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ISOLATION AND CHROMATOGRAPHIC FINGER PRINTING OF RANDIALIC ACID B AN ABORTIFACIENT AGENT ISOLATED FROM BARK OF AYURVEDIC MEDICINAL PLANT *RANDIA SPINOSA* (POIR.) RUBIACEAE

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ABSTRACT

Randialic acid B found in Ayurvedic medicinal plant *Randia spinosa* (Poir.) Rubiaceae exhibit aphrodisiac, emetic, abortifacient, antipyretic, carminative, alexiteric and cures abscesses, ulcers, inflammations, tumors, skin-diseases, piles. Occurance of Randialic acid Bin other plants is so far not reported. Randialic acid B was taken up for chromatographic analysis. Multiphase solvent extraction of stem bark of *R. sapinosa* was done to isolaterandialic acid from stem bark of *Randia spinosa*. Crystallization of Randialic acid B was confirmed by melting point 256°C Identification was done by TLC method. The objective of this work was to determine the standard HPLC chromatograms Randialic acid B found in bark of *Randia spinosa*. This HPLC fingerprint of Randialic acid B could be used as benchmarks for comparison during the qualitative and quantitative analysis of Randialic acid B present in any plant sample.

KEYWORDS

Randialic acid B, *Randia spinosa*, Abortifacient, Radical and Mainphal.

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INTRODUCTION¹

Randia spinosa (Poir.) Rubiaceae, commonly known as 'Mainphal'. In India it is distributed in eastern coastal districts of Andhra Pradesh and Madras and other parts of southern India (Figure No.1). The various parts of *R. spinosa* such as bark, roots and fruits to possess medicinal properties in ethno botanical surveys conducted by ethno botanists and in traditional systems of medicine such as Ayurveda. Most of the parts of Mainphal are of medicinal importance and used traditionally for the treatment of various ailments. The roots of the plant are considered as insecticidal and insect repellent.

The seeds of the plant are used as tonic to induce appetite. The bark is astringent and is given in diarrhoea and dysentery. An infusion of the bark is used as an emetic. It is also reported to be abortifacient. As per Ayurvedic claim, Mainphal is bitter, aphrodisiac, emetic, abortifacient, antipyretic, carminative, alexiteric and cures abscesses, ulcers, inflammations, tumors, skin-diseases, piles etc. The phytochemical studies on the bark of *R. Spinosa* revealed presence of mixture of saponins viz. Randialic acid or acid saponin has been isolated from the bark. The fruits of *R. Spinosa* contain a toxic saponin of oleanolic acid. They also contain leucocyanidin and mannitol. The saponins are concentrated mostly in the pulp. A mixture of two saponins, viz. Randialic or neutral saponin (m.p. 289-290^o C decomp.) and randialic acid or acid saponin (m.p. 260^o C decomp.) has been isolated from the pulp¹⁶⁻¹⁷. The bark contains Randialic acids A and B, scopoletin; catunaregin, epicatunaregin²; ficusal, balanophonin, 5"-methoxy-4"-O-(8-guaiacylglycerol)buddlenol A², 3-(2-hydroxypropyl)-8-hydroxy-3,4-dihydroisocoumarin, 3-deoxyartselaenin C, scoparone, morindolide, pinoresinol, medioresinol, secoisolariciresinol³, Dmannitol'. The saponins on hydrolysis yield glucose, xylose, rhamnose, and two triterpenic acid saponins designated as randialic acid A and randialic acid B.

Isolation of Randialic acid B from bark of *Randia spinosa*

Stem bark of *Randia spinosa* was purchased from local vendor. Bark was dried and grinded. Grinded bark was extracted exhaustively with 95% ethanol (5 times) by maceration. All the extracts were combined and concentrated to 1/25 under reduced pressure at 50^o. The extract was allowed to stand overnight at room temperature to yield brownish crystals of D-mannitol. Extract was filtered and concentrated further to yield a brown viscous mass. Further the obtained viscous mass was dissolved in water and extracted with n-butanol (3 times). Butanol extract was collected and thoroughly washed with water. The solvent was removed under reduced pressure to yield viscous material. A mixture of acetone and

ether (1:1) was added slowly with stirring to obtain buff coloured fluffy precipitate of saponins. Saponin is collected and dried in desiccator due to its hygroscopic nature. Dilute H₂SO₄ (7% v/v) was added to the dried saponins and mixture was heated on a steam bath for 24 h. Mixture was cooled and extracted with diethyl ether (3 times). The combined ether extracts were washed with saturated NaCl solution till the washings were neutral. The ether layer were passed through anhydrous Na₂SO₄ and distilled off to get a mixture of aglycones. The aglycone mixture was repeatedly crystallized from methanol till the white granular mass of melting point 256^oC indicating to be of the Randialic Acid B was not yielded. About 0.004% w/w Randialic Acid B received of dried stem bark of *Randia Spinosa*. Due to photosensitivity it was store in an air tight container in a dark and dry place (Figure No.2).

Identification of Randialic acids B by Thin Layer Chromatography²⁻⁴

Test solution

2 g of powdered drug was extracted with 15 ml of methanol in a Soxhlet apparatus for 18 h. The solvent was removed under reduced pressure at 50^o yielding the crude residue (0.14 g). 5 mg of the residue was dissolved in 5 ml of methanol and used for TLC profiling.

Standard solution

Standard was prepared by dissolving 3 mg of randialic acid B in 5 ml of methanol.

Solvent system

Toluene: Ethyl acetate: Formic acid (7:3:1).
Procedure

Test solution 20 µl and standard solution 5 µl were separately applied on a precoated silica gel 60 F₂₅₄ TLC plate (E. Merck) of uniform thickness of 0.2 mm. The plate was developed in the solvent system till the solvent raised to a distance of 8 cm.

Observation

The plate was visualized under visible light after spraying with anisaldehyde-sulphuric acid reagent and heating at 105^oC for 5 min. R_f value and colour of the resolved bands noted (Table No.1 and Figure No.3).

Evaluation

A violet colored band (R_f 0.55) corresponding to randialic acid B was visible in both the test and standard.

HPLC Assay

Test solution

Accurately weighed 2 g of extract was put with 15 ml of methanol in a Soxhlet apparatus for 18 h. Solvent was removed under reduced pressure at 50° yielding the crude residue. Residue was collected and 10 mg was dissolved in 10 ml of methanol in a 10-ml volumetric flask. Solution was filtered through 0.45 μ membrane and applied. For chromatography.

Standard solution

Accurately weighed 2 mg randialic acid B was dissolved in 10 ml of methanol in a 10-ml volumetric flask. From this stock solution standard solutions of 0.012, 0.025, 0.05 and 0.1 mg/ml were prepared by transferring aliquots (0.62, 1.25, 2.5 and 5 ml) of stock solution to 10-ml volumetric flasks and adjusting the volume of each solution to 10 ml with methanol. Each of the solutions was passed through 0.45 μ membrane before applying for chromatography.

Estimation

The percentage of randialic acid B was found ranges from 0.0091 to 0.010 in the samples (Table No.2 and Figure No.4).

Table No.1: TLC Details of Test Solution of *Randia spinosa* Stem Bark

S.No	R_f value	Colour of the band
1	0.17	Blue
2	0.21	Blue
3	0.29	Greenish black
4	0.33	Aqua green
5	0.39	Yellow
6	0.50	Violet
7	0.55	Violet (<i>randialic acid b</i>)
8	0.64	Aqua green
9	0.67	Blackish grey
10	0.75	Faint violet

Table No.2: Chromatographic conditions

S.No	Instrument	Agilent 1100 series quaternary HPLC with auto sampler
1	Column	Zorbax Eclipse, XDB, C18, 4.6 mm x 150 mm, reverse phase
2	Detection	UV Detector at 254 nm
3	Mobile phase	Methanol : Acetonitrile : Water (10 : 20 : 70)
4	Flow rate	1 ml/min
5	Concentration of standard solution	0.2 mg/ml of methanol
6	Concentration of test solution	0.1 mg/ml of methanol
7	Injection volume	5 μ l



Figure No.1: Fruit of *Randia spinosa*

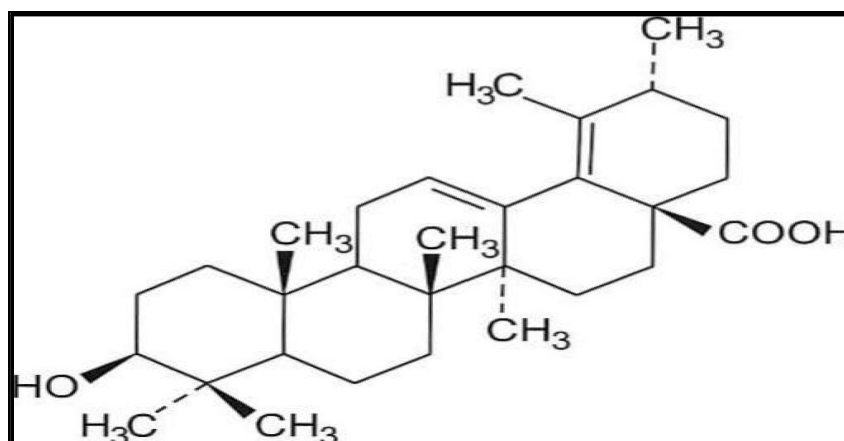


Figure No.2: Randialic acid B 3/3-Hydroxyursa-12, 18-dien-28-oic acid

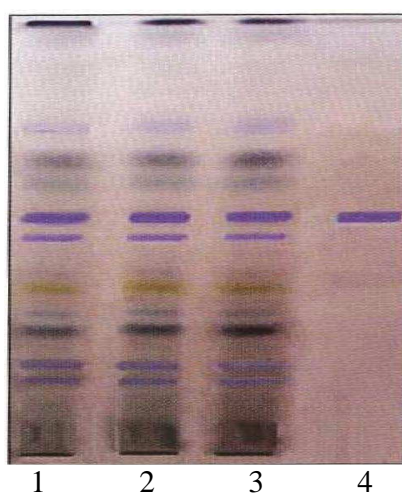


Figure No.3: TLC profile of test solution of *Randia spinosa* stem bark.
1-3: Test solution; 4: Randialic acid B standard

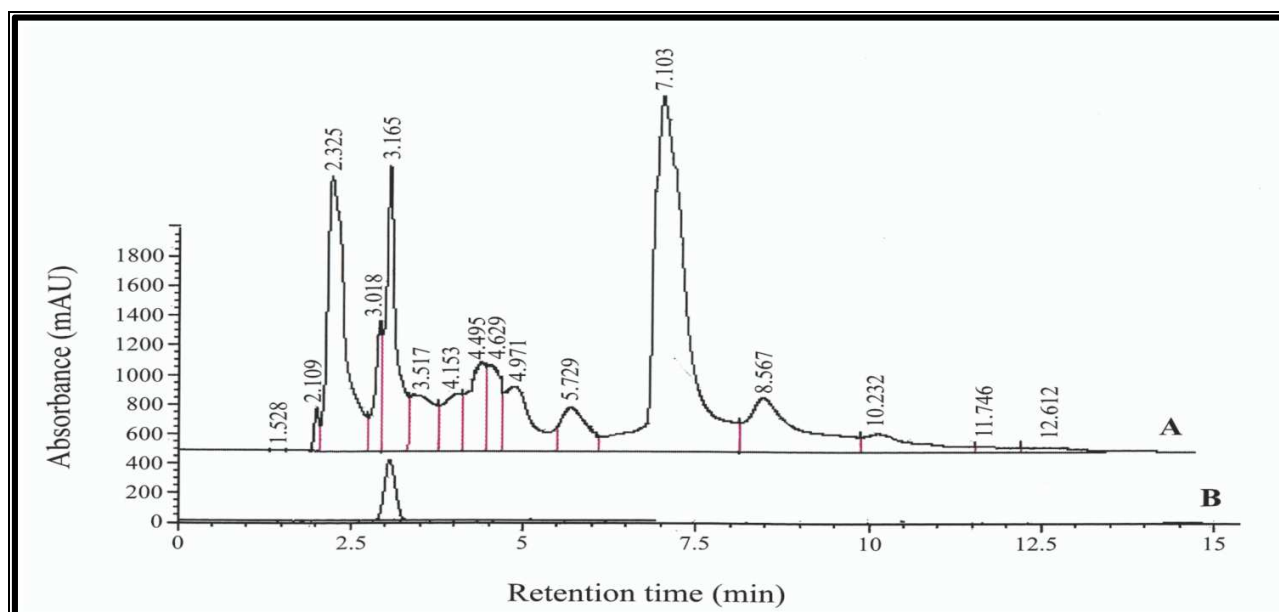


Figure No.4: HPLC Chromatogram of *Randia spinosa* stems bark
A. Test solution; B. Randialic acid B standard

CONCLUSION

The validated HPLC method allows a simple, accurate and precise quantitative analysis of a biologically important molecule Randialic Acid B from *R.sapinosa*. Presence or absence of Randialic Acid B in stem bark extract of *R.sapinosa*, related species and commercial formulations can be confirmed by comparing their retention times (tR) using developed method. This HPLC fingerprint of Randialic acid B could be used as benchmarks for comparison during the qualitative and quantitative analysis of Randialic acid B present in any plant sample.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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