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Qualitative and quantitative estimation of gallic acid in flower galls of *Crataeva religiosa* through FT-IR and HPTLC techniques

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ABSTRACT

In the present study, FT- IR (FourierTransform Infrared spectroscopy) analysis of galled flower was conducted to qualitatively identify gallic acid while comparative HPTLC (High-Performance ThinLayer Chromatography) studies of normal and galled flower extracts confirmed the gallic acid quantitatively. The quantitative estimation of gallic acid was carried out on silica gel 60 F_{254}HPTLC plates (stationary phase). The plates were developed in a solvent system as prepared with Toluene, Acetone and Glacial acetic acid at a ratio of 3:1:2, in a CAMAG glass twin trough chamber of 20X10 cm, at a temperature of 25 ± 2^0C. Detection was carried out densitometrically at ë=220 nm. The FTIR analysis was done with a spectrophotometer system, which was used to identify and analyze the characteristic peaks and their respective functional groups. HPTLC analysis revealed that gallic acid content increased almost two folds in gall tissues as compared to normal tissues. *C. religiosa* flower galls can be utilized medicinally due to enhanced gallic acid.This is the first report of the occurrence of gallic acid in flower galls of *C.religiosa*.

Figure : 00 References : 21 Tables : 02
KEY WORDS : *Crataeva religiosa*, Flower galls, FTIR, Gallic acid, HPTLC

Abbreviations:

FT : FourierTransform
GA : Gallic Acid
ROS : Reactive oxygen species
FTIR : FourierTransform Infrared spectroscopy
HPTLC: High-Performance Thin Layer Chromatography
HPLC : High-Performance Liquid Chromatography
ATR : Attenuated Total Reflection

Introduction

Crataeva religiosa Forst. (Family- Capparidaceae) is found distributed in tropical regions and is one of the herbal drugs, used in urolithiasis. The biotic stress induced by the gall-forming insect Diptera: Cecidomyiidae on the anatomy and physiology of the flowers of *C. religiosa* leads to distinct functionalities of tissues, culminating into a new organogenetic array. Anatomical studies of flower galls showed isodiametric parenchymatous cells with continuous hyperplasia and hypertrophy.

Crataeva religiosa Forst. (Family: Capparidaceae) also known as Baruna or Varuna is a common tree found throughout India, Sri Lanka, Myanmar, Japan, Australia, and several south pacific islands[14]. The Barun trees are economically, commercially and medicinally important because of their timber, fruit and seeds. This plant is well known for its various pharmacological properties like hepatoprotectant, lithotriptic, antirheumatic, antihelminthic, rubefacient, antioxidant, antioxaluric, antiperiodic, antimycotic, contraceptive, antipyretic, anti-inflammatory, laxative, antilithiatic, and vesicant properties[15]. The bark of the *Crataeva religiosa* is useful in removing kidney stones and in curing urinary disorders. The crude drug contains an active triterpenoid *i.e.,* lupeol, which is mainly involved in the pharmacological activities of this plant[2]. The flowers and fruits are unpleasantly distressed by the insect, *Aschistonyx crataevae* Mani, (Order-Diptera). The mature flower galls of *C. religiosa* are yellowish-white,irregular parenchymatous swellings of the complete flower.The floral parts got completely hypertrophied and fused. The normal differentiation of tissue is lacking in the portion of the gall, extensive undifferentiated parenchyma was found. The cells of the gall showed continuous hyperplasia and hypertrophy.

The growth and development of gall are controlled by the insect and therefore represent an extended phenotype of the gall formation[5]. The continuous feeding activity by the insect prompts the plant to respond, which alters the biochemistry of a zoocecidia (Gall). The subsequent feeding and oviposition by the insect elicit a cascade of events including the formation of Reactive oxygen species (ROS). The accumulation of phenols and auxins at gall sites is mediated by controlled levels of ROS.This accumulation contributes to gall formation *via* biochemical alterations and new cell developmental pathways[13]. Plants produce a high diversity of secondary metabolites used for defense and survival, in response to various biotic and abiotic stresses. The central components of host defense prompt the synthesis of secondary metabolites at the site of the attack that sometimes can be harmful or toxic to insects. The higher amount of polyphenols such as tannins, gallic acid (gallotannins), condensed tannins, *etc.* in leaf galls of *Quercus leucotrichophora* and *Lannea coromandelica* may be correlated to a triggered defense mechanism in gall tissues[9,16]. Earlier we have estimated a higher amount of phenols biochemically in flower galls of *Crataeva religiosa* as compared to normal counterparts[20]. The involvement of phenolic compounds in the abnormal growth of plants has been reviewed by several workers[8,11,13].

Production and accumulation of free radicals upon oxidative stress is the leading cause of several degenerative diseases such as cardiovascular diseases,atherosclerosis,aging,cancer,inflammatory diseases, *etc*.[17]. All polyphenols (naturally occurring antioxidants) are known for antiviral, antiulcer, antifungal, antibacterial, anticancer,and anticholesterol activities. Among various polyphenols, gallic acid is a naturally occurring low molecular weight triphenolic compound, which has emerged as a strong antioxidant[1].

Gallic acid (GA) (IUPAC name- 3, 4, 5-trihydroxybenzoic acid) is a polyphenol. The chemical structure of gallic acid represents one benzene ring and two functional groups in the same molecule that are hydroxyl groups and a carboxylic acid group. Gallic acid exists in plant material in the form of esters, free acids, hydrolyzable tannins and catechin derivatives and is one of the most biologically-active phenolic compounds of plant origin[7]. The antioxidant activity of gallic acid and its derivatives has been reported[6]. Quantitative estimation of gallic acid in *Quercus infectoria* Oliv. galls using HPTLC was done.[4] A recent review stated that gallic acid is enormously used in dye making, pharmaceutical,leather making,chemical,and food industries[3]. Gallic acid is characterized as antioxidative, antimicrobial, anticarcinogenic, hypotensive, serum lipid reducing, anti atherogenic, anticariogenic and heavy metal chelating agent[21].

Significant importance of phenolic compounds in the defense system of plants needs to be evaluated further in various plant-insect interactions. The present investigation deals with the quantitative and qualitative estimation of Gallic acid from flower gall of *C. religiosa* and their normal counterpart by using FTIR and HPTLC techniques.

Materials and Methods

2.1 Collection of plant samples

Plants of *Crataeva religiosa* were surveyed in the flowering season *i.e.,* March-April in Jaipur and adjoining areas. The mature healthy flowers and old flower galls of *Crataeva religiosa* were observed and collected from the Central Park of Jaipur. Plant was authenticated (RUBL-13249) by Herbarium, Department of Botany, University of Rajasthan, Jaipur for further studies.

2.2 FTIR Analysis

The Fourier Transform(FT) results in spectrum that is used to identify or quantify specific compounds. This technique is based on interferometry which records information about a material/ compound placed in the IR beam. In the present investigation, normal flower and flower galls were analyzed and different functional groups were identified with the help of wavelength observed in the FTIR interferogram.

0.1 g of the dried crude powder was taken and evaluated directly by Attenuated Total Reflection (ATR) mode, in which an IR beam was directed onto an optically dense crystal. The powdered sample of both extracts was loaded in an FTIR spectroscope, and the functional groups were analyzed using Fourier-transform infrared with a scan range in the region 4000–400 cm^{-1}.

2.3 HPTLC Analysis

2.3.1 Preparation of Plant extracts

Collected plant material (normal and galled flowers) was dried at room temperature under shade, powdered and soxhlet extracted with water for 24 hrs. The extracts were then filtered using Whatman filter paper and dried in a rotary vacuum evaporator.

2.3.2 Reagents and Other Materials

Gallic acid (Sigma Aldrich), toluene, acetone, glacial acetic acid, and methanol (all Reagents of analytical grade, E-Merck) and silica gel 60 F_{254} precoated TLC aluminium plates (E-Merck).

2.3.3 Instrumentation Conditions for HPTLC

A Camag HPTLC ("Linomat5_192428") comprising of Camag TLC Scanner-3, Linomate V automatic sample applicator, Camag Win CAT software, Camag Twin trough

TABLE-1: FTIR spectral peak absorption values and functional groups obtained for flower gall extracts.

Plant Sample	Absorption Ranges (cm-1)	FunctionalGroups Name	Type of Vibration
Flower Gall	3287.42	Phenols and Alcohols Alkanes	O-H stretch
	2935.4	Carboxylic acids Alkenes	H-C-H stretch C=O
	1738.03	Nitro group Ethers and Esters	stretch
	1628.66	Ethers and Esters	C-C=C stretch N=O
	1368.72		bend
	1222.62		C-O and C=O
	1017.16		stretch
			C-O and C=O
			stretch

chamber, Hamilton Syringe, and stationary phase precoated silica gel (60 F_{254}) were used.

2.3.4 HPTLC Quantification of the Extracts

Standard solution of gallic acid was applied in aliquots of 3ìL, 6ìL, 9ìL, 12ìL, 15ìL. Bandwidth was set at 8 mm,distance between bands was10mm. Aliquots were applied in triplicates on a precoated silica gel plates E Merck 60 F_{254} (20 x10cm, 0.2 mm thickness) (stationary phase). Solvent system comprising Toluene, Acetone, and Glacial acetic acid in the ratio of 3:1:2 (v/v/v) was used to run HPTLC for 15 mins at the temperature of 25±2^{0}C.

Developed plates were air-dried and scanned at 220nm using CAMAG TLCscanner-3andWINCATS IV controlling software.The peak areas were recorded and calibration curves were obtained by plotting peak area *vs* concentration.10iL of each of the powdered samples in solutions were applied in duplicate on the same TLC plate using CAMAG Linomat V semiautomatic spotter. The peak areas in developed plates were recorded and the amount of gallic acid was calculated from the respective calibration curves.

2.3.5 Calibration Curve of the Standard:

Calibration curves for the concentration of gallic acid were found to be linear over the concentration range. Standard working solutions were used in duplicate to determine and confirm linearity. The peak area and concentration were subjected for analysis to calculate the calibration equation Y = 0.9342 + 0.0482 X and regression coefficient (r^2) was r =0.98658.The Rf value of standard gallic acid was recorded to be 0.65. Bands of gallic acid in samples were confirmed by overlaying respective UV absorption spectra with that of standards. The average content of the Gallic acid in samples of normal and galled tissues was expressed in microgram(ìg).

Results and Discussion

IR spectrum of wave number (cm-1) *vs* %T gives sufficient information about the structure of compounds or the number of functional groups present in the sample. Observations of FTIR analysis of flower galls are given in interferograms and the pertaining results are given in Table 1. The flower gall extract exhibited absorption band at 3287.42 cm-^{1}(O-H stretch), 2935.40cm-^{1}, 1738.03, 1628.66 cm-^{1}(C=O stretch),1368.72 cm-^{1} (Phenol (or) tertiary alcohol, OH bend),1222.62 cm-1(C-O stretch), and1017.16cm-^{1} (C-C stretch)and revealed the presence of Phenols,Alcohols, Alkanes, Carboxylic acids, Alkenes, Nitro group, Ethers and Esters. Hence the presence of carboxylic acid and –OH groups are indicating the presence of Gallic acid in flower gall extract. Involvement of phenolic compounds in the abnormal growth of plants has been reviewed by several workers[8,11,13.] Similar five peaks which are characteristics of Gallic acid include 3409.38 cm-1(polymeric OH stretch), 1639.45 cm-1(C=O stretch), 1389.62 cm-1(Phenol (or) tertiary alcohol, OH bend), 1276.15 cm-1(C-O stretch), and 1016.36 cm-1(C-C stretch) were recorded to identify gallic acid in *Pseudarthria viscida* root extract[18].

TABLE-2: Chromatographic data for HPTLC of Gallic acid in normal flower and galled flower extracts.

S.No.	Sample	Rf	Maximum Height	Area	Content (ìg)
1.	Standard Gallic acid	0.65	122.37	2993.1	2.500 ìg
2.	Normal Flower	0.63	76.12	1692.6	1.560 ìg
3.	GalledFlower	0.65	384.92	11621.9	2.750 ìg

HPTLC provides a chromatographic fingerprint of phytochemicals and is used to confirm the presence and purity of raw materials of medicinal plants.The active principles (chromatic data) with their respective Rf value, area, maximum height and content in microgram (ìg) obtained for normal flower and flower galls are shown in Table 2. The standard gallic acid and both samples were statistically resolved with Rf values 0.65, 0.63, and 0.65 respectively. The nearly equal Rf values prove the significant presence of Gallic acid.

HPTLC fingerprinting after chromatography of gallic acid in standard and different samples are there . The gallic acid bands in the sample chromatogram were confirmed by the chromatogram obtained from the reference standard solution. HPTLC densitograms for standard gallic acid and both the samples are there. The results showed that the method used in this study revealed good fingerprinting and good resolution of gallic acid from *C. religiosa* normal flower and galled flower tissues samples. The HPTLC analysis of normal and galled flower extracts showed that the gallic acid amount in galled tissues has increased almost twofold due to insect attacks. Similar findings were reported earlier for *Madhuca longifolia* in which galled leaf had a maximum amount of gallic acid (344.4 ng) while normal leaf had less amount of gallic acid (180 ng)[10]. Gallic acid content was found at 1.560ìg/mg in normal flowers and 2.750ìg/mg in galled flowers. Elevated levels of gallic acid were observed in cotton plant as a response to herbivory by *Spodoptera litura*[19].

Conclusions

FTIR and HPTLC analysis confirmed the qualitative presence of Gallic acid in gall extract. Chemical structure of flower gall was detected by IR spectrum that confirmed the presence of Gallic acid. The various functional groups observed in the flower gall extract indicate the presence of phenolic compounds, carbohydrates, amino acids, glycogen, starch, amides, cellulose, and lipids. The good antioxidant and anti-inflammatory activity of flower gall extract could also be correlated with the presence of different functional groups, especially phenols and alcohols. The quantitative estimation showed that biotic stress exerted by insects led to the production of more gallic acid in galls than in the normal flower tissues. This can be concluded from the result that production of polyphenolic compound (gallic acid) is increased due to defense mechanism of host plant as a response to insect attack and hence *C.religiosa* flower galls can be a good source of gallic acid.The gallic acid can be enhanced with the help of biotechnological advances or *in vitro* techniques. Gallic acid is firstly reported and isolated from *C. religiosa* flower galls.

References

1. Badhani B, Sharma N, Kakkar R. Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. *Res Adv.* 2015; **5**: 40-57.
2. Bani S, Kaul A, Khan B, Ahmad SF, Suri KA, Gupta BD, Satti NK, Qazi GN. Suppression of T lymphocyte activity by lupeol isolated from *Crataeva religiosa*. *Phytother Res.* 2006; **20**: 279-287. doi:10.1002/ptr.1852.
3. Choubey S, Varughese LR, Kumar V, Beniwal V. Medicinal importance of gallic acid and its ester derivatives: a patent review. *Pharm Pat Anal.* 2015; **4**(4): 305–315.
4. Dash GK, Ansari MT, Sami F, Majeed S. Proximate analysis and quantitative estimation of gallic acid in *Quercus infectoria*oliv. galls by HPTLC. *Intern Jour PharmacSci Res.* 2016; **7**(11): 4400-06.
5. Egan SP, Comerford MS, Zhang L, Hood GR. Botanical parasitism of an insect by a parasitic vine. *Curr Biol*. 2018; **28**: 863–864.
6. Gramza A, Korczak J, Amarowicz R. Tea polyphenols – their antioxidant properties and biological activity – a review. *Pol J Food Nutr. Sci.* 2005; **14**(55): 219–235.

7. Karamaæ M, Kosiñska A, Pegg RB. Content of gallic acid in selected plant extracts. *Pol J Food & Nutri Sci.* 2006; **15**(1): 8-55.
8. Kumar A, Lakshman K, Jayaveera K, Mani N, Tripathi SN, Satish KV. Estimation of Gallic Acid, Rutin and Quercetin in *Terminalia chebula* by HPTLC. *Jordan J Pharm Sci.* 2010; **3**: 63–68.
9. Kumar S, Tanwer BS, Lodha P. Biochemical analysis of *Lannea coromandelica* L. leaf galls induced by *Odenia diplosisodinae*.*Int J Pharm Sci Rev Res.* 2015; **31**(1): 158–163.
10. Kumkum Mishra P, Meena R, Patni V. Isolation, identification and quantification of gallic acid (gallotannins) through HPTLC in leaf galls of *Madhuca longifolia* (Koenig) j.f. Macb. *Int J Res Pharm Sci.* 2020; **11**(1): 1142-1148.
11. Kuster CV, Costa RU, Fernandes CJC, Isaias RMS, Oliveira D. How Galling Organisms Manipulate the Secondary Metabolites in the Host Plant Tissues? A Histochemical Overview in Neotropical Gall Systems. *Springer Cham.* 2020; 823-842.
12. Mukherjee N, Mukherjee S, Saini P, Roy PP, Sinha BS. Phenolics and Terpenoids; the promising new search for Anthelmintics: A Critical Review. *Bentham Science Publishers.* 2016; **16**(17): 1415-1441.
13. Oliveira DC, Isaias RMS, Fernandes GW, Ferreira BG, Carneiro RGS, Fuzaro L. Manipulation of host plant cells and tissues by gall-inducing insects and adaptive strategies used by different feeding guilds. *J Insect Physiol.* 2016; **84**: 103-113.
14. Parker P. Nematode control of silver leaf nightshade (*Solanum elaeagnifolium*); a biological control pilot project. *Weed Science.* 1986; **34**(S1): 33-34.
15. Patil UH, Gaikwad DK. Medicinal profile of a Scared Drug in Ayurveda: *Crataeva religiosa* a review. *J Pharm Sci & Res.* 2011; **3** (1): 923-929.
16. Patni V, Sharma N, Mishra P. Oak (*Quercus leucotrichophora*) galls, as an intense source of natural gallic acid. *Int Jour Life Sci.* 2012; **1**(3): 186–186.
17. Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *Int J Biomed Sci.* 2008; **4**(2): 89–96.
18. Rajan T, Muthukrishnana S. Characterization of Phenolic Compounds in *Pseudarthria viscida* Root Extract By Hplc And Ft-Ir Analysis. *Asian Journal of Pharmaceutical and Clinical Research.* 2013; **6** (2): 273-275.
19. Rani PU, Pratyusha S. Defensive role of *Gossypium hirsutum* L. anti-oxidative enzymes and phenolic acids in response to *Spodoptera litura* F. feeding. *Journal of Asia-Pacific Entomology.* 2013; **16**(2): 131-136.
20. Sharma S. *In-vitro* and *in-vivo* quantitative estimation of total phenols, ascorbic acid content and antioxidant activity in normal flower and flower gall of *Crataeva religiosa*. *Int J Pharm Sci & Res.* 2019; **10**(11): 5103-08.
21. Verhagen J, Scott TH, Giza BK. Method of inducing sweetness by gallic acid and its applications. US Patent Application Publication. 2002; Pp. 426-548.