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EXTRACTION AND ISOLATION OF FLAVONOIDS FROM AERIAL PART OF
STRYCHNOS POTATORUM LINN

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ABSTRACT

Objective: The current study aims to isolation of flavonoid fractions and identification of flavonoid components through LC-ESI/MS method. The results revealed that methanol extract was found as the best solvent for the isolation of flavonoid fractions and several kinds of flavonoids such as rutin, isoquercitrin, narcissin, quercetin, and isorhamnet in were found as the major flavonols in this plant.

Methods: The powdered flower buds of *Strychnospotatorum Linn.* was extracted with methanol. The filtrate was condensed by evaporation, added a phytochemical studies of medicinal plant *Strychnospotatorum Linn.* The ethyl acetate phase thus retained is used for chromatography. The colour and R_f values of these spots were recorded under ultraviolet light (UV 2540Å).

The chromatographic separation of the methanol extract was carried out by HPLC using a reversed phase C-18 (RP C-18) column.

Result: The methanol extract showed the presence of higher flavonoid content when compared with other solvent extracts. The methanol extract was subjected to fractionalization by column chromatography. The eluted fractions were run in TLC mobile phase with the different solvent ratio. The characterization techniques confirmed that the isolated compound was found to be quercetin.

Conclusion: The flavonoid quercetin was isolated effectively from the leaves of *Strychnospotatorum Linn.*

Keywords: High Performance Liquid Chromatography (HPLC), Ultraviolet Light (UV), Liquid Chromatography-Electrospray Ionization/Multi-Stage Mass Spectrometry(LC-ESI/MS), flavonoids.

I. INTRODUCTION

Strychnospotatorum Linn. Family *Loganiaceae* belong to natural order *strychnaceae* and is known as “Nirmali in Hindi”. It is also known by names like nut tree, tettancottaymorum, and kalaka. The *Loganiaceae* are a family of flowering plants classified in order *Gentianales*. This is the most important genus of the family from medicinal and toxicological point of view, and contains a number of alkaloids which are violent tetanic poisons. Some species contain highly toxic alkaloids, most notably *Strychnosnux-vomica*, the chief source of the drug strychnine for rodent control. Brucine is much less toxic and has no commercial important present in the water clearing nut tree *Strychnospotatorum Linn.*, which is used for clearing muddy water is important from the economical point of view¹.

II. METHODS

Preparation of plant extract

The powdered flower buds of *Strychnospotatorum Linn.* was extracted with 10 ml methanol on water bath (333.15 K/300 Second). The filtrate was condensed by evaporation, added a Phytochemical Studies of Medicinal Plant *Strychnospotatorum Linn* mixture of water and EtOAc (10:1), and mixed thoroughly.

Phytochemical screening

Phytochemical screening for constituents was carried out using standard qualitative methods. Screening test was performed for carbohydrates, tannins, saponins flavonoids, cardiac glycosides, terpenoids, triterpenoids, phlobatannins, anthraquinones, alkaloids, quinones, phenols, coumarins, glycosides, proteins, steroids and phytosteroids by following the method of Harborne².

Chromatography:

The ethyl acetate phase thus retained is used for chromatography. The flavonoid spots were separated using Cyclohexane: Ethyl acetate: Formic (78:20:2), n-Hexane: Ethyl acetate: Formic Acid(80:18:2) and n-Hexane: Ethyl ethanoate: Formic Acid (17:20:5) as a solvent mixture. The colour and R_f values of these spots table-1 were recorded under ultraviolet (UV 254nm) light.

The chromatographic separation of the methanol extract was carried out by HPLC using a reversed phase C-18 (RP C-18) column. The mobile phase consisted of solvent A; water– formic acid (99.5: 0.5, v/v) and solvent B; acetonitrile.

Table 1: TLC of Ethanolic (95%) flower extract of Strychnos potatorum Linn.

S. No	Solvent system	Ratio	N. of spots	Resolution
1	Cyclohexane: Ethyl acetate: Formic Acid	78:20:2	5	Good
2	n-Hexane: Ethyl acetate: Formic Acid	80:18:2	4	Good
3	n-Hexane: Ethyl ethanoate: Formic Acid	17:20:5	5	Excellent

Adsorbent – Activated Silicagel-G ; Detecting agent: Iodine chamber.

	R _f Value		
	Solvent mixture-1	Solvent mixture-2	Solvent mixture-3
Compound –A	0.98	0.48	0.35
Compound –B	0.45	0.28	0.24
Compound –C	0.82	0.33	0.33
Compound –D	0.30	0.20	0.19

III. RESULT AND DISCUSSION

Compound Name: Hesperidin (hespridin -7-rutinoside)

IUPAC Name:

(S)-7-(6-O-(6-deoxy- α -L-mannopyranosyl)- β -D-glucopyranosyloxy)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-4-chromanone

Molecular Formula: C₂₈H₃₄O₁₅

Molecular weight: 610.6 u

UV λ_{max} nm: 283 nm, and 325 nm

IR (KBr) ν_{max} cm⁻¹: Aromatic C=C 1466 cm⁻¹(ϵ_{max} 22) and 1656 (ϵ_{max} 10), O-H 2984(ϵ_{max} 47), and 3418 (ϵ_{max} 4), C-OH deformation 1360 cm⁻¹(ϵ_{max} 9), 1295 cm⁻¹(ϵ_{max} 9) and 1202 cm⁻¹(ϵ_{max} 10), >C=O 1656 cm⁻¹(ϵ_{max} 10) and 1603 cm⁻¹(ϵ_{max} 10)

EI/MS m/z (rel. int.): 302 [M⁺-H](20), 286 (10.41), 285(8.91), 271(4.58), 259(3.89), 179(9.82), 165(19.98), 152(64.89), 150(47.56), 137(100), 135(40.45), 129(12.46), 124(12.49), 111(10.86), 77(21.76), 84(22.36), 57(32.52), 43(15.34).

¹³C-NMR(DMSO, 50.32MHz): (C-1) 196.77 ppm, (C-2) 165.16 ppm, (C-3) 163.08 ppm, (C-4) 162.53 ppm, (C-5) 148.01 ppm, (C-6) 146.45 ppm, (C-7) 130.88 ppm, (C-8) 118.08 ppm, (C-9) 114.15 ppm, (C-10) 112.01 ppm, (C-11) 103.37 ppm, (C-12) 100.62 ppm, (C-13) 99.46 ppm, (C-14) 96.43 ppm, (C-15) 95.62 ppm, (C-16) 78.44 ppm,

(C-17) 76.27 ppm, (C-18) 78.44 ppm, (C-19) 75.54 ppm, (C-20) 73.01 ppm, (C-21) 72.11 ppm, (C-22) 70.73 ppm, (C-23) 69.64 ppm, (C-24) 68.38 ppm, (C-25) 66.06 ppm, (C-26) 55.70 ppm, (C-27) 42.06 ppm, (C-28) 17.88 ppm.

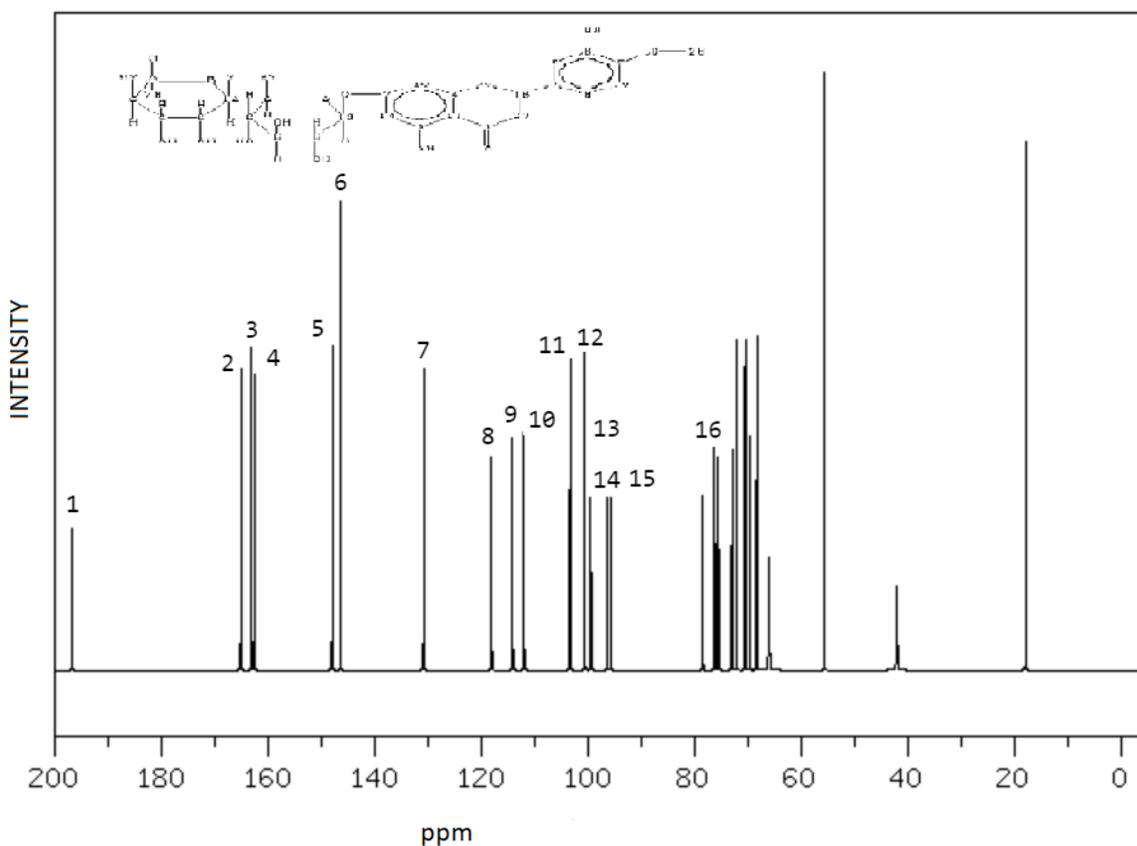
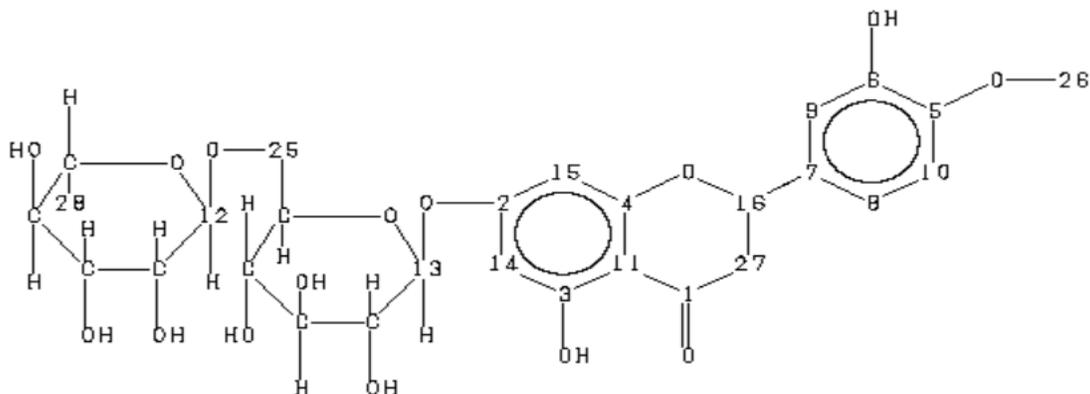
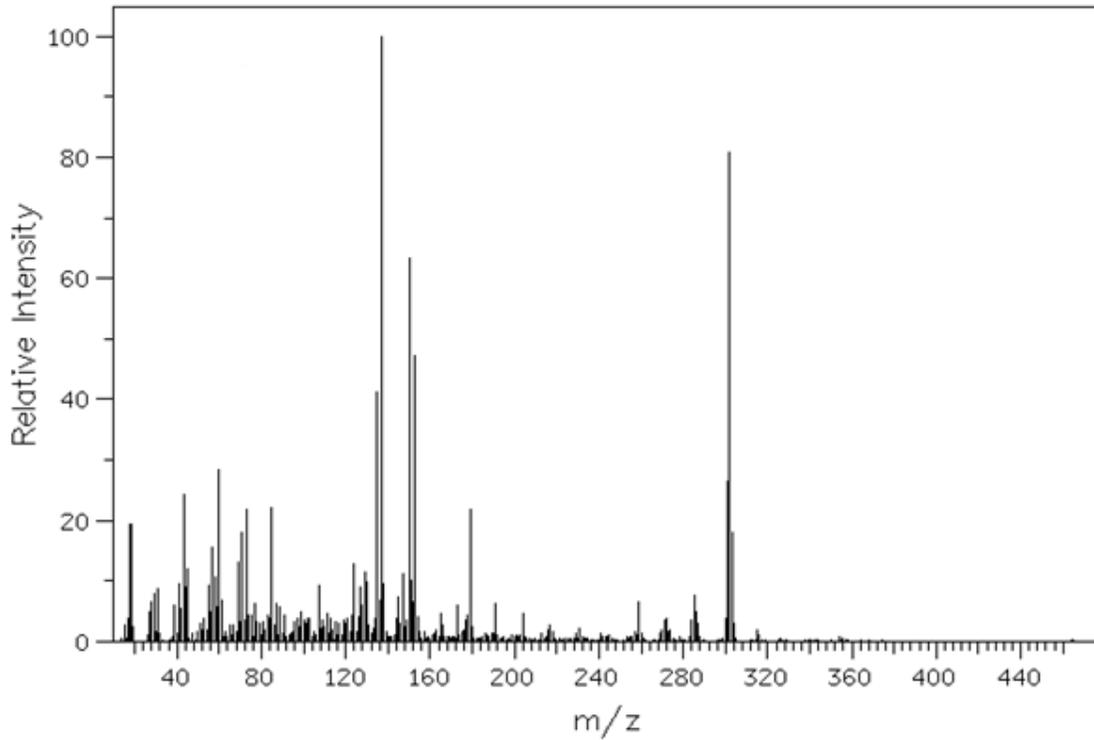


Fig.1 C^{13} -NMR spectra of methanolic extract of Ethanolic (95%) flower extract of *Strychnospotatorum* Linn



Source Temperature: 200 °C

Fig.2 Liquid Chromatography-Electrospray Ionization/Multi-Stage Mass Spectrometry (LC ESI/MS) of Ethanolic (95%) flower extract of *Strychnos potatorum* Linn.

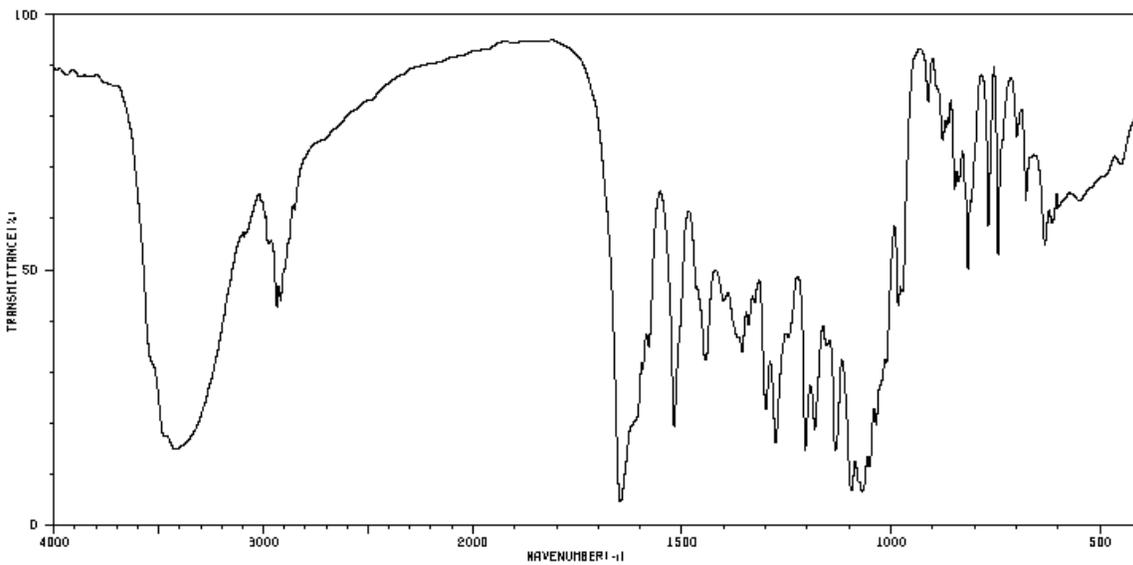


Fig.2 IR spectra of Ethanolic (95%) flower extract of *Strychnos potatorum* Linn

REFERENCES

1. Aherne SA, O'Brien NM (2002) *Dietary flavonols: Chemistry, food content, and metabolism*. *Nutrition* 18: 75-81.
2. Baxter H, Harborne JB (1999) *The handbook of the natural flavonoids*. In: Editor, (ed)[^](eds). *Book The handbook of the natural flavonoids*. Chichester ; New York: John Wiley.
3. Busch KL (2010) *Mass Spectrometry-Mass Spectrometry Retrospective*. *Spectroscopy* 25: 14.
4. Cappiello A, Famiglioni G, Mangani F, Careri M, Lombardi P, Mucchino C (1999) *Liquid chromatographic-mass spectrometric determination of phenolic compounds using a capillary-scale particle beam interface*. *Journal of Chromatography A* 855: 515-527.
5. Careri M, Elviri L, Mangia A (1999) *Validation of a liquid chromatography ionspray mass spectrometry method for the analysis of flavanones, flavones and flavonols*. *Rapid Commun Mass Spectrom* 13: 2399-2405.
6. Cuyckens F, Ma YL, Pocsfalvi G, Claeys M (2000) *Tandem mass spectral strategies for the structural characterization of flavonoid glycosides*. *Analisis* 28: 888-895
7. *Chicester: Wiley-Blackwell Dalluge JJ, Nelson BC, Thomas JB, Sander LC (1998) Selection of column and gradient elution system for the separation of catechins in green tea using high-performance liquid chromatography*. *Journal of Chromatography A* 793: 265-274.
8. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM (1989) *Electrospray Ionization for Mass-Spectrometry of Large Biomolecules*. *Science* 246: 64-71.
9. Harborne JB (1998) *Phytochemical methods : a guide to modern techniques of plant analysis*. In: Editor, (ed)[^](eds). *Book Phytochemical methods : a guide to modern techniques of plant analysis*. 3rd edn. London ; New York: Chapman and Hall
10. Kirkland JJ (2004) *Development of some stationary phases for reversed-phase high-performance liquid chromatography*. *Journal of Chromatography A* 1060: 9-21.
11. McMaster MC (2007) *HPLC, a practical user's guide*. In: Editor, (ed)[^](eds). *Book HPLC, a practical user's guide*. 2nd ed. edn. Hoboken, N.J.: Wiley-Interscience
12. Sanchez-Rabaneda F, Jauregui O, Casals I, Andres-Lacueva C, Izquierdo-Pulido M, Lamuela-Raventos RM (2003a) *Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (Theobroma cacao)*. *J Mass Spectrom* 38: 35-42.