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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 HPLC chromatogram of 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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### INTRODUCTION

*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. 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. Studies determined that the bark contains Randialic acids A and B, scopoletini; catunaregin, epicatunaregin<sup>2</sup>, ficusal, balanophonin, 5"-methoxy-4"-O-(8-guaiacylglycerol) buddlenol A<sup>2</sup>, 3-(2-hydroxypropyl)-8-hydroxy-3,4-dihydroisocoumarin, 3-deoxyartselaenin C, scoparone, morindolide, pinoresinol, medioresinol, secoisolariciresinol<sup>3</sup>, Dmannitol<sup>1</sup>. 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°. 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 H<sub>2</sub>SO<sub>4</sub> (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 Na<sub>2</sub>SO<sub>4</sub> 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°C 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.

#### **Identification of Randialic acids B by Thin Layer Chromatography**

##### **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° 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 20µl and standard solution 5µl were separately applied on a precoated silica gel 60 F<sub>254</sub> 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°C for 5 min. R<sub>f</sub> value and colour of the resolved bands noted.

##### **Evaluation**

A violet colored band (R<sub>f</sub>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 $\mu$  membrane and applied. For chromatography.

#### Standard solution

Accurately weighed 2 mg randialic acid B was dissolved in 10ml 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 $\mu$  membrane before applying for chromatography.

#### Estimation

Randialic acid B percentage was found ranges from 0.0091 to 0.010 in the samples.

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

S.No	R <sub>f</sub> 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 $\mu$ l



Figure No.1: Fruit of *Randia spinosa*

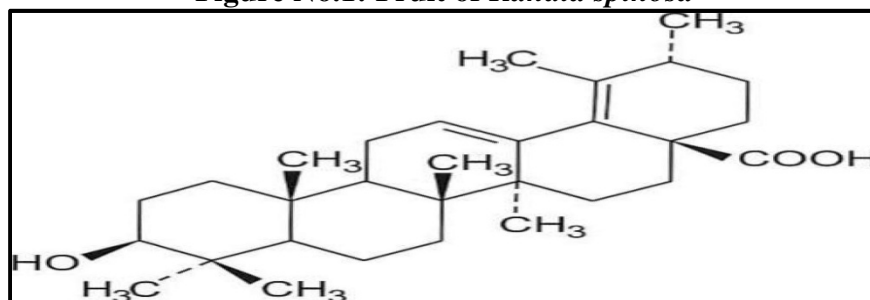


Figure No.2: Randialic acid B 3/3-Hydroxyursa-12, 18-dien-28-oic acid

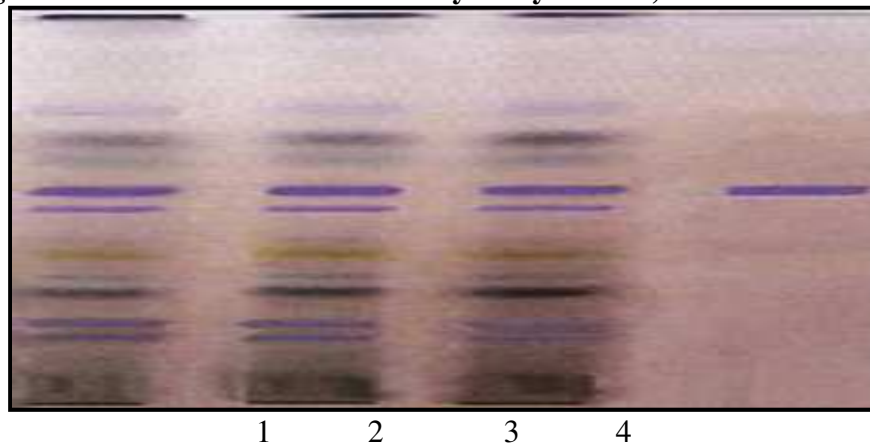


Figure No.3: TLC profile of test solution of *Randia spinos* tem bark, 1-3: Test solution, 4: Randialic acid B standard

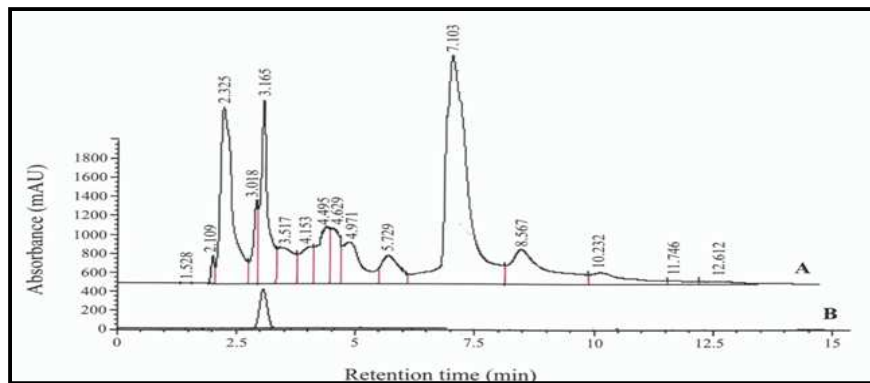


Figure No.4: HPLC Chromatogram of *Randia spinos* tems bark A. Test solution, B. Randialic acid B standard

## 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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