IJPSR (2020), Volume 11, Issue 4 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 26 May 2019; received in revised form, 30 August 2019; accepted, 30 November 2019; published 01 April 2020

IDENTIFICATION, QUANTITATIVE DETERMINATION AND ANTIDEPRESSANT ACTIVITY OF CHLOROGENIC ACID AND GALLIC ACID FROM *MORUS ALBA* LEAVES

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Keywords:

Natural antioxidant, Antidepressant, *Morus alba*, Chlorogenic acid, Gallic acid, HPTLC

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ABSTRACT: Objective: The present study is considered to estimate preliminary phytochemical components, physicochemical parameters**,** HPTLC chromatographic studies and antidepressant activity of chlorogenic acid & gallic acid present in extracts of *Morus alba* leaves. **Methods:** Total phenol content, total flavonoid content, total tannin content, HPTLC fingerprinting analysis for compounds like chlorogenic acid & gallic acid responsible for antioxidant activity and antidepressant activity by TST & FST. **Result:** Preliminary phytochemical studies shows the presence of good phenol (21.75 ± 1.21) and flavonoid (14.83 ± 2.34) content. HPTLC fingerprinting by comparing extract values with standards available of chlorogenic acid and gallic acid also shows the presence of these components in the methanolic and ethanolic extracts of *Morus alba* leaves, which were further used to screen antidepressant activity and shows substantial antidepressant activity in the plant. **Conclusion:** Our results recommend that *Morus alba* leaves are may be demonstrated to be an acceptable natural antioxidant and antidepressant with various bioactive components used for the treatment of numerous other ailments.

INTRODUCTION: Natural plants are used as very good source of nutrition persistent food as well as a source of various chemical constituents operative in curing various diseases that may demand as the biologically active constituents. At present natural plants are very much in petition in the form of drugs because of their fewer side effects, they are considered the potential resources of various bioactive compounds and are also easily available from the natural sources.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.11(4).1683-89

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(4).1683-89

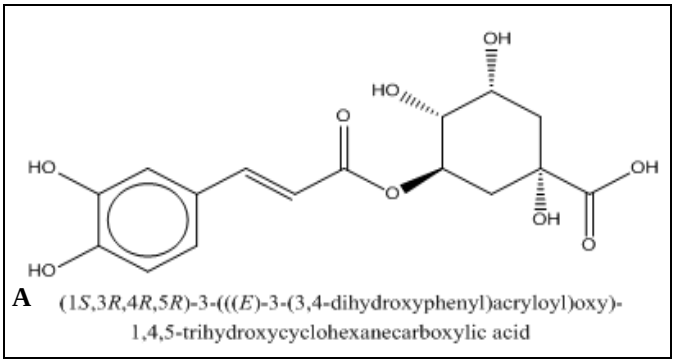
In the same context *Morus alba*, the Mulberry plant which is basically famous for sericulture, the fabrication of silk done through the silkworm and the leaves are also used to diminish the symptoms of diabetes in vernacular medicine as well as for improving cardio-metabolic risks, including anti-hyperglycemic, antihyperlipidaemic, antiobesity, antihypertensive, antioxidative, anti-inflammatory, anti-atherosclerotic and cardioprotective effects [1] in Chinese medicine used to treat constipation, to tonify the blood, prematurely grey hair, cough, edema, to promote urination, fever, headache, dry & sore eyes [2] and so many more, So, the leaves are used further in this study to explore some more about the biological activity of leaves.

Mulberry plant belongs to genus Morus having 68 species which are unisex flowering plants

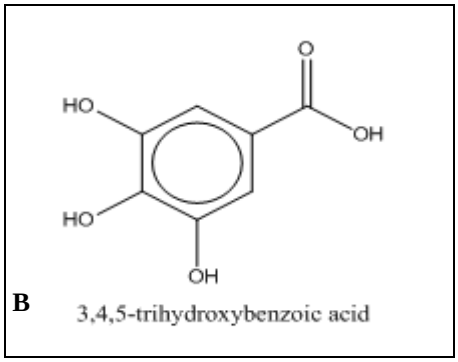
belonging to family Moraceae of the Urticales subclass. The plant is a shrub or tree (20 to 30 feet high) often the size of a small apple tree, having leaves which are thin, glossy, and light green in color with 5 lobes or has one lobe, two lobes, three lobes, or no lobes at all. *Morus alba* L. is also known as white mulberry and can be grown from seed as well as planted from large cuttings of root readily. Commonly, the plantation is upraised in a block foundation with an arrangement of 6 feet × 6 feet, or 8 feet × 8 feet, as plant to plant and row to row spaces. The plants are generally trimmed once a year during the monsoon season (July – August) to a height of 5–6 feet and allowed to grow with a maximum of 8–10 shoots at the top [3]. The plant is widely distributed in India, China, Japan, North Africa, Arabia, South Europe, *etc*.

Morus alba L. leaves had been used as a substantial source of medicine, drink, and functional foods in many countries. It is used in drinks like green tea with several other herbal drugs like tulsi and ashwagandha because of its immune-boosting antioxidants like Chlorogenic acid, rutin, isoquercitrin, and astragalin. Anticancerous alkaloids like 1- deoxynojirimycin, morroles B–F [4], (2R, 3R, 4R)- 2- hydroxymethyl-3, 4-dihydroxy-pyrrolidine-N-propionamide from the root bark and 4-O-R-D-galactopyranosyl-calystegine B2 and 3β, 6β-dihydroxynortropane from the fruits [5], mulbaines A, B & C [6]. Eighteen important amino acids with calcium, potassium, sodium, magnesium, zinc, iron, copper, manganese, chromium, selenium, arsenic, vitamins and it's no caffeine property. Other chemical constituents present in leaves are coumarins, flavonoids, anthocyanins and polyphenols including quercetin 3-(-malonyl-glucoside), rutin, isoquercitin, cyaniding-3-rutinoside apigenin, luteolin, quercetin, morin, caffeic acid, gallic acid, umbelliferone, chlorogenic acid, and kaempferol [7]. The plant extracts rich in polyphenols used as a non-toxic natural healing agent, which also has high prospective applications as skin-whitening agents due to its potent tyrosinase inhibitor property [8].

Chlorogenic acid is a phenolic compound which is an ester of caffeic acid. It is not stable thermally and is readily disintegrated into quinic acid and caffeic acid. Gallic acid also known as 3, 4, 5-trihydroxybenzoic acid is also a phenolic compound which usually bonded to form dimers such as ellagic acid. Both of these phenolics and their derivatives have received much consideration due to recent studies presenting different biological properties of these classes of compounds. Both categories play a very important role as antioxidants, for improving symptoms of diabetes as well as for direct and indirect prevention and cure of various other diseases. Hence both these compounds were chosen in this study to analyze and quantify by HPTLC fingerprinting, spectral analysis, elemental analysis to confirm the structure and to observe its antidepressant activity.



A (1*S*,3*R*,4*R*,5*R*)-3-(((*E*)-3-(3,4-dihydroxyphenyl)acryloyl)oxy)-1,4,5-trihydroxycyclohexanecarboxylic acid



B 3,4,5-trihydroxybenzoic acid

FIG. 1: STRUCTURES OF (A)-CHLOROGENIC ACID, (B)-GALLIC ACID

MATERIALS AND METHODS: Instruments & apparatus like the digital balance of Citizen, UV chamber of Systronics, UV-Visible spectrophotometer of Systronics, Hot air oven of Science Tech, Heating mental of Science Tech and pH meter of Systronics were used at different steps as per requirements. Chemical used like such as petroleum ether, methanol, ethanol, Folin–Ciocalteus reagent, gallic acid, tannic acid, rutin, Na_2CO_3, aluminum chloride, $NaNO_3$, NaOH, were of analytical grade and purchased commercially from SD Fine, Mumbai. Glass wares like Soxhlet apparatus, conical flask, beaker, measuring

cylinder, RBF, separating funnel, volumetric flask, test tube, *etc*. were used of Borosilicate.

Crude Extract Preparation: *Morus alba* leaves were collected from local Lucknow and were authenticated by CSIR-NBRI. Fresh leaves were washed and dried through air at room temperature. After two weeks of air drying, leaves are crushed with the help of a Mixture grinder. The powdered material used for extraction by Soxhlet apparatus using solvents like petroleum ether, ethanol, and methanol successively. During extraction process temperature of 40- 60 ºC was maintained for 6 h. The concentrated product of extracted material was collected and stored in refrigerator for further experimental analysis.

Phytochemical Screening: Preliminary phytochemical screening was done to investigate the phytoconstituents present in petroleum ether, ethanol, and methanol extracts. Performed according to methods & procedures given in Practical Pharmacognosy book of C. K. Kokate [9]. The results were tabulated in **Table 1**. The extract obtained by several extractions gives positive tests for alkaloids, carbohydrate, coumarin, phenolic compounds, tannins, proteins & amino acids, *etc*.

Physicochemical Studies: The physicochemical parameters like extract characteristics (consistency, color in daylight, fluorescence analysis & % yield), moisture content, Chlorophyll content, ash value, *etc*. in extracts were performed using methods reported in AOAC 1990 [10]. Quantitative phytochemical analysis for total phenol content, total flavonoid content, total tannin content was also estimated and results were tabulated. Gallic acid, Rutin and tannic acid were taken as standards for estimation of total phenol, flavonoid, and tannin content respectively.

HPTLC Chromatography Densitometry: Five working standard solutions from methanol were freshly prepared in a concentration ranging from 0.1 – 0.5 mg/ml for calibration curve from stock solution of standards of 1 mg/mL. The working solution of standards (1 mg/mL) and samples (10 mg/ml) were freshly prepared in similar solvent.

The mobile phase used for development of HPTLC plates was toluene: ethyl acetate: formic acid in a ratio of 7: 2.5: 0.5 v/v for gallic acid and ethyl acetate: acetic acid: formic acid: water in a ratio of 10:1.1: 1.1: 2.3 v/v for chlorogenic acid. The analysis was carried out using a Camag HPTLC system equipped with Linomat-V applicator and 100 µl syringe. The samples were spotted against the standards using microliter syringe over the pre-coated silica gel 60 F254 HPTLC plates, and development of the applied plate was carried out in pre-saturated Camag twin-trough chamber. Then the plates are dried and visualized in UV light of 254 nm, and 366 nm wavelength and the results are interrelated in **Table 4-5** and **Fig. 2-3**.

The percentage of chlorogenic acid and gallic acid was calculated by using the formula [11].

(Sample area × standard dilution × purity) × 100 / (Standard area × sample dilution × 100)

Antidepressant Activity:

Animals: Albino mice of either sex were acquired from the Central Animal House, Aryakul College of Pharmacy & Research (Reg. no- 1896/PO/Re/S/16/CPCSEA). Thirty Swiss albino mice (25-35 gm body weight) of either sex were randomly selected and grouped into 5 groups (n = 6). They were acclimatized and housed in an animal house with 12 h: 12 h light-dark cycle at 27 ± 2 °C temperature and 45-55% RH. Food and water delivered *ad libitum*. The work was permitted by the Institutional Animal Ethical Committee (IAEC). Control animals were doped with distilled water. Drugs like imipramine (10 mg/kg), test drug *Morus alba* leaves extract (100 mg, 200 mg, 400 mg/kg) were dissolved in distilled water and doped orally once daily for 7 days (one week). On 8th day tests were reiterated.

Acute Toxicity Studies: The process for acute toxicity of ethanolic leaf extract of *Morus alba* was monitored as per the OECD guideline no. 423 (Acute Toxic Class Method) (OECD, 2002). A dose of 800, 1000, 2000 mg/kg bodyweight is overseen and animals were observed for 15 days.

Tail Suspension Test (TST): A mouse was drooped on a wire in an upside-down position so that its nostrils touch the water surface in a vessel. After initial enthusiastic movement, the mouse undertakes emotionless posture, and the period of motionless posture during five min reflection was noted. This test is consistent and a prompt

screening method for antidepressants, including those involving the serotonergic system [12].

Forced Swimming Test (FST): The rats have placed cylinder (45 × 20 cm) holding 38 cm water (25 ± 2 °C) so that the rat could not touch lowermost part of cylinder with its hind limb or tail or climb over the verge of the chamber. Two swim periods were accompanied, an initial fifteen min pre-test, followed by five minutes test 24 h later. The drug was doped after the pre-test. The period of motionlessness (remained floating in water without harassed and making only those movements necessary to keep its head above water) during five min test period was noted [13].

RESULTS AND DISCUSSION:

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF PET ETHER, ETHANOLIC AND METHANOLIC EXTRACT OF *MORUS ALBA* LEAVES

Constituents	Pet. Ether extract	Ethanolic extract	Methanolic extract
Alkaloids	-	++	+++
Carbohydrates		+	++
Caumarin	+	++	+++
Flavonoids	+	+++	++
Fixed oil	++	-	-
Glycosides	-	+	+
Gums and resins	-	-	-
Mucilages	-	+++	+++
Proteins &amino acid	–	++	+++
Saponins	-	-	-
Steroids	+	-	-
Tannins	-	+++	++

+++ High, ++ Moderate: + Slight: - Negative

TABLE 2: EXTRACT CHARACTERISTICS

Types of solvent	Consistency	Colour in day light	Fluorescence analysis		% w/w
			Short UV	Long UV	
Ethanol	Semi-solid	Brownish black	Reddish black	Brownish black	5.82
Ethanol	Solid	Reddish-brown	Reddish-brown	Brown	6.32
Methanol	Solid	Brown	Greenish brown	Brown	7.14

TABLE 3: PHYSIOCHEMICAL STUDIES AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS

S. no.	Physiochemical Parameters	Values
1	Moisture contents	3.1 ± 0.3%
2	Ash value	12.1 ± 0.04% w/w
3	Chlorophyll content	1.97 ± 0.03
4	Total phenolics (mg gallic acid equivalent/g)*	21.75 ± 1.21
5	Total flavonoid (mg rutin equivalent/g)*	14.83 ± 2.34
6	Total tannin (mg tannic acid equivalent/g)*	7.21 ± 1.75

(*mean ± S.D, n=3)

TABLE 4: HPTLC- PHENOLS PROFILE OF METHANOLIC EXTRACT OF *MORUS ALBA* LEAVES FOR CHLOROGENIC ACID

Peak	R_f	Height (AU)	Area (AU)	Assigned substances
1	0.01	612.1	14992.1	Unknown
2	0.04	603.5	8821.9	Unknown
3	0.06	515.2	26092.5	Chlorogenic acid
4	0.14	328.3	12914.6	Unknown
5	0.20	602.5	20777.9	Unknown
6	0.25	250.1	4616.3	Unknown
7	0.27	450.6	13821.7	Unknown
8	0.31	456.5	11207.6	Unknown
9	0.36	298.3	12070.3	Unknown
10	0.43	166.4	5633.1	Unknown
11	0.47	167.5	4345.4	Unknown
12	0.51	136.3	3452.4	Unknown

13	0.58	133.8	3561.1	Unknown
14	0.61	133.1	4753.1	Unknown
15	0.65	114.3	3271.5	Unknown
16	0.69	151.6	6863.0	Unknown
17	0.75	203.0	10216.0	Unknown
18	0.82	188.1	6897.9	Unknown
19	0.87	192.5	7083.6	Unknown

TABLE 5: HPTLC- PHENOLS PROFILE OF METHANOLIC EXTRACT OF *MORUS ALBA* LEAVES FOR GALLIC ACID

Peak	R_f	Height (AU)	Area (AU)	Assigned substances
1	0.05	27.4	842.4	Unknown
2	0.47	18.7	196.6	Unknown
3	0.67	38.3	959.1	Gallic acid
4	0.71	72.3	1855.8	Unknown
5	0.77	167.7	6291.6	Unknown
6	0.83	192.5	10855.3	Unknown
7	0.93	14.4	346.6	Unknown

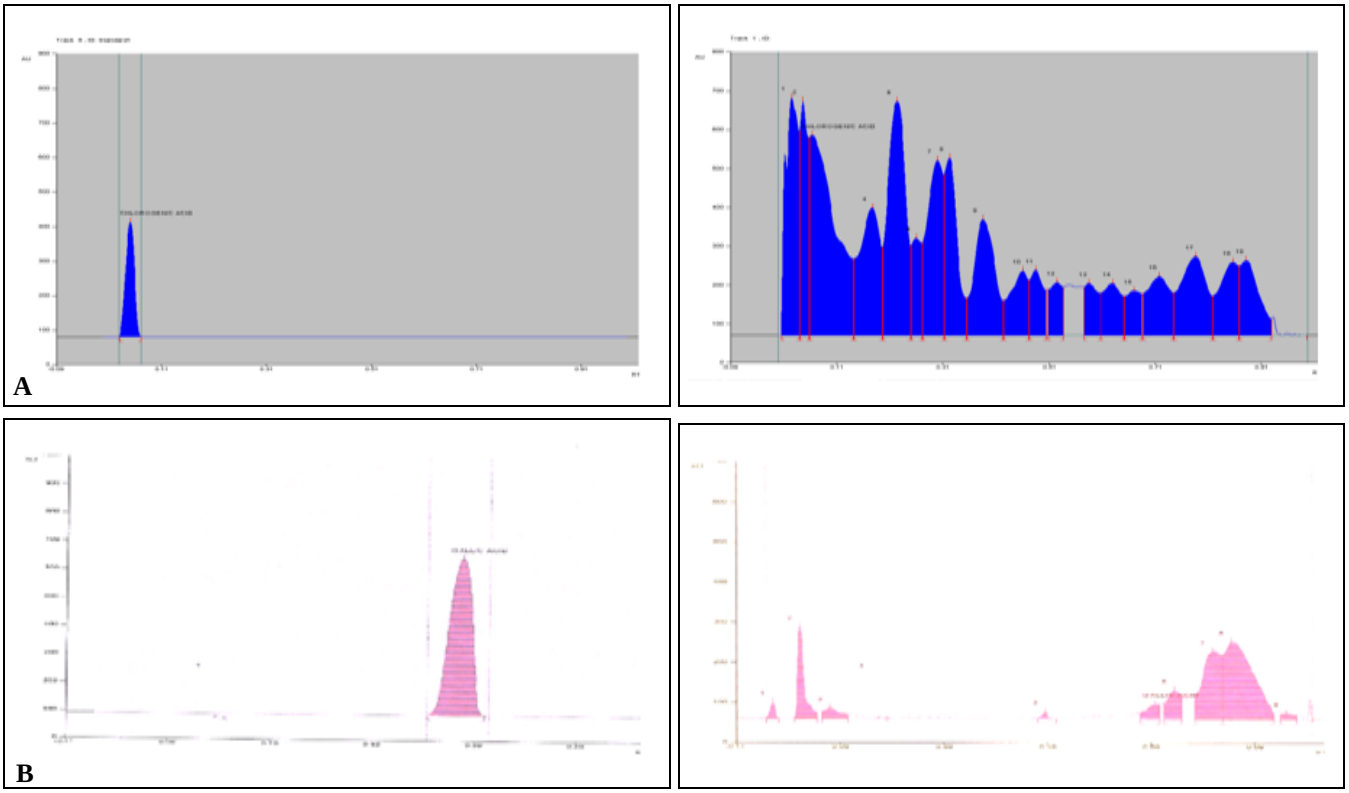
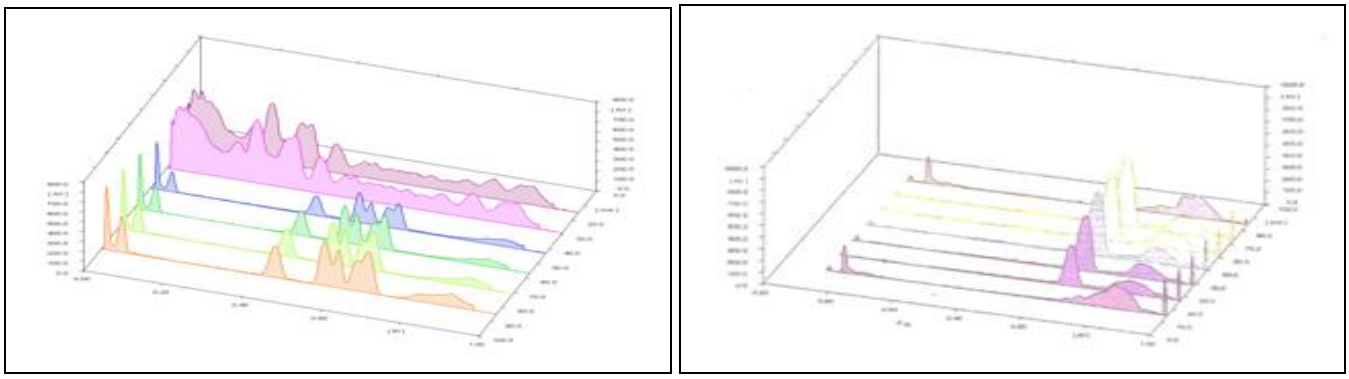


FIG. 2: HPTLC DENSITOGRAM FOR METHANOLIC EXTRACT WITH THEIR RESPECTIVE STANDARDS: A. CHLOROGENIC ACID B. GALLIC ACID



Chlorogenic acid **Gallic acid**

FIG. 3: 3D DIAGRAM OF HPTLC DENSITOGRAMS

Results of Antidepressant Activity:

TABLE 6: EFFECT OF *MORUS ALBA* LEAVES EXTRACT ON IMMOBILITY TIME IN TAIL SUSPENSION TEST

Treatment	Dose (mg/kg)	Immobility time (in sec)
Vehicle control (6)	-	180.40 ± 1.20
M. alba (6)	100	159.0 ± 2.91***
M. alba (6)	200	142.4 ± 0.812***
M. alba (6)	400	122.0 ± 1.37***
Imipramine (6)	10	108.40 ± 0.927***

Values are given as mean ±SEM (N=6 in each group), ***P < 0.001, as compared to control

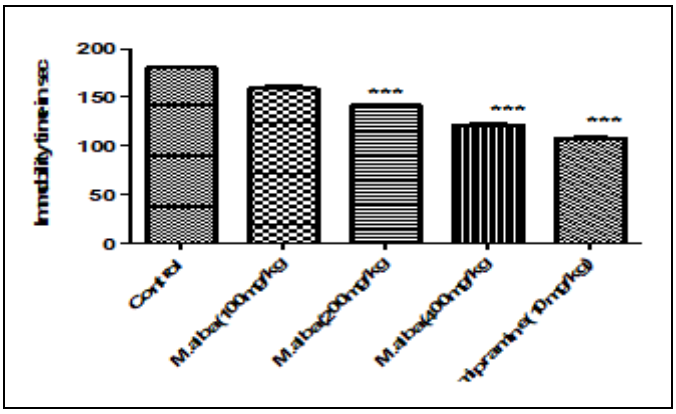
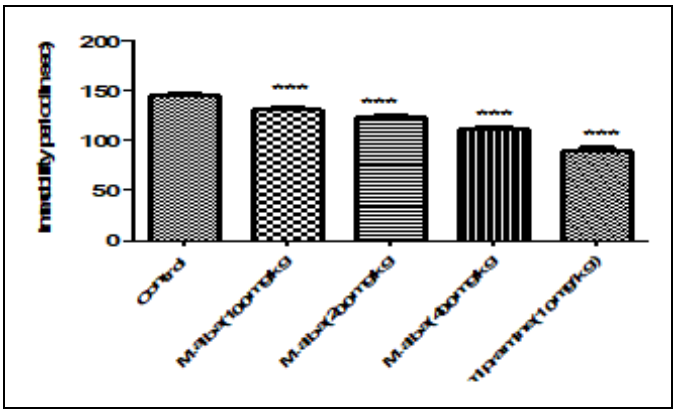


TABLE 7: EFFECT OF *MORUS ALBA* LEAVES EXTRACT ON IMMOBILITY TIME IN FORCED SWIMMING TEST

Treatment	Dose (mg/kg)	Immobility time (in sec)
Vehicle control (6)	-	145.40 ± 1.72
M. alba (6)	100	130.60 ± 1.96***
M. alba (6)	200	123.80 ± 1.35***
M. alba (6)	400	111.20 ± 2.35***
Imipramine (6)	10	89.60 ± 3.26***

Values are given as mean ± SEM (N = 6 in each group), ***P < 0.001, as compared to control



Ample amounts of free radicals are constantly produced in the body through different biochemical reactions; if the level of these free radicals surpasses the normal value, it leads to oxidative impairment in cells as well as tissues leading to various degenerative diseases in the body. Plant extracts are frequently used as natural sources of antioxidants, which can prevent these degenerative disorders. Antioxidants neutralize the free radicals in the body which improves the disease conditions. Chlorogenic and gallic acid are antioxidants present in the plant *Morus alba*, which is demonstrated in the article by using phytochemical screening and HPTLC fingerprinting. These *in-vitro* antioxidants can fight against free radicals produced in the body and hence results in improvements in disease conditions of depression and other ailments like diabetes and cardiovascular diseases. Through our results, it was demonstrated that *Morus alba* leaves are may be acceptable as a natural antioxidant and antidepressant with various bioactive components used for the treatment of numerous other ailments.

CONCLUSION: Our result recommends that *Morus alba* leaves are may be demonstrated to be anacceptable natural antioxidant and antidepressant with various bioactive components used for the treatment of numerous other ailments.

ACKNOWLEDGEMENT: Authors are thankful to CSIR-NBRI Lucknow for providing all the necessary facilities related to the work along with Faculty of Pharmacy, Hygia Institute of Pharmaceutical Education & Research, Lucknow for providing all the necessary support related to the work.

CONFLICTS OF INTEREST: There is no conflicts of interest.

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How to cite this article:
Singh SB and Singh A: Identification, qunatitative determination and antidepressant activity of chlorogenic acid and gallic acid from *Morus alba* leaves. Int J Pharm Sci & Res 2020; 11(4): 1683-89. doi: 10.13040/IJPSR.0975-8232.11(4).1683-89.