecylbenzene	Bark	In cosmetics as surfactant cleansing agents	Becker *et al*., 2010
4	2-Oxo-4-methyl thio butanoic acid	Bark	poultry nutritional supplement, to treat renal failure disease and anti-microbial properties	Masud *et al*., 1994; Tang *et al*., 2011 and Dibner and Buttin 2002
5	Betaxolol	Leaf	Treatment of glaucoma	Yu *et al*., 1999
6	Triparanol	Leaf	Lowering of blood cholesterol level	Steinberg *et al*., 1961
7	Anabasamine	Leaf	Anti-inflammatory activity	Panthong *et al*., 1984
8	Neamine (Neomycin A)	Leaf	Antibiotic	Waksman and Lechevalier, 1949

9	m- Salicylic acid	Leaf	Peeling agent in skin disorders, Anti-inflammatory activity and Antioxidant activity	Kligman and Kligman, 1998; Lee and Kim, 2003, Randjelović *et al*., 2015 and Tasleem, 2015
10	Demeclocycline	Leaf	Antibacterial activity and in the treatment of syndrome of inappropriate antidiuretic hormone (SIADH)	De Troyer and Demanet, 1975, Cherrill *et al*., 1975 and Pasquale and Tan, 2005
11	Tuberonic acid	Leaf	Tuber inducting properties	Shaikh *et al*., 2016
12	Betamethasone dipropionate	Leaf	Anti-inflammatory, immunosuppressive and antiproliferative activity Treatment of Psoriasis	Charney, 1976 and Alam *et al*., 2012
13	Madecassic acid	Leaf	Antidiabetic activity, Anti-colitis effect and Anti-inflammatory activity	Hsu *et al*., 2015; Xu *et al*., 2017b and Won *et al*., 2010
14	Digitoxigenin	Leaf	Cardiotonic activity and Cytotoxic activity	Cornelius *et al*., 2013 and Schneider *et al*., 2018a
15	Convallatoxin	Bark and Leaf	Antitumor effect and inhibit viral infection and replication	Kaushik *et al*., 2017; Schneider *et al*., 2018b; Schneider *et al*., 2017; Anderson and Barton, 2017; Amarelle and Lecuona, 2018
16	Dihydrodeoxy streptomycin	Bark and Leaf	Antimicrobial activity	Patil and Lade, 2018

Table 3: List of compounds identified in the Hydroalcoholic extract of *C. arborea* Roxb. leaves

No.	*Compound Name*	*RT*	*Mass*	*Formula*	*Hits (DB)*
1	Triparanol	1.026	143.0952	$C_7 H_{13} N O_2$	2
2	Anabasamine	1.77	253.1569	$C_{16} H_{19} N_3$	1
3	m-Salicylic acid	4.327	138.0328	$C_7 H_6 O_3$	4
4	Benzenemethanol, 2- (2-aminopropoxy)-3-methyl-	9.016	196.1113	$C_{11} H_{16} O_3$	2
5	Demeclocycline	9.72	464.1003	$C_{21} H_{21} Cl N_2 O_8$	1
6	Tuberonic acid	11.318	226.1227	$C_{12} H_{18} O_4$	4
7	Vanillylmandelic acid	11.474	198.0494	$C_9 H_{10} O_5$	4
8	Betaxolol	11.508	307.2172	$C_{18} H_{29} N O_3$	3
9	Ubiquinone	11.765	250.1201	$C_{14} H_{18} O_4$	3
10	Convallatoxin	11.845	550.2708	$C_{29} H_{42} O_{10}$	1
11	Dihydrodeoxy streptomycin	11.846	567.2964	$C_{21} H_{41} N_7 O_{11}$	1
12	Nostoxanthin sulfate	12.66	702.3691	$C_{40} H_{55} Na O_7 S$	1
13	Undecanedioic acid	13.334	216.1378	$C_{11} H_{20} O_4$	1
14	Cucurbitacin P	13.449	520.3473	$C_{30} H_{48} O_7$	2
15	Madecassic acid	13.787	504.3525	$C_{30} H_{48} O_6$	1
16	Neamine (Neomycin A)	14.692	322.1817	$C_{12} H_{26} N_4 O_6$	3
17	Betamethasone dipropionate	15.566	504.26	$C_{28} H_{37} F O_7$	1
18	2-Methyl- tridecanedioic acid	15.933	258.1865	$C_{14} H_{26} O_4$	4
19	Hydrocortisone-17-butyrate	16.658	432.2537	$C_{25} H_{36} O_6$	2
20	ZK 168281	16.871	510.3392	$C_{32} H_{46} O_5$	3
21	Atocalcitol	17.413	494.3448	$C_{32} H_{46} O_4$	3
22	Digitoxigenin	17.656	374.2474	$C_{23} H_{34} O_4$	2
23	Taxa-4(20),11(12)- dien-5alpha-acetoxy-10beta- ol	17.953	346.2522	$C_{22} H_{34} O_3$	5

24	(22E,24E)-1alpha,25 dihydroxy-26,27-diethyl 22,23,24,24a-tetradehydro- 24a-homovitamin D3 / (22E,24E)	18.201	482.3761	$C_{32}H_{50}O_3$	2
25	1-Hexadecanoyl-sn-glycerol	18.297	330.2826	$C_{19}H_{38}O_4$	2
26	2Alpha-(3- Hydroxypropyl)1alpha,25- dihydroxy-19-norvitamin D3	19.189	462.3774	$C_{29}H_{50}O_4$	1
27	Spheroidene	20.66	568.456	$C_{41}H_{60}O$	2
28	1,2-di-(13Z-docosenoyl)-sn-glycerol	26.292	732.653	$C_{47}H_{88}O_5$	2

Salicylic acid is used in many face washes and scrubs (Vichy Normaderm, Alba botanica, Clean n Clear, Paula's choice, etc.) for acne prone skin types. Triparanol was the first synthetic cholesterol lowering drug which was patented in 1959 (Blohm and MacKenzie, 1959). Demeclocycline is used in the treatment of bacterial infections and a condition which causes a very low level of sodium in the blood, called syndrome of inappropriate secretion of antidiuretic hormone (SIADH) (De Troyer and Demanet, 1975, Cherrill *et al*., 1975 and Pasquale and Tan, 2005). It is available in the market in the form of capsules (Ledemycin, Advanz pharma). Betamethasone dipropionate has immunosuppressive and anti-inflammatory properties (Charney, 1976 and Alam *et al*., 2012). It is available in the form of spray (Sernivo, DPT Lab), cream (Betamil-M, Merck Ltd) and ointment (Diprolene, MSD Lab) to treat discomfort of various skin conditions such as dermatitis, eczema and psoriasis.

Conclusion

The present study investigated and specified the various active metabolites found in the hydroalcoholic bark and leaf extract of *Careya arborea* Roxb. by carrying out HR-LCMS analysis. Using the results of this study, it should be possible to extract, purify, and screen a variety of secondary active metabolites from this traditionally well-known medicinal plant for pharmacological activity.

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