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PRELIMINARY QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF *LAGERSTROEMIA SPECIOSA* ETHANOLIC LEAVES EXTRACT.

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Abstract

Background: Plants are important resources in healthcare as they contain primary and secondary metabolites. These secondary metabolites have shown to possess various beneficial effects, which provide the scientific base for the use of herbs in the traditional medicine where they, contribute significant biological activities such as hypoglycemic, anti-diabetic, antioxidant, anti-microbial, anti-inflammatory, anti-carcinogenic, antimalarial, anticholinergic, anti-leprosy activities etc. Therefore, the active substances present in the medicinal plants have gained much importance because of their versatile applications. *Lagerstroemia speciosa* (L.) Pers (Lythraceae) also known as banaba, possesses several polyphenolic compounds.These phytoconstituents include ellagic acid and its derivatives, triterpenes (tannins), triterpenoids (corosolic acid), quercetin, isoquercitin, flavones and glycosides, with various biological activities.

Aims and Objectives: Objective of this study was to confirm various phytochemicals in ethanolic banaba leaves extract (EBLE) by qualitative analysis and quantitative analysis by high performance thin layer chromatography (HPTLC).

Materials and Methods: Ethanolic leaf extract of *Lagerstroemia speciosa* (L.) Pers., was purchased from Quimico (Batch no. KAN/BE/1801009). Herbal Extract Manufacturer, Bengaluru, India. The preliminary phytochemical analysis was done by method of Harborne, 1973. HPTLC was performed using, aluminum sheets coated with silica gel GF254 (Merck 20 x 20 cm) and were used as adsorbent (stationary phase). The plates were pre- washed by methanol and activated at 600°C for 5 min prior to chromatography.

Results and Conclusion: The qualitative results confirm the presence of primary metabolites and

secondary metabolites in EBLE. Quantitative analysis confirmed. Presence of berberine, corosolic acid, gallic acid and ellagic acid in EBLE extract has been analyzed by HPTLC method.

Keywords: Corosolic acid, banaba extract, Hepatoprotective activity, *Lagerstroemia speciosa,*

INTRODUCTION

Plants are one of the important sources of ethnomedicine. It has been estimated that 14-18% of higher plants were used for medicinal purpose and relatively 74% pharmacologically active plants were discovered after following up on ethnomedicinal usage of the plants (Petrovska, 2012). Plants contain primary and secondary metabolites. Primary metabolites include sugars, amino acids, tricarboxylic acids, proteins, nucleic acids and polysaccharides (Erb and Kliebenstein, 2020). Secondary plant metabolites are numerous chemical compounds produced by the plant cell through metabolic pathways derived from the primary metabolic pathways (Kumar et al, 2021). These secondary plant metabolites are glycosides, flavonoids, phenolics alkaloids, saponins, terpenes, lipids and carbohydrates etc. Particularly, secondary metabolites have shown to possess various beneficial effects, which provide the scientific base for the use of herbs in the traditional medicine in many ancient communities. They have been described as antibiotic, antifungal and antiviral and therefore, they are able to protect plants from pathogens. For instance, phenolic compounds are important natural compounds which have been shown to possess wide range of bioactivities (Rahman et al, 2021)

These secondary metabolites from medicinal plants contribute significant biological activities such as hypoglycemic, anti-diabetic, antioxidant, anti-microbial, anti-inflammatory, anti-carcinogenic, antimalarial, anticholinergic, anti-leprosy activities etc. (Rady et al, 2018). Therefore, the active substances present in the medicinal plants have gained much importance because of their versatile applications (Duangjai, 2018). Therefore, these secondary metabolites serve as drug precursors, drug prototypes, and pharmacological probes (shah et al, 2020). A variety of medicinal plants have been screened and their isolated phytochemicals play a pivotal role in the drug discovery programs worldwide (Wawrosch and Zotchev ,2021) these secondary metabolites can also be used as blue prints for the manufacture of synthetic drugs of a similar structure, or serve as building blocks or starting materials for the productionof semi-synthetic drugs (Wawrosch and Zotchev ,2021).

Lagerstroemia speciosa (L.) Pers (Lythraceae) possesses several polyphenolic compounds (Posadzk et al, 2013). To date, more than 40 phytoconstituents have been identified and isolated from different parts of *L. speciosa* plant. These phytoconstituents include ellagic acid and its derivatives, triterpenes (tannins), triterpenoids (corosolic acid), quercetin, isoquercitin, flavones and glycosides, with various biological activities (Posadzk et al, 2013. Traditionally, tea made from banaba leaves has been used to treat diabetes mellitusin Southeast Asia (rohith singh and Ezhilarasan 2021). *L. speciosa* leaf extract has been reported for its extensive anti-diabetic, anti-obesity (Dina et al. 2009), anti- inflammatory, antioxidant, antiviral, antibacterial (Posadzk et al, 2013), anti- hypertensive (Moure et al., 2001), anti-fibrotic (Mossa et al. 2015) and analgesic (Vladimir et al. 2015) effects. The leaves and flower extract of banaba is reported to have hepatoprotective effects against a variety of liver injury models such as hepatic fibrosis, non-alcoholic steatohepatitis, and oxidative stress in liver (Amresh et al. 2018; Tiwary et al. 2017; Sai Saraswathi et al. 2017). Identification of specific phytochemicals needs special attention because of complex nature ofplant based medicines and the inherent variability of their constituents (Rajani et al. 2008). In view of the above scenario, qualitative and quantitative phytochemical investigation wascarried out to identify the potential phytocompounds in EBLE in the present study.

AIM AND OBJECTIVES

1. To confirm the presence of various phytochemical compounds in EBLE by qualitative analysis

2. To identify the phytochemical compounds present in EBLE quantitatively by high performance thin layer chromatography (HPTLC) method

MATERIALS AND METHODS

Plant material and extraction

Ethanolic leaf extract of *Lagerstroemia speciosa* (L.) Pers., was purchased from Quimico (Batch no. KAN/BE/1801009). Herbal Extract Manufacturer, Bengaluru, India. As per the manufacturer's certificate, ethanolic banaba leaves extract (EBLE) contains 20% corosolic acid, which was analyzed and proved by high performance liquid chromatography assay.

Fig 1 *Lagerstroemia speciosa* (L.) Pers leaves

Fig 2 High Performance Liquid ChromatographyPreliminary

Phytochemical Analysis

The preliminary phytochemical analysis was done by Harborne, 1973. The following preliminary phytochemical tests were performed in EBLE.

Identification test for Alkaloids

a) Dragendorff's test: To the EBLE, diluted hydrochloric acid and Dragendorff's reagent was added. The reddish brown precipitate formation confirms alkaloids presence.
b) Tannic acid test: To the EBLE, diluted hydrochloric acid and tannic acid solution was added. The cream buff colored precipitate formation indicates presence of alkaloids.

Identification test for Amino acids

a) Millon's test: 2 mL of Million's reagent was added to the EBLE, the white color precipitate formation confirms the amino acids.
b) Ninhydrin test: Ninhydrin solution was added to the EBLE and boiled. The violet color formation confirms the amino acid.

Identification test for Carbohydrates

a) Molisch's test: To the EBLE, few drops of alcoholic α-naphthol, and few drops of

concentrated sulphuric acid were added through sides of test tube. Appearance of purple to violet color ring at the junction confirms the presence of carbohydrates.

b) Barfoed's test: 1 mL of EBLE is heated with 1 mL of Barfoed's reagent. Formation of red cupric oxide indicates the presence of monosaccharide. Disaccharides on prolonged heating (about 10 minutes) may also cause reduction, owing to partial hydrolysis to monosaccharides.

c) Seliwinoff's test (Test for ketohexoses)**:** To the EBLE, crystals of resorcinol and equal volume of concentrated hydrochloric acid was added and it was heated on a water bath. Formation of rose color indicates the presence of carbohydrates (eg. fructose, honey).

Identification test for Flavonoids

a) Shinoda test: To the EBLE few magnesium turnings and concentrated hydrochloric acid were added drop wise. Pink scarlet, crimson red or occasionally green to blue color appearance after few minutes indicates the presence of flavonoids.

b) Alkaline reagent test: A few drops of sodium hydroxide solution were added to EBLE. The intense yellow color change to colorless on addition of dilute acid confirms the flavonoids.

Identification test for Glycosides

Legal's test: The EBLE was treated with pyridine and alkaline sodium nitroprusside solution. Appearance of blood red color indicates cardiac glycoside.

Identification test for Phenolic compounds

Ferric chloride test: Treat the extract with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Identification test for Tannins

a) Test for gallotannins: To the extract, potassium iodide solution was added, appearance of pink color indicates the presence of free gallic acid

b) Test for ellagitannins: To the extract, acetic acid and concentrated nitric acid were added. The appearance of pink at first and then purple and blue indicates the presence of ellagitannins

Identification test for proteins

a) Test with trichloroacetic acid (TCA): To the extract, add TCA, precipitate is formed, which indicates the presence of proteins

b) Xanthoproteic test: To the (5 mL) of extract, 1 mL of concentrated nitric acid was added and boil, yellow precipitate is formed. After cooling it, add 40% sodium hydroxide solution, orange color is formed.

Identification test for Steroids

Libermann-Burchard test: To the plant extract few drops of acetic anhydride was added and it was boiled and cooled. Then concentrated sulphuric acid was added from the side of the test

tube, at the junction two layers brown ring was formed and upper layer turns green indicates the presence of steroids and deep red color formation indicates presence of triterpenoids.

High Performance Thin Layer Chromatography

HPTLC plate

HPTLC plates are aluminum sheets coated with silica gel GF254 (Merck 20 x 20 cm) and were used as adsorbent (stationary phase). The plates were pre- washed by methanol and activated at 600°C for 5 min prior to chromatography.

Sample preparation

5 mg of EBLE was weighed and dissolved in 1 mL of methanol and the volume was made up to 10 mL in a volumetric flask. The standards, corosolic acid, berberine, gallic and ellagic acid was weighed at a concentration of 1mg/ml and dissolved in methanol. The stock concentration of the standards was diluted using methanol and a working concentration of 50 µg/mL was used. 10 µL of sample and each standards were used.

Procedure

The method employed silica gel 60 F254 (20 x 10 cm) precoated plates (10×10 cm with 250 µM thickness) (Merck, Darmstadt, Germany) was used as stationary phase. HPLC grade chloroform: methanol (9.5:0.5), isopropanol: formic acid: water (4.5:0.1:0.4), chloroform: ethyl acetate (EA): formic acid (FA) (2.5:2:0.8) and Toluene: EA: FA: methanol (3:3:0.8:0.2) were used as mobile phases for corosolic acid, berberine, gallic acid and ellagic acid respectively. 10 µL of EBLE and the corresponding standards were applied on the HPTLC aluminium sheets as different tracks in the form of 6 mm wide bands by using a semiautomatic Linomat 5 spotter at a distance of 12 mm and 8 mm from the bottom. Nitrogen gas was used for drying bands and drier was used to dry bands The chamber (20 x 10 cm) was saturated using mobile phase (20 ml) in each flat- bottomed twin trough TLC chamber at room temperature (25 ± 2°C and 40% relative humidity), 30 min before the development of the HPTLC plate. The sealed chamber with parafilm was covered with glass lid. The TLC plates were allowed to develop in a twin trough chamber, and the linear ascending development was carried out. The solvent system was selected in a trial and error manner to discriminate the maximum number of compounds as well as to elute the respective marker compounds. Development was done in ascending mode using CAMAG Automatic TLC Sampler 4 (ATS4), which was programmed through winCATS software.

RESULTS

Qualitative phytochemical analysis

Phytochemical screening is necessary to establish the information about the chemical diversity of the plants. Preliminary phytochemicals screening is an essential step for the detection of various bioactive principles present in the medicinal plants and subsequently may

lead to drug discovery and development. Most of these chemical entities are important for the therapeutic usage. The results of qualitative phytochemical analysis of EBLE are given in Table 1. The results confirm the presence of primary metabolites, proteins, amino acids and carbohydrates. The presence of secondary metabolites such as alkaloids, phenolic compounds, tannins was also reported. Presence of steroids and glycosides were also indicated.

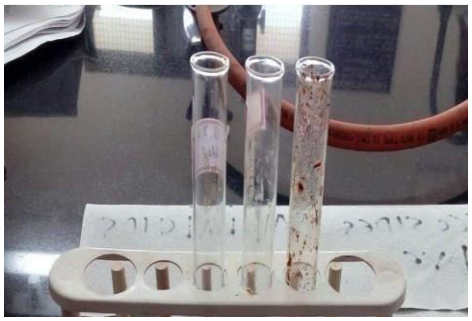


Fig 3 Phytochemical test for Gums

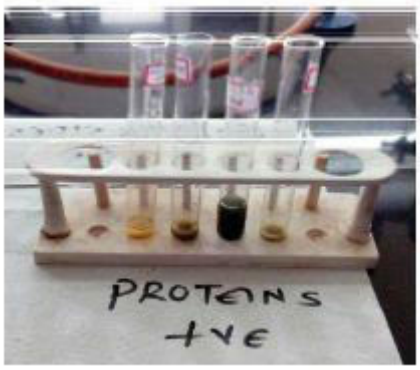


Fig 4 Phytochemical test for Proteins

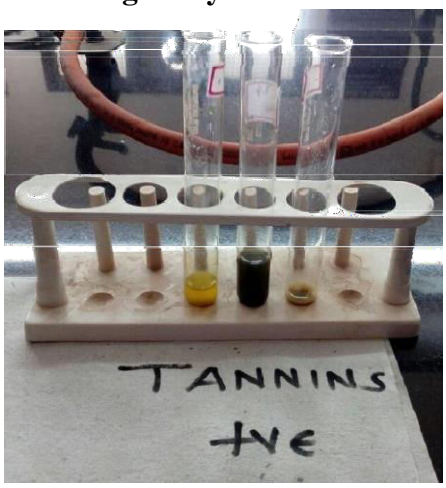


Fig 5 Phytochemical test for Tannins

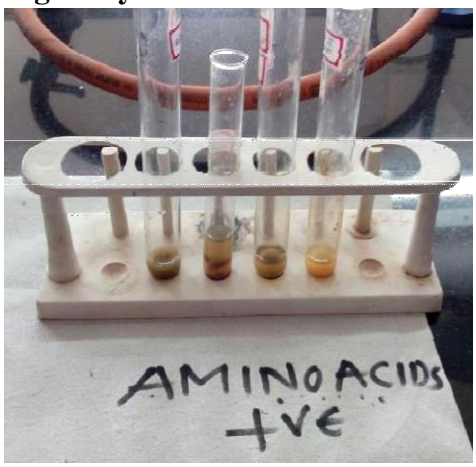


Fig 6 Phytochemical test for Amino acids

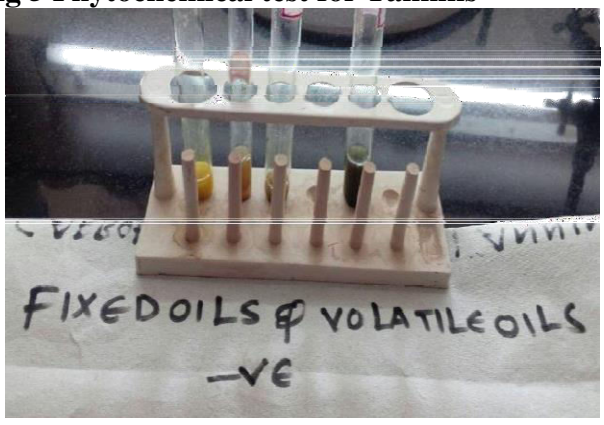


Fig 7 Phytochemical test for Fixed & Volatile oils

Fig 8 Phytochemical test for Carbohydrates

Fig 9 Miscellaneous photos of phytochemical tests

Table 1. Results of qualitative phytochemical analysis of EBLE

S.No	Phytoconstituents	Test Name	Inference
1	Protein	• Trichloroacetic Acid Test • Xanthoprotein Test	Positive
2	Amino acids	• Millons Test • Ninhydrin Test	Positive
3	Carbohydrates	• Molisch Test • Barfoed's Test • Seliwinoff's Test	Positive
4	Alkaloids	• Dragendroff's Test • Tannic acid Test	Positive
5	Flavonoids	• Shinoda Test • Alkaline reagent Test	Positive
6	Phenolic Compounds	• Ferric Chloride Test	Positive
7	Tannins	• Gallotannins test • Ellagitannins test	Positive
8	Steroids	• Libermann-Burchard test	Positive
9	Glycosides	• Legal'test	Positive

10	Saponins	● Foam test	Positive

Quantitative phytochemical analysis

Presence of berberine, corosolic acid, gallic acid and ellagic acid in EBLE extract has been analyzed by HPTLC method. Chromatograms shows the presence of the above phytoconstituents in EBLE with the Rf values of 0.24 vs.0.23 (berberine) (Figure 1), 0.77 vs. 0.79 (corosolic acid) (Figure 2), 0.44 vs. 0.39 (gallic acid) (Figure 3) and 0.32 vs 0.42 (ellagic acid) (Figure 4) of their corresponding standards respectively was detected in EBLE extract. The quantitative analysis showed the presence of corosolic acid (12.87 µg), berberine (3.19 µg), gallic acid (2.94 µg) and ellagic acid (1.14 µg) per mg of EBLE (Table 2- 5).

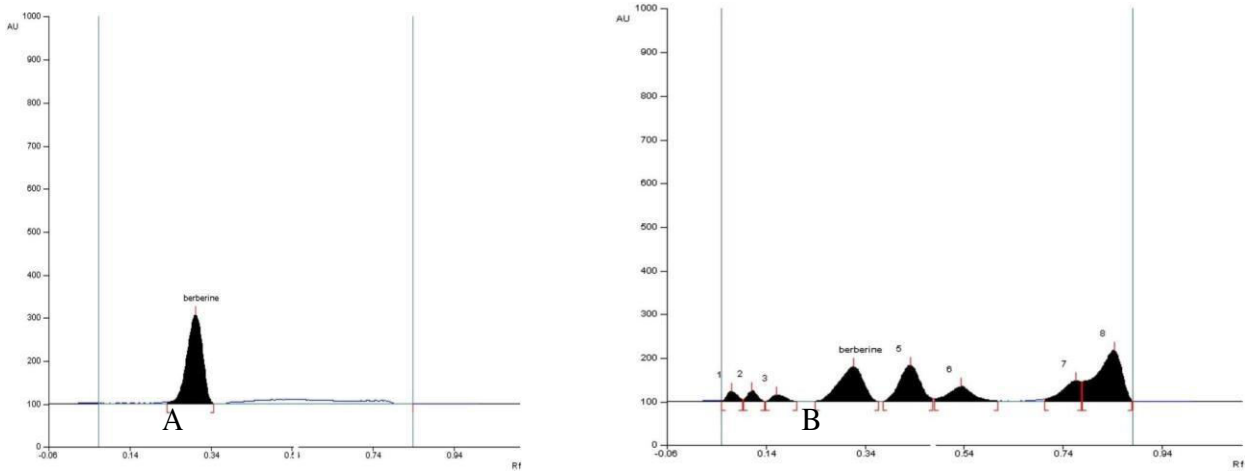
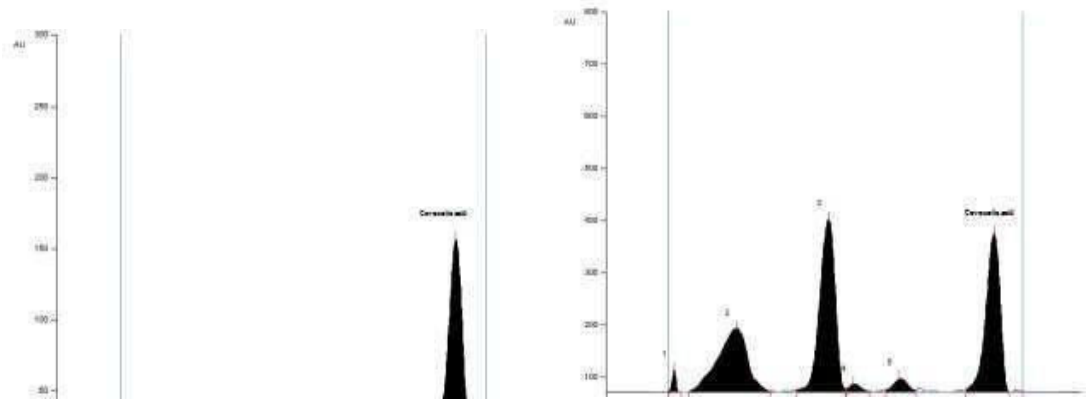
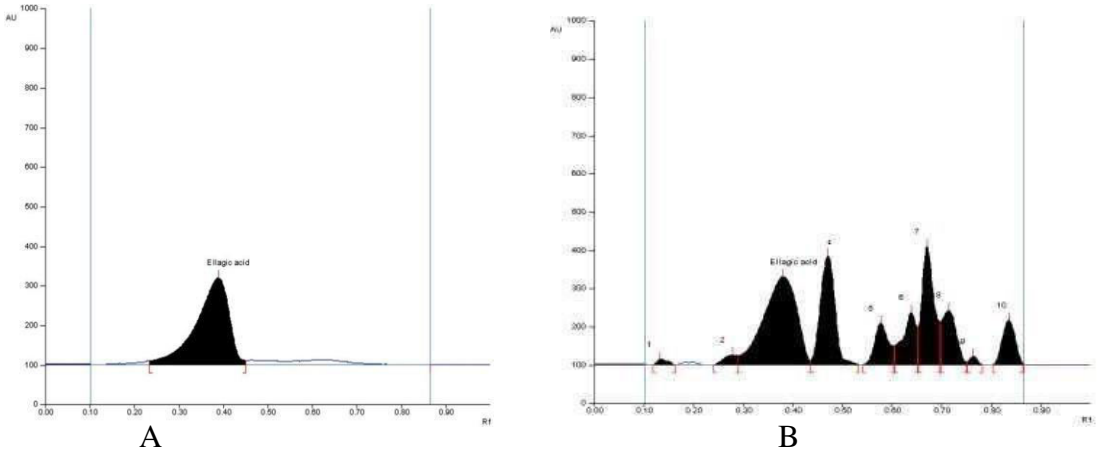


Fig 10. HPTLC chromatogram showing standard and presence of berberine in EBLE. A. HPTLC chromatogram of standard berberine with Rf value 0.23 b. HPTLC chromatogram of berberine in EBLE with Rf value 0.24.



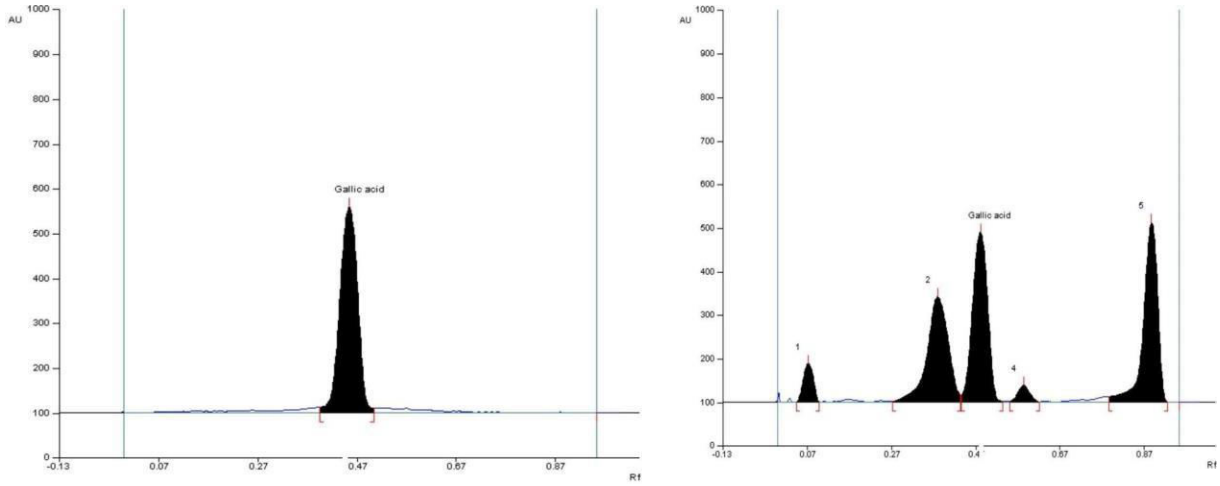
A B

Fig 11. HPTLC chromatogram showing standard and presence of corosolic acid in EBLE. A. HPTLC chromatogram of standard corosolic acid with Rf value 0.79 b. HPTLC chromatogram of corosolic acid in EBLE with Rf value 0.77.



A B

Fig 12. HPTLC chromatogram showing standard and presence of ellagic acid in EBLE. A. HPTLC chromatogram of standard ellagic acid with Rf value 0.30 b. HPTLC chromatogram of ellagic acid in EBLE with Rf value 0.32



A B

Fig 13. HPTLC chromatogram showing standard and presence of gallic acid in EBLE. A. HPTLC chromatogram of standard gallic acid with Rf value 0.39 b. HPTLC chromatogram of gallic acid in EBLE with Rf value 0.44.

DISCUSSION

Medicinal plants possess a variety of secondary metabolites with beneficial biological activity that can be of therapeutic value. The protective effect of herbs has been attributed by the phytochemicals, which are the non-nutrient plant compounds (Rohit Singh and Ezhilarasan, 2020). The phytochemical compounds such as phenols, reducing sugars, flavones, saponins, alkaloids, proteins, glycosides, anthroquinones, triterpenoids and quinines were qualitatively analyzed using standard methods. Each phytochemical possesses a wide range of bioactivities, which may help in hepatoprotection and protection against other chronic diseases like cancer, diabetes, cardiovascular disorders etc. (Choi et al, 2015).The preliminary phytochemical analysis of EBLE showed the presence of terpenes, phenols, flavonoids, tannins, saponins, total proteins, glycosides, carbohydrates, steroids, and alkaloids in considerable amount. It has been reported that saponin, terpenoid, flavonoid, tannin, steroid and alkaloid rich medicinal plants have been reported to have hepatoprotective, antioxidant, analgesic and anti-inflammatory effects (Valan et al, 2010; Gupta et al, 2017; Orhan et al, 2007). The presence of phenols has been responsible for the ability to block specific enzymes that causes inflammation. They also modify the prostaglandin pathways there by protecting platelets from clumping (Akindele and Adeyemi,2007). Tannins are basically astringent and it has wound healing properties and it reduces inflammation of mucous membrane (Ibrahim et al, 2018). Altogether, the major role of the phytochemicals is used for the protection against oxidation (Cilla et al, 2017). Therefore, the presence of the above phytochemicals in EBLE extract may offer several beneficial effect, if it is administered against various drug and chemical induced hepatotoxic agents.

Conclusion

Our preliminary qualitative phytochemical analysis of EBLE revealed the presence of primary metabolites such as proteins, amino acids and carbohydrates and secondary metabolites such as alkaloids, phenolic compounds, tannins, steroids and glycosides. The quantitative analysis showed the presence of corosolic acid (12.87 µg), berberine (3.19 µg), gallic acid (2.94 µg) and ellagic acid (1.14 µg) per mg of EBLE . The results obtained from this study will play a significant role in setting standards for this medicinal plant. Phytochemicals reported from EBLE exerts various therapeutic properties .Corosolic acid which was quantified from the ethanolic banaba leaves extract using HPTLC could be used as a chemical marker for the standardization of L. speciosa.Hence,phytochemicals obtains from EBLE may have beneficial effects like anti-inflammatory, anti-diarrheal, anti-gout effects and a promising hepatoprotective property.

Bibilography

- Petrovska BB. Historical review of medicinal plants' usage. Pharmacogn Rev. 2012;6(11):1-5.
- Erb M, Kliebenstein DJ. Plant Secondary Metabolites as Defenses, Regulators, and Primary Metabolites: The Blurred Functional Trichotomy. Plant Physiol. 2020 Sep;184(1):39-52.
- Kumar M, Kumar Patel M, Kumar N, Bajpai AB, Siddique KHM. Metabolomics and Molecular Approaches Reveal Drought Stress Tolerance in Plants. Int J Mol Sci. 2021 Aug 24;22(17):9108
- Rahman MM, Rahaman MS, Islam MR, Rahman F, Mithi FM, Alqahtani T, Almikhlafi MA, Alghamdi SQ, Alruwaili AS, Hossain MS, Ahmed M, Das R, Emran TB, Uddin MS. Role of Phenolic Compounds in Human Disease: Current Knowledge and Future Prospects. Molecules. 2021 Dec 30;27(1):233.
- Rady I, Bloch MB, Chamcheu RN, Banang Mbeumi S, Anwar MR, Mohamed H, Babatunde AS, Kuiate JR, Noubissi FK, El Sayed KA, Whitfield GK, Chamcheu JC. Anticancer Properties of Graviola (*Annona muricata*): A Comprehensive Mechanistic Review. Oxid Med Cell Longev. 2018 Jul 30;2018:1826170.
- Duangjai, Areeya T, Apinan P and AujanaY. Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. Medicines 2018;5:93.
- Shah SAA, Hassan SSU, Bungau S, Si Y, Xu H, Rahman MH, Behl T, Gitea D, Pavel FM, Corb Aron RA, Pasca B, Nemeth S. Chemically Diverse and Biologically Active Secondary Metabolites from Marine *Phylum chlorophyta*. Mar Drugs. 2020 Sep 26;18(10):493.

- Wawrosch C, Zotchev SB. Production of bioactive plant secondary metabolites through in vitro technologies-status and outlook. Appl Microbiol Biotechnol. 2021 Sep;105(18):6649-6668.
- Posadzki P, Watson LK, Ernst E. Adverse effects of herbal medicines: an overview of systematic reviews. Clin Med (Lond). 2013 Feb;13(1):7-12.
- Rohit Singh T, Ezhilarasan D. Lagerstroemia speciosa (L.) Pers., ethanolic extract attenuates simultaneously administered isoniazid- and dapsone-induced hepatotoxicity in rats. J Food Biochem. 2021 Jun 21:e13830.
- Dina A, Nassima C, Meriem B, Karima A, Hakima L, Hania B. Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chem. 2009; 112:303–09.
- Moure A, Cruz JM, Franco D, Dominguez JM, Sineiro J, Dominguez H, et al. Natural antioxidants from residual sources. Food Chem. 2001; 72:145–71.
- Mousa AM, El-Sammad NM, Abdel-Halim AH, Anwar N, Khalil WKB, Nawwar M, Hashim AN, Elsayed EA, Hassan SK. *Lagerstroemia speciosa* (L.) Pers Leaf Extract Attenuates Lung Tumorigenesis via Alleviating Oxidative Stress, Inflammation and Apoptosis. Biomolecules 2019;9(12):871.
- Vladimir Knezevic S, Cvijanovic O, Blazekovic B, Kindl M, Stefan MB, Domitrovic R. Hepatoprotective effects of Micromeria croatica ethanolic extract against CCl4-induced liver injury in mice. BMC Complement Altern Med. 2015; 15:233.
- Amresh G, Agarwal VK, and Rao CV: Self micro emulsifying formulation of Lagerstroemia speciosa against chemically induced hepatotoxicity. J Tradit Complement Med, 2018;8(1):164–169.
- Tiwary BK, Dutta S, Dey P, Hossain M, Kumar A, Bihani S, Nanda AK, Chaudhuri TK, Chakraborty R. Radical Scavenging Activities of Lagerstroemia speciosa (L.) Pers. Petal Extracts and its hepato-protection in CCl4-intoxicated mice. BMC Complement Altern Med. 2017;17(1):55.
- Sai Saraswathi V, Saravanan D, Santhakumar K. Isolation of quercetin from the methanolic extract of Lagerstroemia speciosa by HPLC technique, its cytotoxicity against MCF-7 cells and photo catalytic activity. J Photochem Photobiol B. 2017; 171:20-26.
- Rajani, M, Kanaki N. S, Ramawat K. G., Merillon, J. M., (Eds.). In Bioactive Molecules and Medicinal Plants. Springer, 2008; 349 – 369.
- Rohit Singh T, Ezhilarasan D. Ethanolic Extract of Lagerstroemia Speciosa (L.)

Pers., Induces Apoptosis and Cell Cycle Arrest in HepG2 Cells. *Nutr Cancer.* Nutr Cancer. 2020;72(1):146-156.

- Choi J, Cho JY, Choi SJ, Jeon H, Kim YD, Htwe KM, Chin YW, Lee WS, Kim J, Yoon KD. Two new phenolic glucosides from Lagerstroemia speciosa. Molecules. 2015;20(3):4483-91.

- Valan MF, John De Britto A, Venkataraman R. Phytoconstituents with hepatoprotective activity. Int. J. Chem. Sci.: 8(3), 2010, 1421-1432.

- Gupta A, Agrawal VK, Rao CV. Exploration of Analgesic and Antiinflammatory potential of Lagerstroemia speciosa. J App Pharm Sci, 2017;7(02):156-161

- Orhan I, Kupeli E, Sener B, Yesilada E. Appraisal of anti-inflammatory potential of the clubmoss, Lycopodium clavatum L. J Ethnopharmacol. 2007;109(1):146-50.

- Akindele AJ, Adeyemi OO. Anti-inflammatory activity of the aqueous leaf extract of Byrsocarpus coccineus. Fitoterapia. 2007;78(1):25-8.

- Ibrahim N', Wong SK, Mohamed IN, Mohamed N, Chin KY, Ima-Nirwana S, Shuid AN. Wound Healing Properties of Selected Natural Products. Int J Environ Res Public Health. 2018 Oct 25;15(11):2360.

- Cilla A, Alegría A, Attanzio A, Garcia-Llatas G, Tesoriere L, Livrea MA. Dietary phytochemicals in the protection against oxysterol-induced damage. Chem Phys Lipids. 2017 Oct; 207(Pt B):19

Table 2. Analysis of peak table from HPTLC studies of berberine

Sample ID	Peak	Start Rf	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
Standard	**1**	**0.23**	**0.3**	**206.8**	**86.43**	**0.34**	**0.3**	**6365.7**	**100**	**Berberine**
Ext	1	0.05	0.07	23	5.41	0.09	4.5	383	2.67	unknown*
Ext	2	0.09	0.11	24.9	5.86	0.13	0.1	390.8	2.72	unknown*
Ext	3	0.14	0.16	15.2	3.58	0.2	0.6	328	2.29	unknown*
Ext	**4**	**0.24**	**0.31**	**79.3**	**18.66**	**0.36**	**0.2**	**3111.3**	**21.68**	**Berberine**
Ext	5	0.37	0.43	82.6	19.45	0.47	6.9	2709.5	18.88	unknown*
Ext	6	0.48	0.53	34.6	8.14	0.61	0.5	1322	9.21	unknown*
Ext	7	0.7	0.76	47.2	11.12	0.77	45.9	1393.3	9.71	unknown*
Ext	8	0.78	0.84	118	27.79	0.88	1.8	4716	32.86	unknown*

Rf- Retention factor, EXT- Extract (ethanolic banaba leaves extract).

Table 3. Analysis of peak table from HPTLC studies of corosolic acid

Sample ID	Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
Ext	1	0.05	2.2	0.06	42.2	5.02	0.08	0	350.1	1.16	unknown *
Ext	2	0.1	0.1	0.22	122	14.51	0.3	1	7952.4	26.37	unknown *
Ext	3	0.36	4.7	0.44	331.7	39.45	0.48	6.6	11182.1	37.08	unknown *
Ext	4	0.48	6.8	0.5	15	1.78	0.54	1.6	394.7	1.31	unknown *
Ext	5	0.58	3.9	0.61	25.6	3.05	0.65	3	805.6	2.67	unknown *
Ext	**6**	**0.77**	**4.4**	**0.84**	**304.3**	**36.19**	**0.88**	**0.1**	**9474.5**	**31.41**	**Corosolic acid**
Standard	**1**	**0.79**	**4.8**	**0.84**	**137**	**100**	**0.88**	**0.9**	**3893.4**	**100**	**Corosolic acid**

Rf- Retention factor, EXT- Extract (ethanolic banaba leaves extract).

Table 4. Analysis of peak table from HPTLC studies of gallic acid

Sample ID	Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
Ext	1	0.04	0.1	0.07	89.3	7.61	0.1	0.2	1906.2	4.9	unknown *
Ext	2	0.27	1.1	0.38	241.4	20.6	0.43	15.8	10908.9	28.06	unknown *
Ext	**3**	**0.44**	**16**	**0.48**	**391.1**	**33.37**	**0.53**	**1.6**	**12571.8**	**32.34**	**Gallic acid**
Ext	4	0.55	2.2	0.58	38.5	3.29	0.62	0.9	985.4	2.53	unknown *
Ext	5	0.79	12.5	0.89	411.7	35.13	0.92	0.4	12503.5	32.16	unknown *
Standard	**1**	**0.39**	**12.4**	**0.45**	**459**	**100**	**0.5**	**9.6**	**14744.5**	**100**	**Gallic acid**

Rf- Retention factor, EXT- Extract (ethanolic banaba leaves extract).

Table 5. Analysis of peak table from HPTLC studies of ellagic acid

Sample ID	Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
Standard	**1**	**0.30**	**30.1**	**0.43**	**523**	**59.21**	**0.45**	**52**	**11132.8**	**100**	**Ellagic acid**
Ext	1	0.11	0.7	0.1	18.5	4.93	0.11	0.3	158.7	1.41	unknown *
Ext	2	0.26	8	0.29	31.2	8.31	0.32	2.5	710.8	6.31	unknown *
Ext	**3**	**0.32**	**30.1**	**0.42**	**405**	**59.21**	**0.45**	**52**	**12777.6**	**55.88**	**Ellagic acid**
Ext	4	0.47	20.3	0.51	60.8	16.15	0.54	31.5	2143.3	19.01	unknown *
Ext	5	0.62	30.2	0.58	62.3	6.88	0.6	36.7	1951.8	7	unknown *
Ext	6	0.65	57.9	0.73	66.1	7.3	0.79	1.6	1807.7	6.48	unknown *
Ext	7	0.68	37	0.66	142.3	15.72	0.7	59.5	5682.4	20.36	unknown *
Ext	8	0.71	73.7	0.69	500.8	27.32	0.73	71.6	1601.5	34.43	unknown *
Ext	9	0.73	71.8	0.76	209.6	11.43	0.8	0.2	521	9.21	unknown *
Ext	10	0.72	57.9	0.73	66.1	7.3	0.79	1.6	1807.7	6.48	unknown *

Rf- Retention factor, EXT- Extract (ethanolic banaba leaves extract