REPORT

QUANTITATIVE ESTIMATION OF β-SITOSTEROL, LUPEOL, QUERCETIN AND QUERCETIN GLYCOSIDES FROM LEAFLETS OF *SOYMIDA FEBRIFUGA* USING HPTLC TECHNIQUE

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ABSTRACT

Soymida febrifuga (Meliaceae) dried leaflets (10 gm) were extracted with petroleum ether. Unsaponifiable matter quantitatively used for sample preparation, labeled as SF-U. Another 10 gm leaflet powder was extracted with methanol and quantitatively used for sample preparation labeled as SF-A. Sample and standard solution were dosage on three different plates and developed in its respective mobile phase plates were scanned using TLC scanner III and estimated using integration software CATs 4.05. Calculations for percentage were done considering standard and sample R_f, AUC and dilution factor. Estimation of β Sitosterol, Lupeol, Quercetin, Quercetin-3-O-galactoside, Quercetin-3-O-xyloside and Quercetin-3-O-rutinoside were determined as 0.02146 % w/w, 0.0377 % w/w, 0.4079 % w/w, 0.6197 % w/w, 2.974 % w/w and 3.235 % w/w respectively with the help of HPLC techniques.

Keyword: *Soymida febrifuga,* HPTLC, β-Sitosterol, Lupeol, Quercetin-3-O-xyloside and Quercetin-3-O-galactoside, Rutin.

INTRODUCTION

Soymida febrifuga or *Swietenia rubra* belonging to Meliaceae is known as Rohuna, Patranga, Chandravallabha, Indian red wood tree, Bastard Cedar, in various regional languages.

It is lofty deciduous tree found frequently on dry stony hills and laterite soil. It is distributed in the dry forests of Merwara, Chota Nagpur, Kerala, Gujrat, Uttar Pradesh, Bihar and Ceylon. Various tribal in Bhandara and Gadchiroli district of Manharastra use this plant for various ailments. In Ayurveda it is used to remove '*vata*', cures for tridosha, fever, cough, asthma, blood impurities, ulcers, leprosy and dysentery. In Unani system it is astringent to bowels and useful in fever. It is bitter tonic, antiperiodic and antimalerials. It is used in the form of decoction for rheumatic swellings (Kirtikar, 1984; Nadkarni, 1976).

LITERATURE SURVEY

Many scientist in India and abroad have worked on bark, fruit, seeds and leaves. Literature survey showed that methyl angolensate and steryl glycoside (Adesida *et al*., 1971; Adesogan *et al*., 1972 and Ambaye *et al*., 1971) were isolated from bark. Sitosterol, Obtusifoliol, Syringetin, dihydrosyringetin (Pardhasardhi and Sindhu, 1972), quercetin, myrecetin, dihydromyrecetin, naringenin, febrinin A, febrinin B (Rao *et al*., 1979) and febrifugin (Murali *et al*., 1978) were isolated from root heartwood. Epoxyfebrinin B, 14,15-dihydroepoxyfebrinin B and febrinolide (Mallavarpu *et al*, 1985; Nair and Subramanian, 1975) have been isolated from fruit pericarp. Quercetin-3-O-rhamnoside and Quercetin-3-O-rutinoside (Nair and Subramanian, 1975) were isolated from leaves. Palmitic, steric, oleic, linoleic and lenolenic acids, sitosterol and lupeol were isolated from seed (Lakshmi, 1987). Anti-inflammatory activity of Soymida febrifuga in rats and mice showed dose dependant inhibition of rat paw oedema (Divan and Singh, 1993).

Isolation and Characterization of Compounds

Dried leaflets were extracted consecutively with petroleum ether, chloroform and methanol. Unsaponifiable matters were separated from saponified petroleum ether extract. Two pure compounds were isolated by column chromatography and preparative TLC from unsaponifiable matter. These were characterized as β-Sitosterol and Lupeol by FTIR, U.V., Co-TLC and melting point. Methanolic extract was fractionated by extraction with ethyl acetate. Four pure compounds were isolated by column chromatography and preparative TLC of ethyl acetate extract. These were characterized as Quercetin, Quercetin-3-O-galactoside, Quercetin-3-O-xyloside and Quercetin-3-O-rutinoside (Rutin), by FTIR, U.V.(spectral shift), after hydrolysis
Co-TLC and osazone formation.

The present work was emphasized on quantification of these isolated compounds by HPTLC technique.

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MATERIALS AND METHODS

The leaflets were collected from Rahuri (Ahemadnagar Dist., Maharastra). Conformation of plant was done by Mr. Kapse, Plantation Officer, Samjic Vanikaran Vibhag, Nasik and by Prof. Mahajan, HOD, Botany Department, KTHM College, Nasik.

For extraction Soxhlet apparatus was used. Reagents and solvents were of analytical grade. HPTLC was performed at Anchrom lab. (Mumbai). Instrument specification are as follows: Camag HPTLC System equipment with sample application Linomat IV, Twin trough Chamber, TLC Scanner III, Integration software CATS 4.05. Adsorbent used as HPTLC Aluminum precoated plates i.e., Silica gel 60 F_{254} (E.Merck). Standards were used from Anchrom and Sigma Lab.

Sample Preparation

1. Dried leaflets (10gm) were exhaustively extracted with petroleum ether (60-80°C) Unsaponifiable matters were separated from saponified petroleum ether extract (I.P. 96). It was diluted with benzene as concentration of 2 mg/ml (SF-U).
2. Dried leaflets (10gm) were exhaustively extracted with methanol. Dried extract was diluted with methanol as concentration of 20 mg/ml (SF-A).

Standard Preparation

Lupeol (L), β sitosterol (Bs) concentration were prepared in benzene as 2.5 µg/µl and 2 µg/µl respectively. Rutin (R), Quercetin-3-o-xyloside (Q-G1), Quercetin (Q), Quercetin-3-o-galactoside (M-A2) concentration were prepared in methanol as 47.3 µg/µl, 1.4 µg/µl, 2.5 µg/µl and 2.1 µg/µl respectively.

Sample dosage, Solvent System (Stahl.1996) and Chromatogram development

Plate 1- Standard Q-G1, Sample SF-A and Standard R were dosed in 5µl, 5µl and 1µl quantity on 1st, 2nd, 3rd track respectively and developed in- Ethyl acetate : Ethyl methyl ketone : Formic acid : water (5:3:1:1) system.

Plate 2- Standard MA-2, Sample SF-A and Standard Q were dosed in 5µl each on 1st, 2nd, 3rd track respectively and developed in-Toluene : Ethyl acetate : Formic acid (5: 4 :1) system.

Plate 3- Standard BS, Sample SF-U and Standard L were dosed in 5µl each on 1st, 2nd, 3rd track respectively and developed in – Benzene : Methanol (9:1)

Chromatogram evaluation and estimation

Plates for glycoside and aglycone were scanned at – 254 nm and for sterol and triterpene was scanned at - 200 nm using TLC Scanner III. Estimation of retention factor (Rf) and Area Under Curve (AUC) were done by Integration Software 4.05. Integrated legends are presented as figs. 1-9.

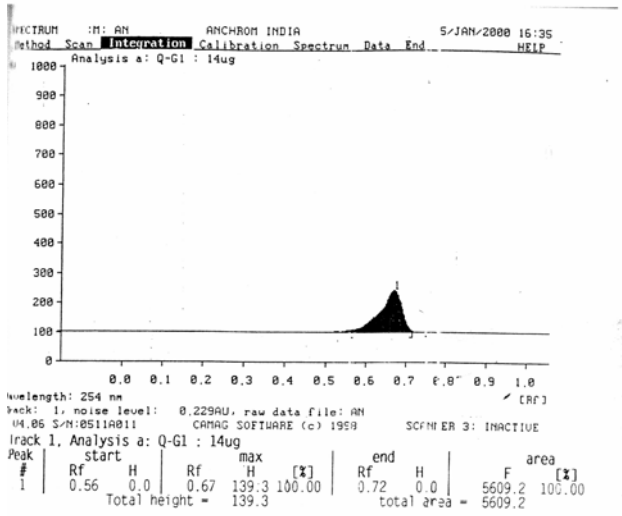

Fig. 1: Plate-1: Integration of AUC of standard Q-G1

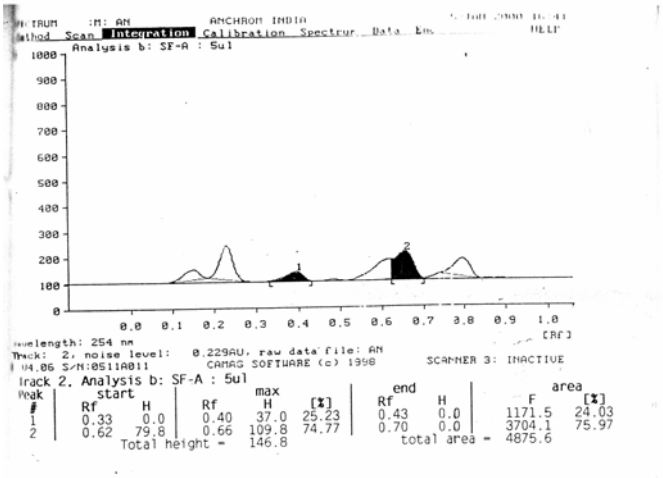

Fig. 2: Plate-1: Integration of AUC of sample SF-A

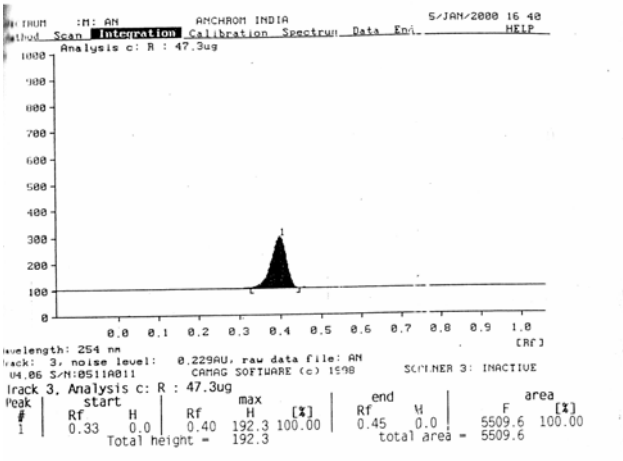

Fig. 3: Plate-1: Integration of AUC of standard R

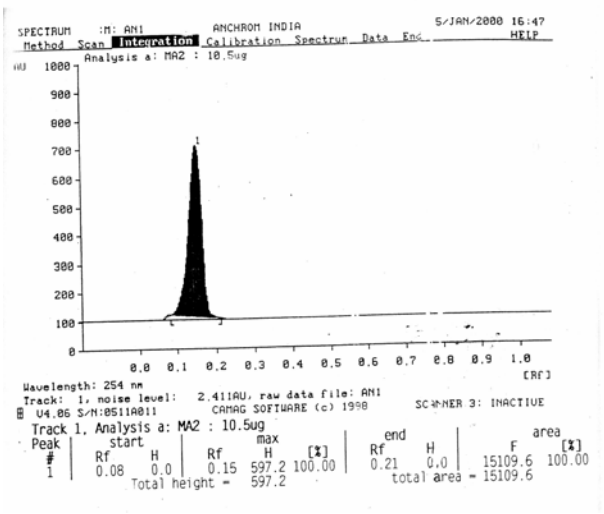

Fig. 4: Plate-2: Integration of AUC of standard MA2.

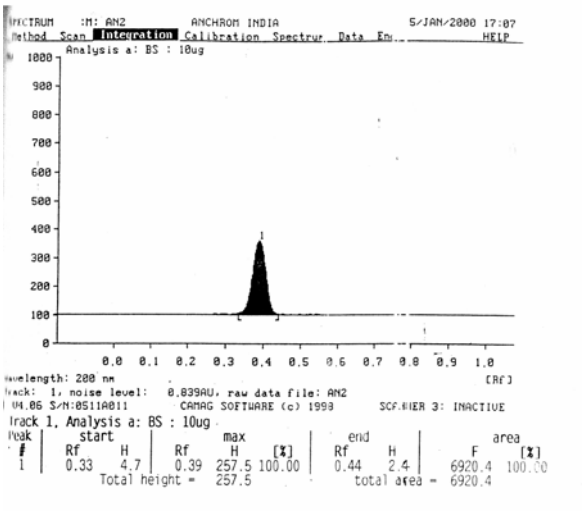

Fig. 7: Plate-3: Integration of AUC of standard BS

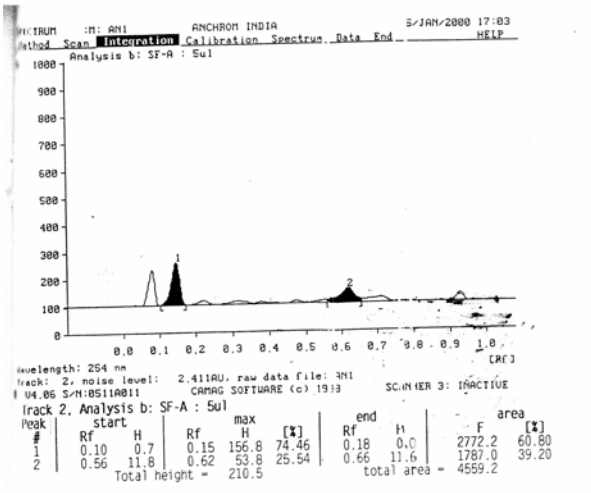

Fig. 5: Plate-2: Integration of AUC of Sample SF-A

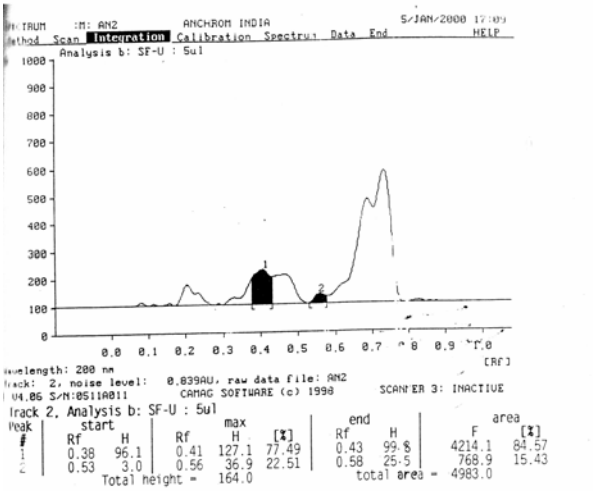

Fig. 8: Plate-3: Integration of AUC of sample SF-U

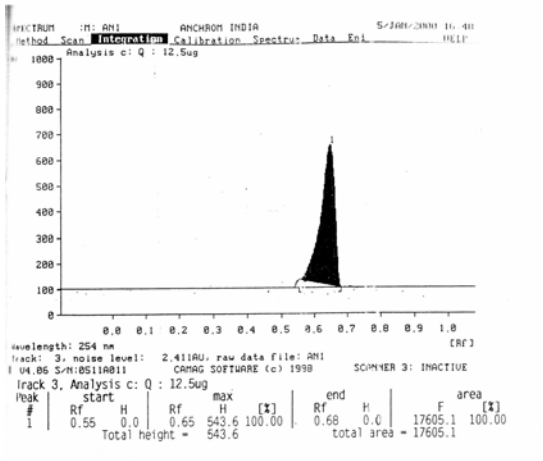

Fig. 6: Plate-2: Integration Of AUC of Standard Q

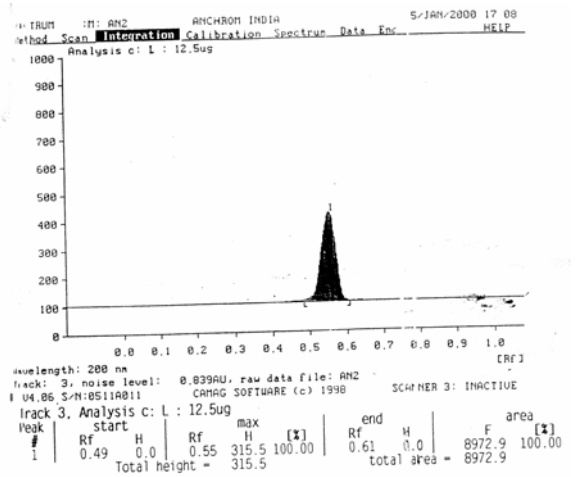

Fig. 9: Plate-3: Integration of AUC of standard L

RESULTS AND DISCUSSION

The present manuscript mainly focus on quantification of new compounds isolated from leaflets.

Percentage of β sitosterol, Lupeol, Quercetin, Quercetin-3-O-galactoside, Quercetin-3-O-xyloside and Quercetin-3-O-rutinoside were determined as 0.02146 % w/w, 0.0377 % w/w, 0.4079 % w/w, 0.6197 % w/w, 2.974 % w/w and 3.235 % w/w respectively. As value of Rutin is quite high so it can serve as commercial source. Drug also shows good amount of flavonols and their glycoside, therefore it will prove better in antioxidant profile also.

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