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Isolation, identification and quantification of gallic acid (gallotannins) through HPTLC in leaf galls of *Madhuca longifolia* (Koenig) j.f. Macb

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ABSTRACT

Plant galls (cecidia) are pathologically produced cells, tissues, or organs of plants that have developed by hypertrophy and hyperplasia of plant tissues under the effect of gall causing organisms. *Madhuca Longifolia* (Koenig) j.f Macb. is an economically and medicinally very important plant of the family Sapotaceae. It is a tropical mixed deciduous tree. Various galls due to insect infestation are found on almost all tree species. Leaf galls of *Madhuca longifolia* induced by insects of order Diptera and Hymenoptera represent a unique pattern of chemical perturbations which normally do not occur in normal leaf. During the present investigation, an accurate, fast and easy HPTLC method was followed for quantification of gallic acid occur in the normal leaf and dried leaf galls of the *Madhuca longifolia* plant. The protocol followed in this study resulted in an intense peak and was able to give a good resolution of gallic acid from normal leaf and galled tissues of *Madhuca longifolia* (Koenig) j.f. Macb. Adaxial leaf gall induced by insect *Mohwadiplosis Orientalis* Rao belonging to Diptera class, Marginal gall induced by another Dipteran and vein gall by chalcidoiid Hymenopteran class of insect. Variation in Gallic acid present in normal leaf and leaf galls of *Madhuca longifolia* was critically reported. It was found that Gallic acid content increased almost two folds in gall tissues as compared to normal leaf tissue. Galled leaf (Dipteran adaxial gall) had a maximum amount of Gallic acid (344.4 ng) while in normal leaf and another leaf galls had less amount of gallic acid was found (180 ng). This study is of practical importance because gallic acid is the most important active phenolic acid, which may be correlated with post-infection biochemical defense. Compound gallic acid has been reported for the first time from leaf galls of *Madhuca longifolia* (Koenig) j.f. Macb. Plant in the study.

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INTRODUCTION

Madhuca longifolia (Koenig) j.f. Macb. known as Madhuca or mahua is a large, tropical evergreen tree mainly distributed in Nepal, India and Srilanka (Saluja *et al.*, 2011). (Figure 2 & Figure 3). In India, the Madhuca tree is widely distributed in various states of India, such as Madhya-Pradesh, Bihar, Gujrat, Jharkhand, Andhra-Pradesh, Uttar-Pradesh Rajasthan and Chhattisgarh. It is a multipurpose forest tree of India that provides food, fodder, fuel and medicines (Ramadan *et al.*, 2006). The leaves of *Madhuca longifolia* have several medicinal proper-

ties due to the presence of beta- carotene and

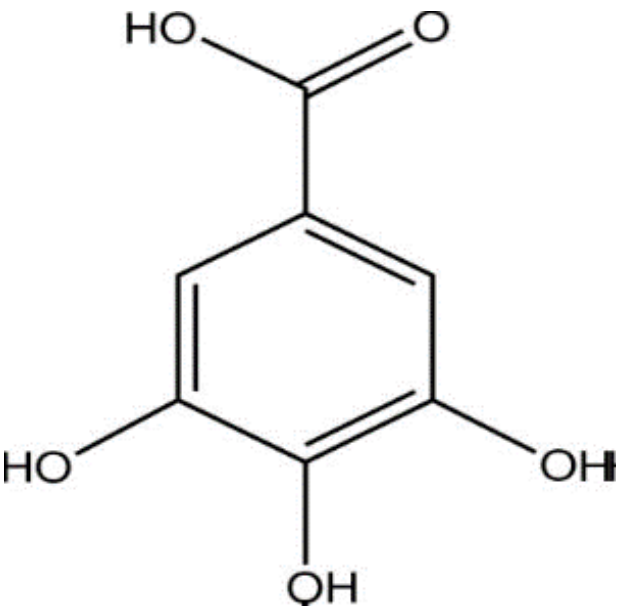

Figure 1: Chemical Structure of Gallic Acid

xanthophylls; erthrodiol, palmitic acid, oleic acid, myricetin, sitosterol, quercetin and many more. So that it is used for chronic bronchitis and Cushings disease and to cure eczema (Prajapati *et al.*, 2003). The oil extracted from the seeds of *Madhuca longifolia* is utilized for cooking purposes by local peoples. Madhuca seeds powder has various therapeutic uses; therefore, the seed oil of Madhuca used as biodiesel (Bindu and Ringmichon, 2016). Apart from these some insect pest and diseases has been reported from such an important multipurpose tree, but leaf galls has not been reported so-far from this tree. The insects galls (Zoocecidia) are unique examples of complex interactions and mutual adaptation between the host plant and gall maker insects, characterized by cellular hypertrophy and hyperplasia. The galls on the leaves of *Madhuca longifolia* are caused by insects of order diptera and Hymenoptera. Three different types of galls of different shapes and sizes on different areas of leaves were seen. Three different types of galls are as following,

Dipteran Adaxial gall (DAG)

This pustule like, greenish- yellowish gall was found on the adaxial surface of Madhuca leaf, which is caused by an insect *Mohwadiplosis Orientalis* Rao of cecidomyiid of order Diptera (Figure 4) .

Dipteran Marginal gall (DMG)

This frill or fringe-like brownish galls occurred on the margins of the whole lamina of the leaf of *Madhuca longifolia* and caused by an insect of cecidomyiid (Gall midge) of order Diptera (Figure 5).

Hymenopteran vein gall (HVG)

These fusiform, unilocular and dark brownish galls are found on the abaxial surface, on every vein of a leaf of *Madhuca longifolia*, caused by insect chalcidoiid of order Hymenoptera. All of these three galls (DAG, DMG and HVG) looks different in their size, shape, and colour (Figure 6). These gall-inducing insects cause biotic stress to the host plant. Stress can leads to various results. Stress can have a devastating impact on plant growth and yield (Suzuki *et al.*, 2014). These secondary metabolites have the capacity to induce the changes into plant cell thats helps to overcome the stress (Mazid *et al.*, 2011).

The qualitative and quantitative analysis of leaf galls of *Quercus leucotrichophora* and *Lannea coromandelica* revealed the presence of a higher amount of various phenolics such as Phenol, orthodihydroxy phenol, tannins, gallotannins (gallic acid). The increase in levels of phenolics may be attributed to a defense mechanism (Mishra and Patni, 2008; Kumar *et al.*, 2015).

Gallic acid (trihydroxybenzoic acid) is a most important phenolic acid which is obtained from nutgalls and other plants or by the hydrolysis of tannic acid. The IUPAC (chemical) name of Gallic acid is 3,4,5 trihydroxy benzoic acid. The chemical structure of gallic acid (Figure 1) has one benzene ring and two functional groups in the same molecule that's are hydroxyl groups and a carboxylic acid group. Gallic acid and its derivatives are used in making dyes and inks photographic developers and has been used as astringents in medicine. Some gallets are used as antioxidants in foods. The Hydrolysis of Hydrolyzable tannins provides gallic acid and ellagic acid, which is known as gallotannins and ellagitannins, respectively (Ritzer and Sundermann, 2000). Gallic acid plays an important role in the pharmaceutical industry (Waterhouse, 1999). Gallic acid used as a standard for determining the phenol content of various analytes by the Folin-Ciocalteau assay (Fernandes and Salgado, 2016). The name Gallic acid is derived from oak galls, which were historically used to prepare tannic acid. Oak galls are relatively rich in tannin (as ellagic acid. Gallic acid and ellagic acid has been reported from many plant species viz. Nymphaea stellata Willd (Rakesh *et al.*, 2009), *Lantana camara* (Jaafar *et al.*, 2018), and *Woodfordia fruticosa* (Grover *et al.*, 2014). The leaf galls of oak ((*Quercus leucotrichophora*) have been reported as an intense source of gallic acid (Patni *et al.*, 2012) . Quantitative estimation of gallic acid is important to decode the defense responses taking place in the

host tissues. In the present study, isolation, identification and quantitative estimation of gallic acid con-

Figure 2: Tree of *Madhuca longifolia* (Habit)

tent from leaf galls of *Madhuca longifolia* (Koenig) j. f. Macb. was carried out.

MATERIALS AND METHODS

Collection of plant samples

The normal and galled leaves of *Madhuca longifolia* were collected from the tehsil weir of district Bharatpur, Rajasthan and authenticated by the Herbarium of Dept. of Botany of the university of Rajasthan Jaipur. (RUBL20635).

Preparation of plant extracts

The collected normal leaf samples and galled tissues of *Madhuca longifolia* used in the present investigation were washed with tap water to remove dust and sand, and dried at room temperature. The dried plant materials were grinded into powder. These powdered materials were used for further HPTLC analysis.

Equipment and glassware

Linomats TLC Applicator, Oven, TLC developing chamber, visualizing chamber, pre-coated aluminum plates with silica gel 60f254, beaker, measuring cylinder with stoppered and glass pippets, Camang TLC scanner and photographic chamber.

Reagent and other Chemicals used

Toluene, ethyle acetate, formic acid and Gallic acid. Reference standard Gallic acid was purchased from Sigma Chemicals.

Instrumentation Conditions for HPTLC

Stationary Phase

Precoated silica gel plates Merck 60 F254 (15 x 10cm, 0.2 mm thickness)

Mobile Phase

Toluene: ethyl acetate: formic acid, 5:5:1 (v/v/v)

Chamber saturation time

20 mins.

Development Mode

Camang TLC scanner and photographic chamber.

Preparation of standard and test solution

The standard solution of gallic acid was made by dissolving 25 mg of gallic acid in 25 ml of methanol and takes 0.5 ml of the above solution and diluted up to 10 ml of methanol. To prepare the test samples, 5gm of normal leaf and galled tissues of *Madhuca longifolia* plant was extracted by cold maceration in methanol and then filter the liquid extract. Made the volume up to 50 ml with methanol.

Procedure for HPTLC analysis of gallic acid

Previously dried TLC plates were taken and dimention was fixed at X position and marked from the base with the help of pencil at 10 mm and 90 mm. and also left 15mm from both sides of the plate. The reference standard solution of gallic acid 2,4,6,8 and 10 ul was applied in the forms of bands with the programming of Linomats applicator. The test sample solution 2ul in triplicate was applied in the forms of bands with the programming of Linomats applicator. The solvent was evaporated and the plates were placed vertically in the saturated tank

that's by bands or spots obtained above the level of the mobile phase. After that the tank was closed and allowed to stand at room temperature until the mobile phase ascended to the marked line. The plate was removed, dried and visualized as in UV light at 254 nm. The scanning programme was prepared for completely dried plates at 300 nm. The calibration curve was ploted by scan data of different spots of standard gallic acid varied for concentration and concentration of unknown test samples bands (Normal leaf and galled tissues) was calculated by linear plot of a calibration curve.

Figure 3: Normal Leaf of *Madhuca Longifolia*

Figure 4: Dipteran adaxial Gall (DAG)

Figure 5: Dipteran Marginal Gall (DMG)

Figure 6: Hymenopteran Vein Gall (HVG)

HPTLC Quantification of the normal leaf and leaf galls extracts

The Gallic acid content of various extracts (normal leaf and galled tissues) was determined by comparing the area of chromatogram with the calibration curve of concentration of standards. The Rf value of standard Gallic acid (0.70) was compared with the Rf value of the normal leaf and leaf galls of *Madhuca longifolia* plant. Quantitative estimation of the plate was performed in the remission/absorption mode at 254 nm, with the following conditions slit width 6.00x0.30mm, micro scanning speed 20mm/s and data resolution 100 μm step. Calibration parameters were as follows: calibration mode- single level, statistics mode-cv, evolution mode- peak height. The average content of the Gallic acid in different samples of normal and galled tissues of Madhuca *longifolia* was expressed in nanogram (ng).

Table 1: Chromatographic data for HPTLC of Gallic acid in normal leaf and galled tissues of *Madhuca longifolia*

S.No.	Type of Sample	Rf	Maximum Height	Area	Content in (ng)
1	Standard gallic acid	0.70	205.8	5368.8	100ng
2	Normal Leaf	0.70	24.8	571.0	180.00ng
3	DAG	0.70	123.4	3092.7	344.4ng
4	DMG	0.70	15.2	330.4	180.00ng
5	HVG	0.73	38.5	907.5	180.00ng

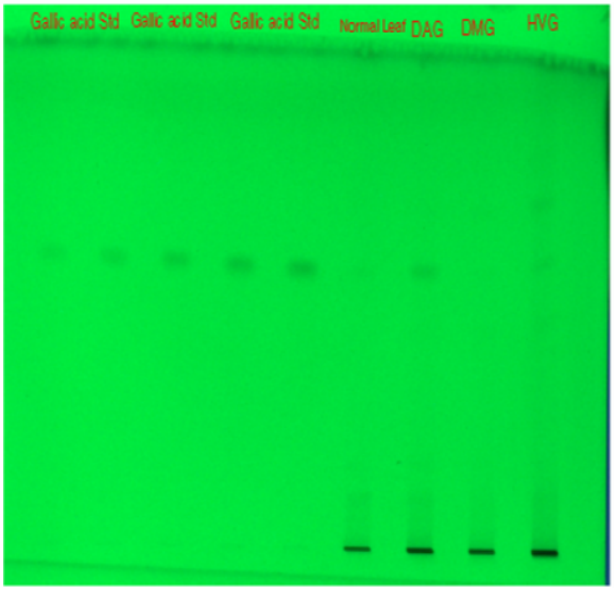

Figure 7: HPTLC Finger Printing of Standard and Galled Leaf

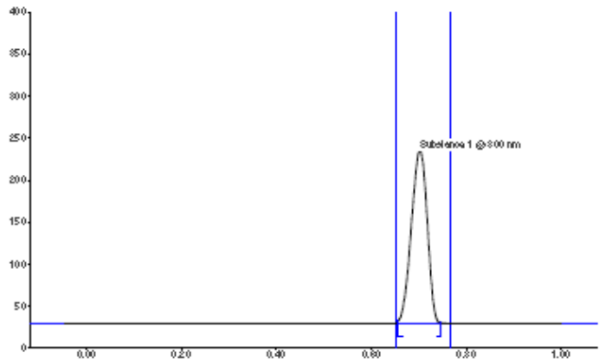

Figure 8: HPTLC chromatogram of normal leaf and galled standard gallic acid

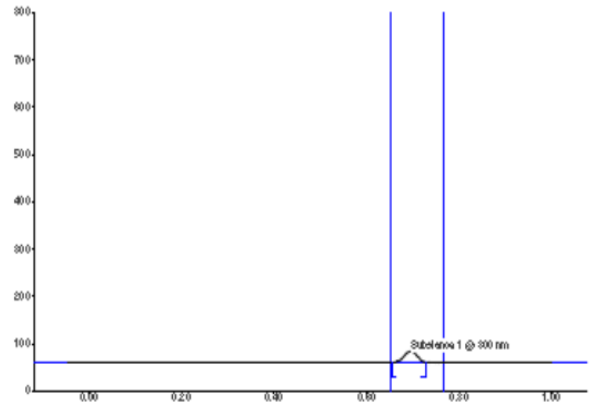

Figure 9: HPTLC chromatogram of normal leaf

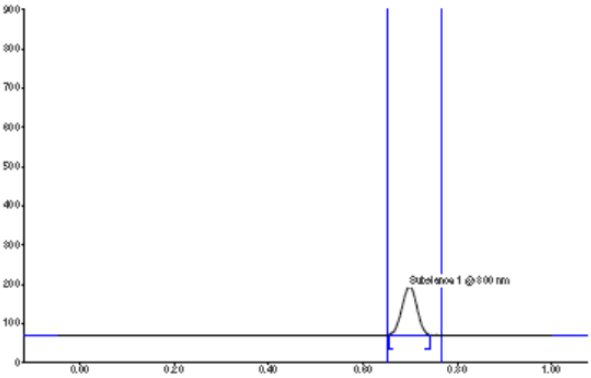

Figure 10: HPTLC chromatogram of DAG

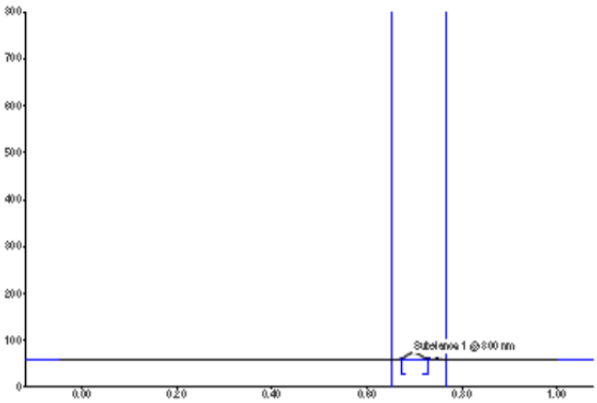

Figure 11: HPTLC chromatogram of DMG

RESULTS AND DISCUSSION

In this present study, the normal leaf and leaf galls of *Madhuca longifolia* were analyzed for isolation, identification and quantification of gallic acid through the HPTLC method. Result of HPTLC fingerprinting after chromatography of gallic acid standard and a methanolic extract of the normal leaf and galled tissue DAG (Dipteran adaxial gall), DMG

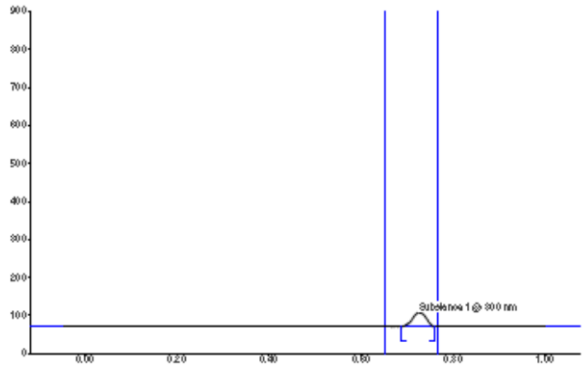

Figure 12: HPTLC chromatogram of HVG

(Dipteran marginal gall) and HVG (Hymenopteran vein gall) samples of *Madhuca longifolia* (Koenig) j. f. Macb. is shown in (Figure 7) The identity of the gallic acid bands in the sample chromatogram was confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution. The chromatogram of standard gallic acid is shown in (Figure 8) and that of gallic acid identified in normal leaf (Figure 9) and leaf galls sample (DAG, DMG and HVG) are shown in (Figures 10 and 11 & Figure 12) respectively. The active principles (chromatic data) with their respective Rf value, area, maximum height and content in ng obtained for normal leaf and leaf galls are shown in Table 1. The peak corresponding to Gallic acid in plants normal and galled samples (0.70, 0.70, 0.70 & 0.73), respectively from the sample solution had almost the same retention factor as that of standard Gallic acid (0.70). Quantitative analysis is an important tool to provide information of the composition and level of the active components contained in a plant material (Fraisse *et al.*, 2011), in which the major one are generally responsible for some particular pharmacological effects including antioxidant effect, antimicrobial, anti-inflammatory, anticancer, cardio- protective, gastro- protective, and neuro- protective effects (Song *et al.*, 2007; Choubey *et al.*, 2015).

The result showed that the method used in this study revealed good fingerprinting and good resolution of gallic acid from *Madhuca longifolia* normal leaf and galled tissues samples. Gallic acid was identified through HPTLC in both normal leaf and galled tissues of *Madhuca longifolia* plant. Different amount of Gallic acid in normal leaf and galled tissues samples in *Madhuca longifolia* was observed. The HPTLC analysis of normal and galled leaf (DAG, DMG and HVG) of *Madhuca longifolia* (Koenig) j. f. Macb. Showed that Dipteran adaxial gall (DAG) had a maximum amount of gallic acid (344.43 ng)) while normal leaf had less amount (180.o ng) amount. The another galled tissue DMG (Dipteran marginal gall) had 180.0 ng and Hymenopteran vein gall (HVG) had a similar amount 180.0 ng of gallic acid.

CONCLUSIONS

This study showed that the Galled Leaf (under Stressed condition) led to the production of more gallic acid than the normal leaf (under non-stressed condition) of *the Madhuca longifolia* plant. On the basis of the result, it is concluded that elevated quantity of condensed gallotannins (GALLIC ACID) founded in gall tissues rather than a normal leaf, can be overexpressed with the help of biotechnological advances and can play an important role in commercial production of gallic acid and its different derivatives. The result obtained from the study may be used to diagnose this gallic acid (gallotannins) Which is the key bioactive compound found in this plant need enhancement treatment to improve the quantity of bioactive compound. Gallic acid is firstly reported and isolated from leaf galls of *Madhuca longifolia* (Koenig) j. f. Macb. for the first time.

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