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Determination of flavonoid (quercetin) from *Bryonopsis laciniosa* fruit extract by HPTLC analysis

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Abstract: *Quercetin has been reported to be effective in inflammation, arteriosclerosis, bleeding, allergy and swellings. It is also known to be associated with reduced risk of certain types of Cancers. Quercetin is present in edible fruits and vegetables. It is present in man's daily diet and is known for biological activities such as antioxidant, antiviral, anticancer, anti-microbial, anti-inflammatory and many more. It is not only used as such but also its various derivatized forms have potentials for development into drugs for the treatment of diseases caused by oxidative stress and lethal viruses. HPTLC technique was carried out to establish the flavonoid Quercetin profile of Bryonopsis laciniosa fruit Extract. High Performance Thin Layer Chromatography is an important tool that can be used as quantitatively for checking the purity and identity of crude drug and also for quality control of finished product. The results demonstrates that the fruit extract possess rich content of Quercetin (58.59%).Therefore, the data generated from this experiment provide the Quercetin as therapeutic agent for treating various ailments. This study offers a platform for using flavonoid of Bryonopsis laciniosa fruit as herbal alternative for various diseases.*

Key Words: *Bryonia laciniosa, Quercetin, HPTLC, Flavonoid, Densitogram.*

1. INTRODUCTION:

India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicines and generally Indians have rich knowledge in traditional medicine [(1)]. Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicines, nutraceuticals, intermediates and chemical entities for synthetic drugs [(2)].Herbs are used in various traditional systems of medicines and plays a major role in the process of drug discovery through the identification of active compounds. Phytochemicals are natural bioactive compounds found in plant foods that works with dietary nutrients to protect our body against disease.[(3,4)].Traditional medicine is widely used because of its cultural acceptability, economic affordability and compatibility with the human body, lesser side effects and efficacy against certain types of diseases as compared to modern medicines [(5)].

Phytochemicals are non- nutritive plant chemicals, working together with nutrients found in leaves, fruits, vegetables and nuts may help to slow the aging process and reduce the risk of many diseases [(6)]. Flavonoids are highly effective phytochemical which one of the important components is in the human diet [(7)]. They are present in foods generally as O-glycosides with sugars bound at C3 position. Biomolecular Activities of Flavonoids are Antioxidative effects, inactivation of oxygen radicals, Binding of electrophils, Induction of protective enzymes, Apoptosis rate increase, Cell proliferation inhibition, Lipid peroxidation inhibition, Angiogenesis inhibition, H-Donation, DNA oxidation inhibition. Quercetin reserves the topmost sight in flavonoids which provides many health promoting benefits, including improvement of cardiovascular health, eye diseases, allergic disorders, arthritis, reducing risk for cancers and many more [(8)].

1.1. Bryonopsis laciniosa:

Bryonopsis laciniosa is genus of about 6 species of annual slender plants in the family cucurbitaceae, native to throughout India from the Himalayas to Ceylon, tropical Africa, Australia and is one of the most versatile medicinal plants having a wide spectrum of biological activity *Bryonopsis laciniosa* is a highly valuable medicinal cucurbit commonly known as lollipop climber and it is called as "Shivlingi" in India [(9,10)].Plant leaves and fruits are cooked as vegetables so they are edible in nature [(11)]. The juices of leaves are used in treatment of jaundice [(12)].Leaves and seeds are anti-inflammatory [(13)], antimicrobial [(14)] and febrifuge and release the stress [(15)]. They are used to treat flatulence, fever and reduce inflammation [(16)]. The seeds are used in Homeopathy and Ayurveda as a tonic for females and they rejuvenate female reproductive system and promotes conception of child. In males the seeds promote spermatogenesis and increase sperm count [(17)]. The seeds are also used as antidote for snake bite [(18)] and antibacterial and anti-fungal [(19)]. In Homeopathy, a tincture made from the roots is the lollipop plant is prescribed for the treatment of inflammation of uterus, vaginal disorders and other urinary genital problems [(20)]. Whole plant is used to treat ailments such as diabetes

[9], asthma, cough and bronchitis. *Bryonopsis laciniosa* is widely employed as herbal drug for the treatment of gastrointestinal, analgesic [21], respiratory, rheumatic and metabolic disorders, as well as for liver and infectious diseases [14]. The aim of this study is to obtain a further understanding of the reported beneficial health effects of Quercetin, its pharmacological effects and also to evaluate its safety in *Bryonopsis laciniosa (Linn)* fruit extract with aid of HPTLC technique which may provide an insight in its use in traditional medicine.

2. MATERIALS AND METHODS:

2.1 Collection and preparation of B*ryonopsis laciniosa* fruit extract:

Bryonopsis laciniosa (Linn) is an annual slender herb and is widely spread in India. This plant fruit was collected from Ramanathapuram District. Each specimen was washed under running tap water, labelled, weighed and annotated with the date of collection. Each specimen dried at 37^0C for 15 days, powdered and stored in air tight container.2gm of dried finely powdered plant material was taken in a beaker. 30ml of distilled water and 70ml of methanol was added. The mixture was shaken by continuous stirring at room temperature for 30 minutes and kept for 2 days [22]. Then the solvent was allowed to evaporate and the extract was used for the analysis.

2.2 HPTLC analysis of flavonoid (quercetin) profile:

HPTLC studies were carried out by the standard method [23]

Sample preparation and application: The 25mg of sample was weighed accurately, dissolved in 125µl of 70% Methanol solvent and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.5 µl of test solution and 2 µl of standard solution were loaded as 5mm band length in the 2 x 10 Silica gel $60F_{254}$ TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. Quercetin standard was used as a reference marker.

Developing solvent system and development of chromatogram: Toluene-Acetone-Formic acid in the ratio of 4.5: 4.5: 1 was used as mobile phase. After the application of sample, the chromatogram was developed in Twin trough glass chamber 10x10 cm saturated with solvent Toluene-Acetone-Formic acid in the above ratio for 15 min.

Spot development and photo documentation: The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapour) with respective mobile phase (Flavonoid) and the plate was developed up to 90mm.The developed plate was dried by hot air to evaporate solvents from the plate. FolinCio-Calteu reagent is used as spraying reagent. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at visible light, UV 254nm and UV366nm.

Scanning and derivatization: The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 254 nm. The Peak table, Peak display and Peak Densitogram were noted. The software used was win CATS 1.3.4 version. The developed plate was sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented in Visible light mode using Photo-documentation (CAMAG REPROSTAR 3) chamber.

3. RESULTS AND DISCUSSION:

HPTLC analysis of the methanol fruit extract of *Bryonopsis laciniosa* was carried out along with the standard flavonoid quercetin. Blue, Brown coloured zone at Visible mode was present in the track, it was observed from the chromatogram after derivatization, which confirmed the Presence of Quercetin in the fruit extract. The identity of the bands of quercetin in the methanol extract was confirmed by comparing the UV-Vis absorption spectra with those of standards using a CAMAG TLC scanner 3. The standard quercetin has RF value of 0.78 and the sample RF shows 0.80 (Peak table 1).The peak purity of quercetin was assessed by comparing the spectra at peak start, peak apex and peak end positions of the spot. HPLC spectra of standard Quercetin shows peak at height of 674.1 in mobile phase and fruit extract shows peak at the height of 475.3 as compared to standard Quercetin, this shows the method specificity. By comparing the area of sample and standard, calculated the percentage of Quercetin in sample using the formula.*Bryonopsis laciniosa* fruit extract contains 58.59% of Quercetin

$$Percentage\ of\ Quercetin = \frac{Area\ of\ BL\ Fruit\ Extract\ Sample}{Area\ of\ Standard} \times 100$$

$$Percentage\ of\ Quercetin = \frac{12699.8}{21675.4} \times 100$$

$$Percentage\ of\ Quercetin = 58.59\%$$

HPTLC technique could be considered as an accurate and precise method for the determination of flavonoid [23]. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots were well resolved [24].It is common knowledge that flavonoid are related to various beneficial effects exerted on human health. Among flavonoids, quercetin (Qu) is considered an excellent free-radical scavenging antioxidant. Apart from antioxidant activity, Quercetin also exerts a direct, pro-apoptotic effect in tumour cells, and can indeed block the growth of several human cancer cell lines at different phases of the cell cycle [25]. Quercetin is one of the naturally occurring dietary flavonol compounds present abundantly in plants and has chemo preventive and anticancer effects [26].Quercetin is a versatile molecule with many

pharmacological properties including antioxidant, neurological, antiviral, anticancer, cardiovascular, antimicrobial, anti-inflammatory, hepatoprotective, protective of the reproductive system and anti-obesity agent [27].The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry and plant systematic studies. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials, and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. The HPTLC studies conducted with the methanol fruit extract of Bryonopsis laciniosa revealed a rich amount of flavonoid quercetin, which confirms its medicinal value.

Peak Table 3.1

Track	Peak	Rf	Height	Area	Assigned substance
Sample BL	1	0.09	110.4	2781.6	Flavonoid 1
Sample BL	2	0.15	84.9	1254.1	Unknown
Sample BL	3	0.18	12.2	129.4	Unknown
Sample BL	4	0.21	21.9	234.1	Unknown
Sample BL	5	0.25	83.7	2184.5	Unknown
Sample BL	6	0.80	475.3	12699.8	**Quercetin**
Sample BL	7	0.83	296.4	28332.7	Flavonoid 2
STD	1	0.78	674.1	21675.4	**Quercetin standard**

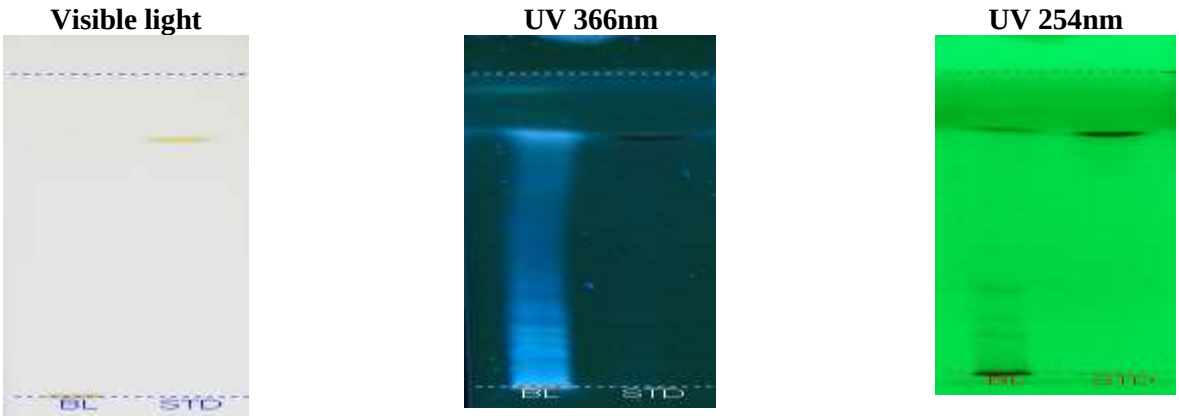


Figure 1. Chromatogram- Before derivatization

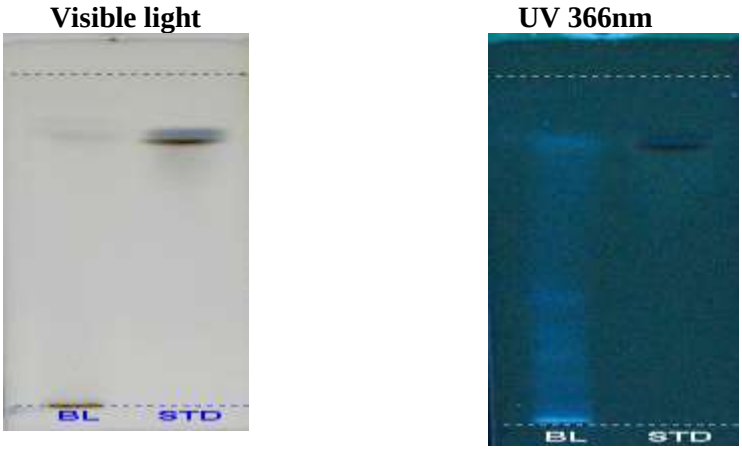
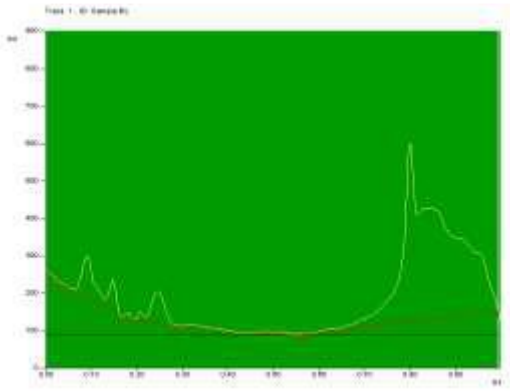
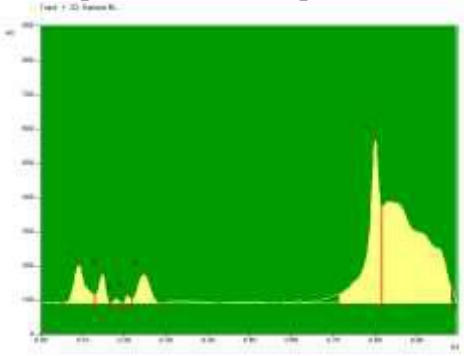


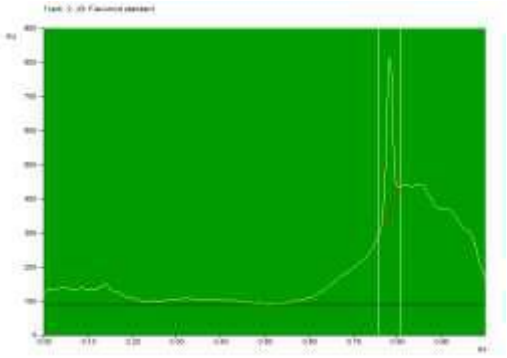
Figure 2. Chromatogram -After derivatization



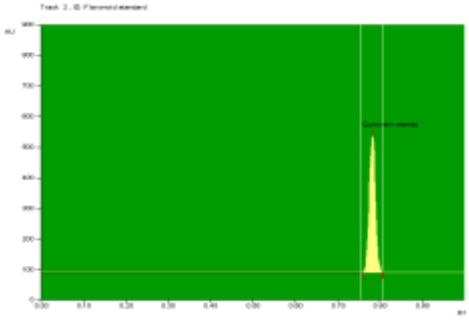
Graph 3.1 Track 1 – Sample BL plant extract sample-Baseline display (Scanned at 254 nm



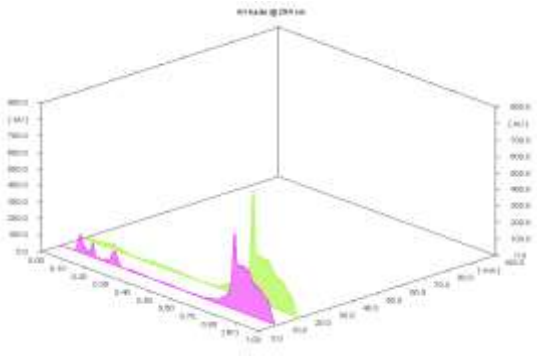
Graph 3.2 Track 1 – Sample BL plant extract sample –Peak Densitogram display (Scanned at 254 nm)



Graph 3.3 Track STD – Quercetin standard Baseline display (Scanned at 254 nm)



Graph 3.4 Track STD – Quercetin standard Peak densitogram display (Scanned at 254 nm)



Graph 3.5 3D display of all Tracks

4. CONCLUSION:

HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. The HPTLC studies conducted with the methanol fruit extract of Bryonopsis laciniosa revealed a rich amount of flavonoid quercetin, which confirms its medicinal value.

Conflict of interest: This work has no conflict of interest

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REFERENCES

1. Hamedeyazdan S, Fathiazad F, Sharifi S, Nazemiyeh H. (20120. Ant proliferative Activity of *Marrubiumpersicum* Extract in the MCF-7 Human Breast Cancer Cell Line. Asian Pac. J. Cancer Prev; 13(11): 5843-5848.
2. Ncube NS, Afolayan AJ, Okoh AI. (2008).Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotechnol; 7(12): 1797-1806.
3. Ramya B, Malarvili T, Velavan S. (2015a).Micronutrients and Vitamin Analysis of *Bryonopsis laciniosa* Fruits. Int J Pharm Bio Sci; 6(4): 265-273.
4. Christother L, Ankamah M. (2014).Botanicals for Managing Cardiovascular disorders: A review of medicinal weeds on knust campus 2014; 3 (9): 349 - 358.
5. Anthonio OA, Roland NN. (2013).Phytochemical analysis and antibacterial evaluation of the ethyl acetate extract of the stem bark of *Brideliamicrantha*. Pharmacogn Mag; 9(33): 45-50.
6. Pal D, Banerjee S, Ghosh AK. (2012). Dietary-induced cancer prevention: An expanding research arena of emerging diet related to healthcare system. J Adv Pharm Tech Res 2012; 3(1): 16-21.
7. Yao LH, Jiang YM, Shi J, Tomás-Barberán FA, Datta N .(2004) Flavonoids in food and their health benefits. Plant Foods Hum Nutr**,** 59(3): 113-122.
8. ParulLakhanpal, and Deepak Kumar Rai (2007).Quercetin: a versatile flavonoid. IJMU; 2 (2):22-37.
9. Ujjwala Supe. (2016). In Vitro characteristics of callus induction of *Bryonia Laciniosa*– A medicinal plant. International Journal of Innovations in Engineering and Science; l (1)2: 7-10
10. Alekhya M, Prathiba G, Nazmi A, Sultana R, Dayala D (2014). Pharmacognostic and Preliminary Phytochemical Screening of Plant *Bryonopsis laciniosa* Linn. Leaves. J Scientific Res Pharmacy; 3(1): 45-48.
11. Bandyopadhyay S, Mukherjee SK. (2009).Wild edible plants of Koch Bihar district, West Bengal. Nat. Prod. Radiance; 8: 64-72.
12. Vhotracharcho C Chironji b .Banaushadhi, vol. 2, 4th Edn. Ananda PublisheresPtv. Ltd.: Calcutta 1987; 96-100.
13. Gupta M, Mazumdar UK, Sivakumar T, Vamsi ML, Karki SS, Sambathkumar R (2003) .Evaluation of anti-inflammatory activity of chloroform extract of *Bryonia laciniosa* in experimental animal models. Biol Pharm Bull; 26: 1342-1344.
14. Singh V, Malviya T, Tripathi DN, Naraian U. (2009). An Escherichia coli antimicrobial effect of arabinoglucomannan from fruit of *Bryonia lacinosa*. CarbohydrPolym; 75: 534-537.
15. Gabrielian SE, Gevorgovich A (1997) .Bryonia, as novel plant adoptogen, for prevention and treatment of stress induced disorders. Promising Res Abstr; PRA- 5003:1-8.

16. Moghe AS, Gangal SG, Shilkar PR (2011). In vitro cytotoxicity of *Bryonia laciniosa* (Linn.) on human cancer cell lines. Ind J Nat Prod Res; 322-329.
17. Chauhan NS, Dixit VK. (2010). Effects of *Bryonia laciniosa* seeds on sexual behaviour of male rats. Int J Impotence Res; 22: 190-195.
18. Bonyadi E, Awad V, Nirichan K. (2009).Antimicrobial activity of Ethanolic extract of *Bryonopsis laciniosa* leaf, stem, fruit and seed. Afr J Biotechnol; 8(15): 3565-3567.
19. Saxena N, Balyari N, Srivastva A. (2004). Pharmacological studies of novel pharmaceutical saponin molecules of seeds of *Bryonia laciniosa*. IUPAC. Internationational conference on biodiversity, natural product chemistry and medicinal applications, New Delhi; 26-31: 368.
20. Mosaddik MA, Haque ME. (2003). Cytotoxicity and Antimicrobial Activity of Goniothalamin Isolated from *Bryonopsis laciniosa*. Phytother Res; 17: 1155-1157.
21. Reddy JD, Gnanasekaran D, Vijay D, Ranganathan TV.(2010) In vitro studies on anti-asthmatic, analgesic and anti convulsant activities of the medicinal plant *Bryonia laciniosa*. Linn. Int J Drug Discovery; 2: 1-10.
22. Ramya B, Malarvili T, Velavan S. (2015b) GC-MS Analysis of Bioactive compounds in *Bryonopsis Laciniosa* Fruits. IJPSR; 6(8): 1000-1005.
23. Mythili T and Ravindhran R (2013).Determination of Quercetin by HPTLC Method in SesbaniaSesban (L.) Merr. Stem Extract.Int J Adv Pharm Biol Chem; 2 (1):113-118.
24. Sushma G S, B. Archana Devi, CH. Madhulatha, K. Uday Kumar, P. Harathi, N. Siva Subramanian and M. Ramadevi (2013).Preliminary phytochemical screening and HPTLC fingerprinting of leaf extracts of *Ficus nervosa Heyne ex Roth.* J Chem Pharm Res; 5(3):98-104
25. Lara Gibellini, Marcello Pinti, Milena Nasi, Jonas P.Montagna, Sara De Biasi, Erika Roat, Linda Bertoncelli, Edwin L. Cooper, and Andrea Cossarizza.(2011). Quercetin and Cancer Chemoprevention. Evid *Based* Complement Alternat Med.2011; 1-15
26. GuominNiu, Songmei Yin, ShuangfengXie, Yiqing Li, DanianNie, Liping Ma, XiujuWang, and Yudan Wu. (2011).Quercetin induces apoptosis by activating caspase-3 and regulating Bcl-2 and cyclooxygenase-2 pathways in human HL-60 cells. Acta Biochim Biophys; 43: 30–37
27. AneelaMaalik, Farhan A. Khan, Amara Mumtaz, AdeemMehmood, SairaAzhar, Muhammad Atif, Sabiha Karim, YasirAltaf ,Imran Tariq (2014). Pharmacological Applications of Quercetin and its Derivatives: A Short Review. Trop J Pharm Res; 13 (9): 1561-1566.