



Research Article

PRELIMINARY PHYTOCHEMICAL SCREENING AND HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY[HPTLC] DETECTION OF PHENOLIC ACIDS IN *LANATA CAMARA* LEAVES CULTIVATED IN IRAQ

Noor S Jaafar, Maha N Hamad, Dhuha A. Alshammaa *, Maryam R Abd

Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq

*Corresponding Author Email: alshammaadhuha6@gmail.com

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ABSTRACT

Objective: the objective of this work was to investigate the phytochemical groups and to detect phenolic acids in *L. camara* leaves using high-performance thin-layer chromatography (HPTLC). Methods: Leaves of *L. camara* were macerated thrice a week in petroleum ether, then in ethanol. Powdered leaves and ethanolic extract were subjected to phytochemical investigation. Acidic hydrolysis were performed on the extract, and then fractionated by petroleum ether, chloroform, ethyl acetate, and n-butanol. The chloroform and ethyl acetate fractions were analyzed by HPTLC for their phenolic acid contents. Result: *L. camara* leaves devoid of alkaloids. Three different phenolic acids were detected in the leaf extract. Conclusion: Gallic acid, caffeic acid and p-coumaric acid were detected in *L. camara* leaves

Keywords: *Lantana camara*, preliminary screening, phenolic acids, HPTLC.

INTRODUCTION

Lantana camara L. (Family: Verbenaceae) sometimes also known as wild or red sage¹, is an ornamental flowering evergreen shrub whose indigenous origin from America and Africa, it spreads over fifty countries^{2,3}. Because *L. camara* predominates the native types and result in biodiversity loss so considered as a noxious weed⁴, nevertheless it is recognized as one of the important medicinal plants⁵. *Lantana camara* L is a perennial, struggling, erect, aromatic odor shrub with diverse flower color as orange, red, pink, white and violet, it is growing to a height of about 3 meters and 2.5 meters width^{6,7}, this shrub mainly uses as an herbal medicine, but also as a source of firewood and mulch⁸.

A great variation in *L. Camera* chemical constituents have been demonstrated according to geographical regions or climate⁹. Phyosterols, saponins, flavonoids, tannins, coumarins, anthocyanins, alkaloids, essential oil and glycosides have been identified in *Lantana camara* fruits and leaves^{5,7,10-12}.

The whole plant is traditionally used for numerous illness¹³ were it's used as a remedy respiratory problems as bronchitis⁷ for rheumatism, wounds, headache, toothache, for malaria, epilepsy, urinary stone, skin problems and others¹⁴.

The leaves have antimicrobial¹⁵, antiurolithiatic, antioxidant^{16,17}, hypoglycemic¹⁷, antiulcer, antispasmodic¹⁸, antimotility, anthelmintic¹⁹, cytotoxic²⁰, analgesic¹⁴, antipyretic, hypolipidemic, wound healing²¹ and larvicidal effects²².

Phenolic acids (phenolcarboxylic acids) are non-flavonoids poly phenolic compounds [23], considered as a subclass of the larger phenolics family, designated as phenols having one carboxylic acid functionality²⁴. Biosynthetically phenolic acids produced from shikmic acid via the phenylpropanoid pathway²⁵.

Predominantly phenolic acids are of plant origin, but some of them are of microbial origin²⁶. Based on the constitutive carbon frameworks, two classes of phenolic acids are distinguished hydroxycinnamic (C6 - C3) and hydroxybenzoic (C6- C1) structures²⁷. The number and the position of the hydroxyl functions on aromatic ring create the variation in phenolic acids, though the basic skeleton remains the same²⁸. The hydroxycinnamic acids as (p-coumaric, caffeic, sinapic and ferulic) are more common than the other class (hydroxybenzoic acids) and mostly occur as esters of quinic acid and glucose such as chlorogenic acid (an ester of caffeic acid and quinic acid). Inedible plants hydroxybenzoic acids (vanillic, p-hydroxy benzoic and protocatechuic acid) content is usually low, except onions, black radish and certain red fruits. Hydroxybenzoic acid derivatives predominantly occur as glycosides^{29,30}.

Pharmacologically phenolic acids act as antioxidants³¹, anti-inflammatory³² antiviral and antibacterial³³, anti-allergic and other effects³⁴.

The aim of this work was to investigate the phytochemicals and to detect phenolic acids in *L. camara* leaves using HPTLC.

MATERIAL AND METHODS

Collection of plant materials

Leaves of *Lanata camara* were collected from garden of college of pharmacy/Baghdad University in November 2017, the plant was authenticated by specialist botanist in our pharmacy college without specimen number.

The leaves were thoroughly washed under running tap water, then distilled water, shade dried at room temperature for two weeks, grind in an electric, grinder to get fine powder.

Equipment and chemicals

The instruments used were rotary evaporator (BÜCHI Rotavapor R-205, Swiss) and HPTLC (Eike Reich/CAMAG–Laborator, Switzerland). All chemicals and solvents (ethanol, petroleum ether 60-80 °C, chloroform, ethyl acetate, n-butanol, formic acid and hydrochloric acid) used were of analytical grade and obtained from Riedel-de Haen, Germany. The standard Gallic acid, Caffeic acid and P-Coumaric acid were purchased from Chengdu Biopurify phytochemicals, China (purity>97). HPTLC plates silica gel 60 F 254 (20 x 10cm) from E. MERCK KGaA was used in the analysis.

Extraction

Fifty grams of leaves were macerated thrice a week in 500 ml petroleum ether 60-80 °C with occasional shaking, then filtration, the dried marc was macerated thrice a week in 500 ml ethanol, and the filtrates were dried under reduced pressure to get dry extracts.

Phytochemical investigation for crude extract

Chemical tests are used to identify the presence of phytochemicals. The crude extract and powdered leaves were subjected to phytochemical investigation for flavonoids, tannins, alkaloids, saponins and phytosterols.

Test for flavonoids (alkaline reagent test)

The crude leaves extract was mixed with 2 ml of 2% sodium hydroxide solution. An intense yellow color which vanishes upon addition of a few drops of dilutes acid indicate the presence of flavonoids³⁵.

Test for tannins

In a test tube contains 0.5 g of extract, 10 ml of water was added and heated till boiling then filtration. A few drops of 0.1 % ferric chloride solution were added to filtrate. The presence of tannins was confirmed through the formation of brownish green or a blue-black color³⁶.

Test for alkaloids

A test tube containing 0.5 g of powdered leaves, 1.5 ml of ammonia solution was added to it, allowed to stand for minutes. 5 ml of chloroform was added to this mixture, which was shaken, filtered to remove the powder. The chloroform was evaporated by water bath. 1 ml of Mayer's reagent was added. The presence of alkaloid was confirmed through the formation of creamy colored precipitate³⁷.

Test for saponins

A pinch of the dried powdered leaves was added to a test tube contains 2-3 ml of distilled water. The mixture was shaken vigorously. The presence of saponin is indicated by foam formation³⁸.

Test for phytosterols

Fifty mg of the extract was dissolved in 2 ml of acetic anhydride, then into this mixture a few drops of concentrated sulfuric acid were added slowly along the sides of each test tube, the presence of phytosterol is indicating an array of color changes³⁹.

Hydrolysis of crude ethanolic extract

Three grams of the crude extract were boiled with 150ml of 5% HCl solution for seven hours using reflux. The solution was cooled and partitioned with 150ml x 2 of petroleum ether 60-80 °C, chloroform, ethyl acetate and n-butanol successively. The first three fractions were dried over anhydrous sodium sulfate, filtered and evaporated to dryness under reduced pressure using a rotary evaporator.

HPTLC analysis

Chloroform and ethyl acetate fractions were also analyzed for their phenolic acid contents utilizing HPTLC (Eike Reich/CAMAG–Laboratory, Switzerland), using HPTLC plates silica gel 60 F 254, 20 x 10cm, developed in twin trough chamber (20 x 10cm) previously saturated with a mobile phase composed chloroform: ethyl acetate: formic acid (25:20:5) for 20 min. The examination was done at 254, 366 nm wavelength, daylight and after spraying with alcoholic KOH.

Standard solutions of Gallic acid, caffeic acid and p-coumaric acid and samples (chloroform and ethyl acetate fractions) were prepared by dissolving 1 mg of each in 1 ml of methanol.

RESULT AND DISCUSSION

Preliminary phytochemical screening tests are useful in the recognition of bioactive principles and consequently may lead to drug or medicine discovery and development. In this study, several phytochemical constituents have been identified. Preliminary phytochemical screening of *Lanata camara* leaves revealed the presence of flavonoids, tannins, steroids and saponins, while alkaloids are absent^{9,40}. These phytochemicals might be responsible for various medicinal characteristics¹³. The result of the phytochemical analysis is listed in table 1.

HPTLC becoming a popular method for analysis and quantification of compounds present in plant extract, because of better resolution, short analysis duration, small amount of mobile phase required and several samples can be analyzed simultaneously (i.e. On the same plate)⁴¹.

HPTLC chromatograms of chloroform and ethyl acetate fractions at UV 254 and 366 nm revealed that all the sample constituents were obviously separated without any tailing and diffuseness. After spraying with alcoholic KOH the color of separated spots was changed or intensified give an indication for the presence of phenolic compounds. Chloroform and ethyl acetate fractions contain five and eight peaks respectively, which indicate the presence of five and eight constituents successively. The R_f values ranged from 0.02 to 0.62, and from 0.02 to 0.52 for separated constituents in chloroform and ethyl acetate respectively.

By comparing the R_f values of standards with the R_f values of the separated components in both fractions, caffeic, p-coumaric acids were detected in both fractions while Gallic acid was only detected in ethyl acetate fraction. The HPTLC profile of the analyzed fractions is shown in table 2.

Previous studies reported the presence of chlorogenic acid in the *L. camara* leaves⁴², which upon hydrolysis yield caffeic acid and quinic acid⁴³. Also caffeic acid is generated from p-coumaric acid via hydroxylation⁴⁴. Gallic acid (3,4,5 -trihydroxybenzoic acid) is found both as part of the tannin molecule and as a free state. Since Gallic acid has a carboxylic acid functionality and the hydroxyl groups in the same molecule, two of its molecules can interact

with one another to form a digallic acid (an ester), acidic hydrolysis of digallic or tannic acid yield Gallic acid⁴⁵. The max R_f values of standards (Gallic acid, caffeic acid, p-coumaric acid) and detected phenolic acids in analyzing fractions are shown in

table 3. The chemical structures of detecting phenolic acids are shown in figure 1, HPTLC chromatograms of standards and analyzed fractions are shown in figure 2 and figure 3.

Table 1: phytochemical analysis of *Lanata camara* leaves

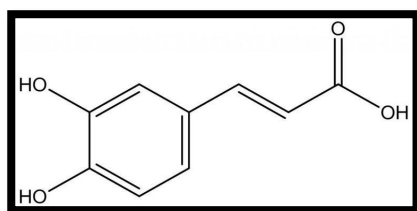
<i>L. camara</i> leaves	Test				
	flavonoids	Tannins	alkaloids	saponins	phyosterols
	+	+	-	+	+

Table 2: Phenolic acid content of *Lanata camara* leaves

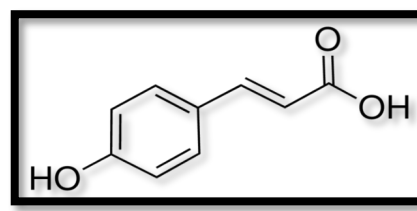
Sample	Phenolic acids
Chloroform fraction	Caffeic acid, p-coumaric acid
Ethyl acetate fraction	Gallic acid, caffeic acid, p-coumaric acid

Table 3: The max R_f values of standards and detected phenolic acids in analyzing fractions

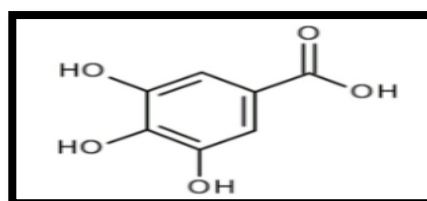
Sample	Max R _f of Gallic acid	Max R _f of p-coumaric acid	Max R _f of caffeic acid
Standard	0.29	0.52	0.44
Chloroform fraction	----	0.49	0.46
Ethylacetate fraction	0.29	0.52	0.43



a

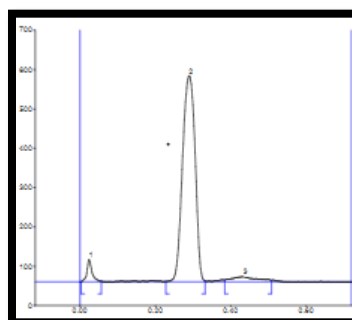


b



c

Fig. 1: Chemical structures of (a: caffeic acid, b: p-coumaric acid, c: gallic acid)



Peak	Start R _f	Start Height	Max R _f	Max Height	Max %	End R _f	End Height	Area	Area %
1	0.00	0.7	0.02	57.0	9.56	0.06	1.9	624.6	4.16
2	0.23	1.5	0.29	525.1	88.15	0.33	3.1	13653.4	90.89
3	0.39	5.2	0.43	13.6	2.29	0.51	4.6	744.6	4.96

Track 1: Gallic acid standard

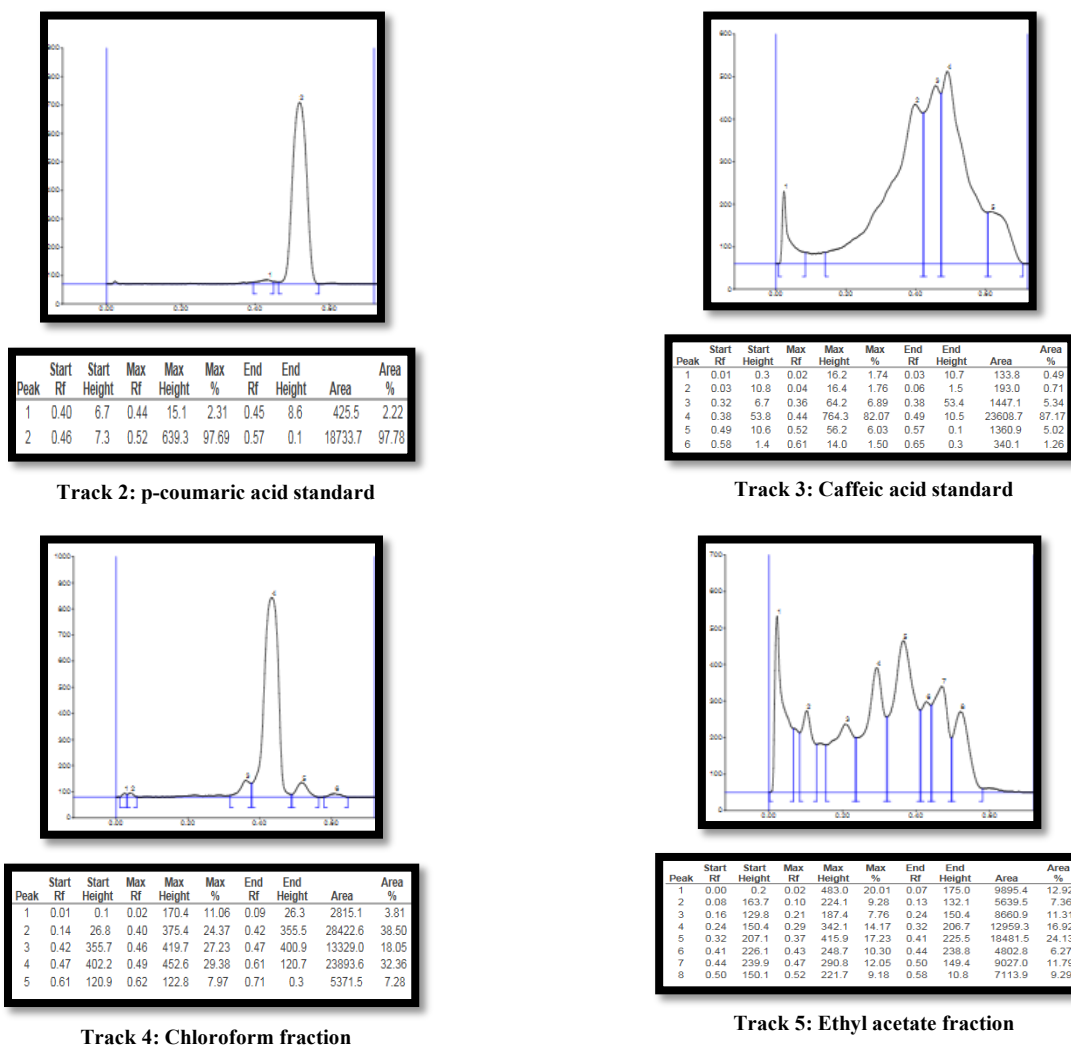


Fig 2: HPTLC chromatograms showing max retardation factor values of standard phenolic acids and analyzed fractions

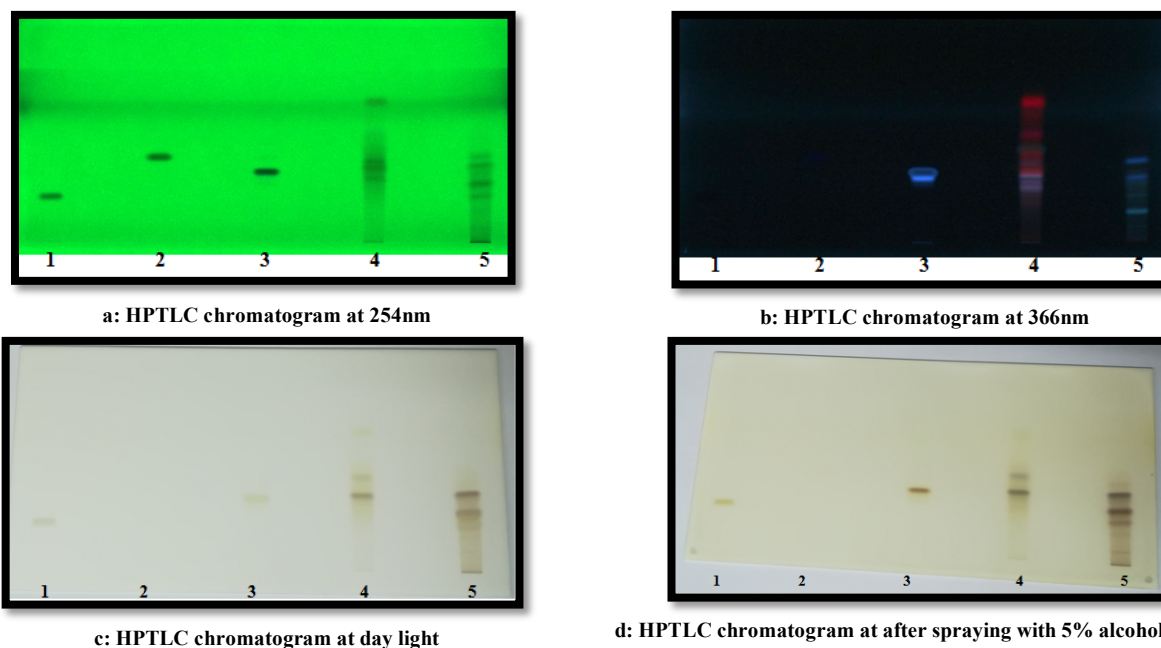


Fig 3: (a,b,c and d) HPTLC plates of analyzed fractions with reference standards, detection under UV light (a) at 254 nm, (b) 366 nm, (c) at day light and (d) after spraying with alcoholic KOH (1: gallic acid, 2: p-coumaric acid, 3: caffeic acid, 4: chloroform fraction, 5: ethyl acetate fraction)

CONCLUSION

The present study provides enough information concerning numerous phytoconstituents present in Iraqi *L. camara* leaves extract and more precise information concerning the type of phenolic acids in chloroform and ethyl acetate fraction. Three different phenolic acids (gallic, caffeic and p-coumaric acid) were detected in the leaf extract by HPTLC. Gallic acid only detected in ethyl acetate fraction.

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