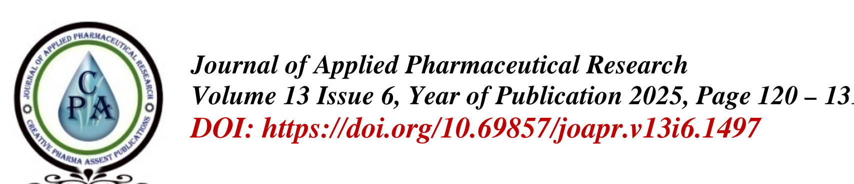

Research Article

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# PHYTOCHEMICAL CHARACTERIZATION AND INVESTIGATION OF BIOACTIVITIES OF MELOCHIA CORCHORIFOLIA L.

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*ABSTRACT*

**Background:** *Melochia corchorifolia L.* (MC) is traditionally used to treat various ailments. This study investigated its phytochemical content and evaluated its antioxidant, analgesic, anti-inflammatory, and hypoglycemic potential. **Methodology:** Standard phytochemical screening was conducted, and advanced chemical analyses were performed using GC-MS and HPLC. In vitro antioxidant effects were investigated using various methods. The in vivo analgesic effect was evaluated using the writhing and paw-licking tests. The anti-inflammatory effect was studied using the ear edema & granuluma formation tests. Hypoglycemic effect was assessed using OGTT. **Results and Discussion:** The standard screening test detected carbohydrates, glycosides, alkaloids, flavonoids, saponins, tannins, steroids, and phytosterols. Advanced analyses using GC-MS identified 75 compounds, including 8 major constituents, and HPLC quantified 5 polyphenols: catechin hydrate, (-)-epicatechin, vanillic acid, rutin hydrate, and rosmarinic acid. Significant levels of flavonoids and phenolics were present in the extract, which demonstrated moderate antioxidant capability in the DPPH assay ($IC_{50}$: 287.35 µg/mL). A significant reduction in pain was observed with the acetic acid ($p<0.01$) and formalin ($p<0.01$) methods. The extract showed significant anti-inflammatory activity in the xylene- and croton oil-induced ear edema tests ($p< 0.001$ and $p < 0.01$), moderately suppressed granuloma formation, and significantly reduced blood glucose levels in the oral glucose tolerance test ($p < 0.001$). **Conclusion:** *M. corchorifolia* contains multiple bioactive compounds with promising antioxidant, analgesic, anti-inflammatory, and hypoglycemic properties, supporting its traditional medicinal use and suggesting its potential for further therapeutic research.

## INTRODUCTION

Medicinal plants have been integral to human health and well-being since ancient times. Early humans relied on these plants in various forms, including whole plants, powders, pastes, juices, infusions, and decoctions, to address a range of health concerns [1]. Over generations, the knowledge of using plants for healing

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has been passed down, forming the foundation for many traditional medicine systems and contributing significantly to the development of modern pharmaceuticals [2]. Bangladesh is particularly rich in medicinal plant diversity, with numerous species thriving in its forests, rural areas, and natural shrub lands. This abundance offers immense potential for scientific research and the discovery of new, effective medicinal compounds that could benefit both local & global healthcare [3]. According to projections from the World Health Organization (WHO), approximately 80 out of 100 individuals worldwide, particularly those in rural and developing regions, rely on plant-based therapies to meet their basic medical needs. The WHO supports the use of traditional herbal medicines in national healthcare systems, emphasizing their accessibility, affordability, safety, and the trust they inspire among communities [4]. One notable medicinal plant is *Melochia corchorifolia L.*, commonly known as Chocolate Weed. This member of the *Malvaceae* family is a common sight in open areas and wastelands, such as roadsides [5]. It is distributed across various regions, including South and Southeast Asia, Africa, Australia, and the Pacific Islands. The plant is recognized by its ovate leaves, slender petioles, and distinctive leaf veins [6]. Research has revealed that *Melochia corchorifolia* contains a range of valuable phytochemicals, including triterpenes, flavonoids, glycosides, alkaloids, and other bioactive compounds [7]. Traditional medicine has long used various parts of this plant to treat ailments such as digestive issues, abdominal swelling, skin sores, smallpox, dysentery, vomiting, urinary problems, snake bites, headaches, and intestinal worms [6]. Various research on M. corchorifolia has been documented. Still, no data were found on GC-MS & HPLC analyses, as well as on bioactivities such as analgesic, anti-inflammatory, and hypoglycemic effects. Therefore, the present investigation could be a new avenue to address this research gap. The present study focuses on extracting and identifying active compounds from the whole plant of *Melochia corchorifolia* using ethanol. The research further evaluates the plant's antioxidant capacity and its potential analgesic, anti-inflammatory, and hypoglycemic effects (Figure 1). These investigations underscore the continued importance of medicinal plants in the search for new therapeutic agents and the need to conserve this valuable natural heritage.

## *MATERIALS AND METHODS*

### Collection of plants and preparation of the extract

The total part of the plant of *Melochia corchorifolia* (MC) was gathered from the Savar region. The species was verified and authenticated by experts at the National Herbarium in Mirpur, Dhaka (Accession Number: DACB 40843). Following harvest, the collected plant was sliced into small pieces and thoroughly rinsed with water to remove contaminants. The resulting pieces were ground into a fine powder and allowed to air dry. Subsequently, Soxhlet extraction was performed on the powdered plant material. The resulting extract was carefully collected, dried at low temperature and reduced pressure, stored, and used for subsequent analyses. 970 g powder was extracted using 5 L of ethanol (96%) into several fractions of the total amount. Each fraction was continued for 3-4 hours, and after drying, 117 g of extract was obtained. The yield value of the extract was found 12.06% w/w.

**Preliminary Phytochemical Screening:** Experiments to identify phytochemicals were conducted using validated test procedures as described below [8, 9].

| Chemical Group | Procedure | Observation and Inference |
|---|---|---|
| **Carbohydrates** | Extract, Molisch's reagent & conc. $H_2SO_4$ was added | A red or purple ring was developed. |
| **Alkaloids** | Extract+ 1% HCl+ Mayer's reagent. | Formation of white or creamy white ppt. |
| **Flavonoids** | Extract+2 ml ethanol+3/4 Pieces of Mg chips+ conc. HCl. | Development of a pink, orange, or purple color |
| **Glycosides** | Extract+2 ml acetic acid, $FeCl_3$ solution & conc. $H_2SO_4$ was added. | In the middle, a reddish-brown color appeared. |
| **Saponin** | 5 ml of extract + 5 ml of distilled water & shaken vigorously for 10-15 min. | Production of a persistent frothing of a 1 cm layer. |
| **Tannins** | Extract (5 ml) + $FeCl_3$ solution (2-3 drops). | Formation of a green, like/ blue/ blue green ppt. |
| **Steroids** | Extract+1 ml acetic acid + 1 ml $H_2SO_4$. | Violet to bluish green color. |
| **Phytosterols** | 2 ml Extract+1 ml chloroform +1 ml of conc. $H_2SO_4$. | Appearance of golden red/golden yellow color. |

### Isolation of Compounds by GC-MS

Gas chromatography-mass spectrometry (GC-MS) was employed to characterize the ethanolic crude extract of *Melochia*

*corchorifolia*. A capillary column (DB-5ms, 30 m long and 0.25 mm in diameter) carrying helium gas at a steady pressure of 53.5 kPa was used in the analysis. The injector temperature was maintained at 250°C, and the starting column temperature was set to 50°C (held for 1 minute). A 5:1 splitting ratio was employed for the splitting of injection of ethanol-dissolved samples [10]. The biologically active components in the whole-plant extract were identified and quantified using GC-MS. The National Institute of Standards and Technology (NIST) database, having more than 62,000 reference spectra, was used to analyze mass spectral data [11]. Mass spectra and retention times of unknown compounds were compared with those of known substances in the NIST collection to identify them. Key parameters, including retention time, percentage composition (based on peak area), and compound names, were documented for further analysis.

### HPLC Profiling of Bioactive Constituents

High-performance liquid chromatography with diode array detection (HPLC-DAD) was employed to characterize the polyphenolic composition of *Melochia corchorifolia*. The analytical system comprised a binary gradient pump, an automated sample injector, a thermostatically controlled column compartment, and a multi-wavelength detector, all synchronized via LC Solution software for real-time monitoring and data processing. A reversed-phase Luna C18 column (5 µm particle size, Phenomenex) kept at 33°C was used to separate the phytochemicals. Two eluents composed solvent A (acetonitrile with 1% glacial acetic acid) and mobile phase B (ultrapure water acidified with 1% acetic acid). For optimal polyphenol quantification, the analytical parameters were set to a 20 µL injection volume, an isocratic flow rate of 0.5 mL/min, and detection at 270 nm [12,13].

A calibration series was constructed using methanolic solutions of 15 reference standards to enable accurate quantification. Catechin hydrate (50 µg/mL) supplied a baseline for flavonoid derivatives, while gallic acid (20 µg/mL) and 3,4-Dihydroxybenzoic acid (15 µg/mL) functioned as high-concentration indicators. Rosmarinic acid (30 µg/mL), catechol, and (-)-epicatechin were all present at intermediate amounts. The calibration hierarchy was completed by Myricetin and Kaempferol (8 µg/mL each) and trans-Cinnamic acid (4 µg/mL). Lower-tier standards included Quercetin, Rutin hydrate, Syringic acid, Vanillic acid, p-Coumaric acid, trans-Ferulic acid, and Caffeic acid (10 µg/mL each) [14].

### DPPH Free Radical Scavenging Assay

The antioxidant efficacy of the extract was examined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. The procedure involved mixing 2 mL of freshly made DPPH methanol solution with 200 µL of the plant extract or established antioxidant (at varying concentrations) in test tubes. To ensure that the antioxidants and free radicals fully interacted, the mixture was left to stand at room temperature for 30 minutes under carefully monitored conditions [15].

Absorbance measurements were recorded at 515 nm using a spectrophotometer, with a solvent-only blank used for baseline correction. A control sample containing DPPH solution without any extract or standard was prepared in parallel [16]. The formula below was utilized to ascertain the percentage inhibition of free radicals:

$$\% \, Inhibition = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

*Where A1 indicates the absorbance of the sample or standard, and $A_O$ stands for the absorbance of the control. Plotting inhibition percentages against the logarithm of sample concentrations produced dose-response curves, which enabled calculation of the half-maximal inhibitory concentration ($IC_{50}$).*

### Quantification of Total Phenolic Constituents

For analysis, 1 mL of the extract (200 µg/mL) or a standard solution of gallic acid was combined with Folin-Ciocalteu reagent (5 mL; 1:1 dilution), and sodium carbonate (4 mL; 7.5% w/v) was added. The absorbance at 765 nm was measured relative to a solvent blank after 60 minutes of incubation at 20°C [17]. Phenolic content was calculated as:

$$C = \frac{c \times V}{m}$$

*Where C represents gallic acid equivalents (mg GAE/g extract), c represents gallic acid concentration (mg/mL) obtained using a calibration curve, V represents volume (mL), and m represents the dry extract mass (g).*

### Evaluation of Total Flavonoid Content

Flavonoid quantification utilized the colorimetric method using $AlCl_3$, which targets flavonoid-metal chelate formation at specific hydroxyl and keto groups. 3 mL of methanol, 200 µL of $AlCl_3$ (10% w/v), 200 µL of potassium acetate (1 M), and 5.6 mL of distilled water were mixed with 1 mL of an extract (200 µg/mL) or a quercetin standard. After incubating for 30 minutes at 25°C, the absorbance was measured at 415 nm [18]. Total flavonoid content was determined using:

$$C = \frac{c \times V}{m}$$

*Where C denotes quercetin equivalents (mg QE/g extract), c represents quercetin concentration (mg/mL), V represents the extract volume (mL), and m represents extract mass (g).*

**Assessment of Total Antioxidant Capacity**

To evaluate the antioxidant properties, a temperature-dependent phosphomolybdenum reduction test was employed [19]. In this procedure, 3 mL of reagent solution (4 mM ammonium molybdate, 28 mM sodium phosphate, and 0.6 M sulfuric acid) was mixed with 200 µL of the extract or ascorbic acid standard. A blank (300 µL of solvent + 3 mL of reagent) was used to measure absorbance at 695 nm after 90 minutes at 95°C. The capacity for antioxidants was stated as:

$$A = \frac{c \times V}{m}$$

*Where A represents ascorbic acid equivalents (mg AAE/g extract), c represents the ascorbic acid concentration (mg/mL), V represents the extract volume (mL), and m represents the extract mass (g). Each assay was performed in triplicate, and calibration curves were validated for linearity ($R2 > 0.99$). Method specificity was ensured by maintaining controlled pH and reagent stoichiometry.*

**Animal Models and Ethical Considerations**

The present study employed Swiss albino mice (25-30 g) and Sprague-Dawley rats (150-170 g) of both sexes, procured from the Animal Research Facility of the Pharmacy Department at Jahangirnagar University. The subjects were housed in regulated conditions (27.0 ± 1.0°C, 55-65% humidity, equal cycle of dark and light for 12 hours), having free food and water access. Before the tests began, a seven-day acclimatization phase was conducted. IEC provides ethical clearance [Ref. Number. BBEC, JU/M 2924/11(141)] and all methods followed institutional animal ethical requirements.

**Acute Toxicity Profiling**

An acute oral toxicity investigation in Swiss albino mice (20-25 g) revealed no adverse effects up to doses of 5,000 mg/kg b.w. p. o., establishing a safety profilefor the experimental extract for subsequent studies [20].

**Acetic Acid-Induced Visceral Pain Model**

Adapted from Koster's methodology, four mouse cohorts (n=6/group) received either saline (10 mL/kg, served as control), diclofenac sodium (100 mg/kg), or *Melochia corchorifolia* ethanolic extract (250/500 mg/kg). Intraperitoneal administration of 0.7% acetic acid (10 mL/kg) at 45 minutes post-treatment induced abdominal constrictions[21]. Writhes were quantified over a 5-minute observation window starting 15 minutes post-injection. Analgesic efficacy was calculated as:

$$\% \textit{ Inhibition} = \frac{\textit{Control writhes} - \textit{Treated writhes}}{\textit{Control writhes}} \times 100$$

**Formalin-Induced Nociception Assessment**

Following Hunskaar's protocol, mice (n = 6/group) were pretreated with saline (control), diclofenac (100 mg/kg), or the extract (250/500 mg/kg). Sub-plantar formalin injection (2.7%, 20 µL) triggered biphasic pain responses. Licking durations were recorded during acute (0–5 min) and tonic (20–25 min) phases [22,23].

**ANTI-INFLAMMATORY EVALUATIONS**

**Xylene-Induced Ear Edema**

Mice (n=6/group) received oral treatments in three groups (control, STD, and therapy with MC at 250 & 500 mg/kg extract), followed by bilateral application of xylene (20 µL/ear). One hour post-exposure, 3-mm ear biopsies were weighed. Edema inhibition was derived from mass differentials between treated and untreated ears [22].

**Croton Oil Dermatitis Model**

Mice (n=6/group) received oral treatments in different groups (control, STD, and therapy with MC 250 & 500 mg/kg extract), followed by topical croton oil (15 µL in acetone), which elicited ear inflammation. Extract-pretreated mice (n=6/group) were euthanized 1 hour post-application for biopsy mass comparisons with vehicle controls [24].

**Subcutaneous Granuloma Formation**

Sterile cotton pellets (10 ± 1 mg) were implanted subcutaneously in anesthetized mice. Seven-day oral administration in three groups (control, STD, and treatment with MC extract at 250 & 500 mg/kg) was followed by pellet excision, desiccation (60°C, 24 h), and dry-mass measurement. A reduction in granuloma mass indicated antiproliferative activity [25].

**Hypoglycemic Activity Assessment**

Oral glucose tolerance tests (OGTT) utilized overnight-fasted rats (n=6,150–200 g) receiving either water (control), Glibenclamide (10 mg/kg), or extract (250/500 mg/kg). At 0, 60, and 120 minutes following the glucose challenge (3 g/kg), blood glucose levels were measured [26].

**Statistical Interpretation**

Microsoft Excel 2007 was used for processing quantitative data. One-way ANOVA and Dunnett's post hoc comparison were used to compare groups in SPSS v16.0. The mean ± SEM/SDwas used to express the results. p<0.05, 0.01, & 0.001were the thresholds for the levels of significance.

## *RESULT AND DISCUSSION*

### Results

**Preliminary Phytochemical Screening**

Initial qualitative profiling of the ethanolic extract of Melochia corchorifolia (MC) identified multiple therapeutically active constituents, including carbohydrates, glycosides, alkaloids, flavonoids, saponins, tannins & phytosterols (Table 1). These phytochemical classes are pharmacologically significant and may contribute to the plant's observed therapeutic properties.

**GC-MS Metabolic Fingerprinting**

Gas chromatography-mass spectrometry (GC-MS) analysis identified 75 distinct compounds, with eight accounting for the majority of the phytochemical profile (Tables 2 & 4). Spectral matching against the NIST database (62,000+ reference entries) enabled compound identification, with retention time and relative abundance (%) prioritized for major constituents. Trace-level metabolites, though present, fell below quantitative thresholds.

**HPLC-DAD Quantification of Phenolics**

HPLC-DAD revealed five polyphenolic markers: catechin hydrate, (-)-epicatechin, vanillic acid, rutin hydrate, and rosmarinic acid (Tables 3 and 4). Rutin hydrate was identified as the most abundant compound (124.70 mg/100 g dry extract), suggesting a potential role in the plant's bioactivity.

**Acute Toxicity Profile**

Oral administration at 5.0 g/kg body weight induced no mortality or behavioral abnormalities (e.g., lethargy, hyperexcitability) in mice during a 72-hour observation period. This safety margin supports further pharmacological exploration without concerns about acute toxicity. These findings establish *M. corchorifolia* as an excellent source of bioactives with antioxidant potential, which is consistent with its traditional therapeutic uses.

**Antioxidant Capacity Evaluation**

The extract exhibited a moderate ability to mitigate the free radicals in the DPPH test ($IC_{50}$ = 287.35 µg/mL). Quantitative phytochemical profiling revealed substantial phenolic (98.70 mg gallic acid equivalents/g), flavonoid (154.67 mg quercetin equivalents/g), and total antioxidant (50.00 mg ascorbic acid equivalents/g) content (Table 5 & Figure 1).

**Table 1: Preliminary Phytochemical screening**

| Chemical Constituents | Result |
|---|---|
| Carbohydrates | + |
| Glycosides | + |
| Alkaloids | ++ |
| Flavonoids | ++ |
| Saponins | + |
| Tannins | + |
| Steroids | - |
| Phytosterol | + |

***[NB: + = Presence, ++ = Moderate Presence, +++ = Strong presence, - = Absence***

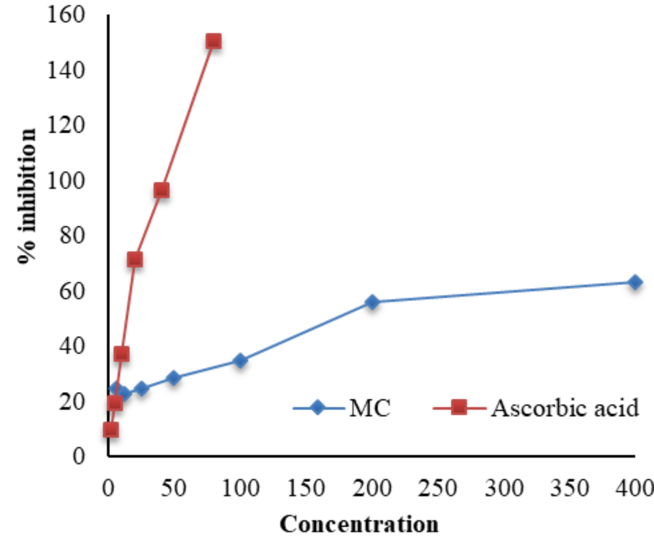


**Figure 1: DPPH radical scavenging activity of the STD (Ascorbic acid) and *M. corchorifolia* extract.**

**Table 2a: List of major compounds isolated using GC-MS.**

| S N | Retention Time | Area % | Name |
|---|---|---|---|
| 1 | 3.581 | 10.23 | 2-fluoro-1-phenylethanone |
| 2 | 3.636 | 4.65 | Glycerin |
| 3 | 4.055 | 2.64 | Ethylene glycol, TMS derivative |
| 4 | 17.321 | 3.65 | Hexadecanoic acid, ethyl ester |
| 5 | 19.114 | 3.51 | 8,11,14-Docosatrienoic acid, methyl ester |
| 6 | 19.264 | 2.81 | Phytol |
| 7 | 20.211 | 3.68 | Ethyl Oleate |
| 8 | 23.506 | 15.29 | `Palmitoleamide |

**Table 2b: List of minor compounds isolated using GC-MS.**

| Sl. No. | Retention Time | Area% | Name |
|---|---|---|---|
| 1 | 3.53 | 0.85 | d-Galactoseoxime |
| 2 | 3.705 | 1.29 | (Chloromethyl) fluorodimethylsilane |
| 3 | 3.777 | 0.93 | 2-Propenoic acid, ethenyl ester |
| 4 | 3.815 | 1.73 | Propionic acid, 2-mercapto-, allyl ester |
| 5 | 3.901 | 1.15 | Ethane, (methylthio)- |
| 6 | 3.93 | 0.77 | 3-Amino-2-oxazolidinone |
| 7 | 3.996 | 1.76 | Butanoic acid, ethyl ester |
| 8 | 4.108 | 0.42 | 2-Propanol, 1,1-dimethoxy- |
| 9 | 4.22 | 0.29 | Pentanoic acid, 4-methyl- |
| 10 | 4.248 | 0.2 | Methyl 2-hydroxy-2-methoxyacetate |
| 11 | 4.314 | 0.33 | 2-Cyclopenten-1-one |
| 12 | 4.455 | 0.35 | Benzenemethanol, 2-nitro- |
| 13 | 4.475 | 0.21 | 1,1,3,3-Tetramethyl-3-(1-methylpropoxy)disiloxan-1-ol |
| 14 | 4.706 | 0.3 | 4-Cyclopentene-1,3-dione |
| 15 | 4.834 | 0.29 | 3-Isopropoxy alanine |
| 16 | 4.873 | 0.28 | Oxime-, methoxy-phenyl-_ |
| 17 | 4.971 | 0.27 | 2(5H)-Furanone |
| 18 | 5.077 | 0.27 | 1,2-Cyclopentanedione |
| 19 | 5.53 | 0.56 | Mesitylene |
| 20 | 5.57 | 0.47 | Phenol |
| 21 | 5.596 | 0.51 | 1-Butyl(dimethyl)silyloxypropane |
| 22 | 5.675 | 0.63 | 1-Decene |
| 23 | 5.745 | 0.71 | 2-Propen-1-ol |
| 24 | 5.814 | 0.58 | Cyanoacetylurea |
| 25 | 6.043 | 0.22 | Dipropylene glycol (isomer 1) |
| 26 | 6.168 | 0.22 | D-Limonene |
| 27 | 7.825 | 0.37 | 1-Dodecene |
| 28 | 7.934 | 1.17 | 1-Tetradecene |
| 29 | 8.675 | 0.23 | Ethanol, 2-(2-methoxyethoxy)- |
| 30 | 8.897 | 0.2 | butanedioic acid, mono[4-(1,1-dimethylethyl)phenyl] ester |
| 31 | 9.201 | 0.31 | 2-Propanol, 1,1'-[(1-methyl-1,2-ethanediyl)bis(oxy)]bis- |
| 32 | 9.268 | 0.44 | 2-Methoxy-4-vinylphenol |
| 33 | 9.999 | 0.92 | 1-Tridecene |
| 34 | 10.446 | 0.7 | 2-(Isobutoxymethyl)oxirane |
| 35 | 10.5 | 0.24 | 1,3,5-Triazine-2,4(1H,3H)-dione, 6-(ethylamino)- |
| 36 | 11.57 | 1.65 | Ethanol, 2-[2-(2-methoxyethoxy)ethoxy]- |
| 37 | 11.7 | 0.67 | .alpha.-D-Glucopyranoside, methyl |
| 38 | 11.901 | 0.84 | 1-Pentadecene |
| 39 | 11.966 | 0.52 | Diethyl Phthalate |
| 40 | 12.174 | 0.33 | 5-Ethyl-1,3-dioxane-5-methanol, tert-butyldimethylsilyl ether |
| 41 | 12.533 | 0.62 | .beta.-D-Glucopyranose, 4-O-.beta.-D-galactopyranosyl- |
| 42 | 12.672 | 0.39 | 3,6,9-Trioxa-2-silapentadecane, 2,2-dimethyl- |
| 43 | 14.316 | 0.27 | n-Heptadecanol-1 |
| 44 | 14.732 | 0.22 | Isopropyl myristate |
| 45 | 14.934 | 1.23 | Neophytadiene |
| 46 | 15.02 | 0.25 | Z-25-Tetratriaconten-2-one |
| 47 | 15.061 | 0.61 | Hexaethylene glycol dimethyl ether |
| 48 | 15.569 | 0.31 | 3,7,11,15-Tetramethyl-2-hexadecen-1-ol |
| 49 | 19.002 | 1.54 | 9,12-Octadecadienoic acid, methyl ester |
| 50 | 19.517 | 1.01 | Methyl stearate |
| 51 | 20.314 | 0.44 | Ethyl 9-hexadecenoate |
| 52 | 20.633 | 1.43 | Octadecanoic acid, ethyl ester |
| 53 | 20.736 | 0.6 | 6-Hydroxy-9a-methoxy-3,8a-dimethyl-5-methylidene-4,4a,6,7,8,9-hexahydrobenzo[f][1]benzofuran-2-one acetate |

| Sl. No. | Retention Time | Area% | Name |
|---|---|---|---|
| 54 | 22.38 | 0.21 | Myristic acid glycidyl ester |
| 55 | 23.41 | 0.85 | 9,11-Octadecadienoic acid, methyl ester, (E,E)- |
| 56 | 23.655 | 0.65 | Deoxyspergualin |
| 57 | 23.775 | 0.32 | Pyridine, 1-acetyl-1,2,3,4-tetrahydro-5-(2-piperidinyl)- |
| 58 | 23.91 | 0.47 | Octadecanamide |
| 59 | 23.99 | 0.33 | 2-Nonenal, (E)- |
| 60 | 24.15 | 0.22 | Hexacontane |
| 61 | 25.758 | 0.24 | 2-Methylhexacosane |
| 62 | 25.91 | 0.2 | 2- Bromopropionic acid, tridecyl ester |
| 63 | 27.721 | 0.21 | 2- Bromopropionic acid, octadecyl ester |
| 64 | 30.012 | 0.28 | 13-Docosenamide, (Z)- |
| 65 | 30.466 | 0.23 | Squalene |
| 66 | 35.056 | 0.51 | alpha.-Tocopheryl acetate |
| 67 | 36.943 | 0.23 | 9,19-Cyclolanostan-3-ol, acetate, (3.beta.)- |

**Table 3: List of compounds isolated using HPLC.**

| Name of Standard | *M. corchorifolia* (mg/100 g dry extract) |
|---|---|
| Gallic acid | ND |
| 3,4 dihyrdoxy benzoic acid | ND |
| Catechin hydrate | 63.77±0.39 |
| Catechol | ND |
| (-) Epicatechin | 47.99±0.36 |
| Caffeic acid | ND |
| Vanillic acid | 27.25±0.17 |
| Syringic acid | ND |
| Rutin hydrate | 124.70±1.33 |
| p-Coumaric acid | ND |
| Trans-Ferulic acid | ND |
| Rosmarinic acid | 10.25±0.30 |
| Myricetin | ND |
| Quercetin | ND |
| Trans-Cinnamic acid | ND |
| Kaempferol | ND |

**Table 4: Major compounds found from *M. corchorifolia* extract in GC-MS and HPLC analysis**

| | | |
|---|---|---|
| 2-fluoro-1-phenylethanone | Glycerin | Ethylene glycol |
| Hexadecanoic acid, ethyl ester | Phytol | Ethyl Oleate |
| Palmitoleamide | Catechin hydrate | (-) Epicatechin |

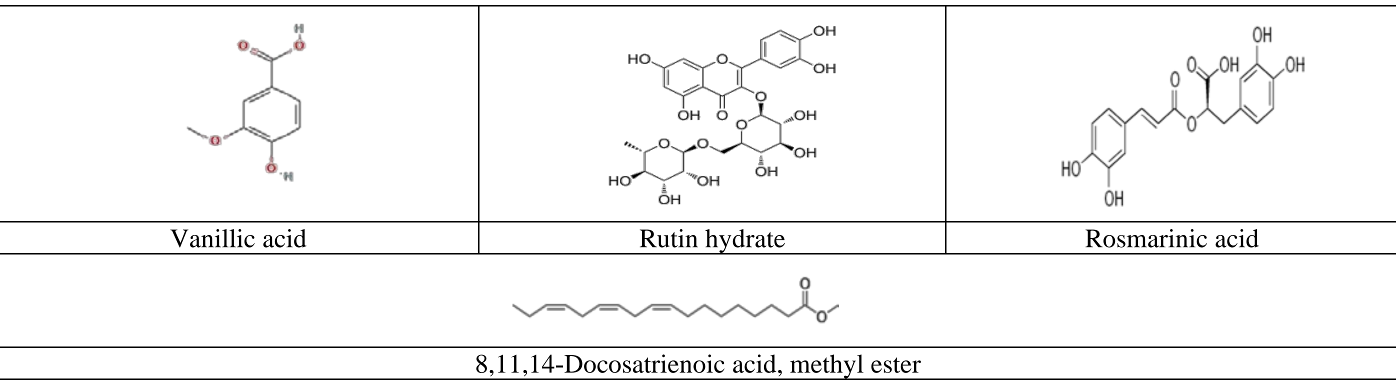


**Table 5: $IC_{50}$ values in DPPH radical scavenging assay, total phenolic content, total flavonoid content and total antioxidant capacity of *M. corchorifolia* extract**

| Sample Name | **$IC_{50}$ value** (µg/ml) in DPPH radical scavenging assay | **Total Phenolic content** (mgGAE/gm) (Mean±SD) | **Total Flavonoid content**(mgQE/gm) (Mean±SD) | **Total antioxidant capacity** (mg AAE/ gm) (Mean±SD) |
|---|---|---|---|---|
| ***M. corchorifolia*** | **287.35** | **98.70±6.20** | **154.67±2.52** | **50.0±7.12** |

*Values represent the mean of triplicate tests with mean ± SD*

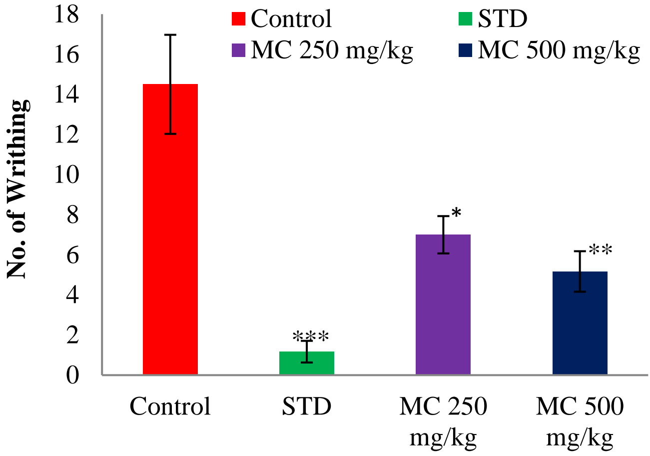


**Figure 2: The effect of standard, MC 250 mg/kg and MC 500 mg/kg in the writhing responseinduced by acetic acid.**

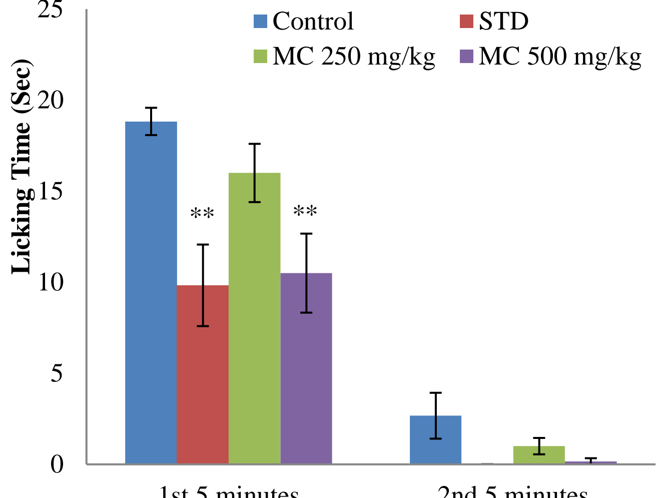


**Figure 3: The effect of standard (Diclofenac-Na), MC 250 mg/kg and MC 500 mg/kg in the formalin induced paw licking test (1st and 2nd 5 minutes).**

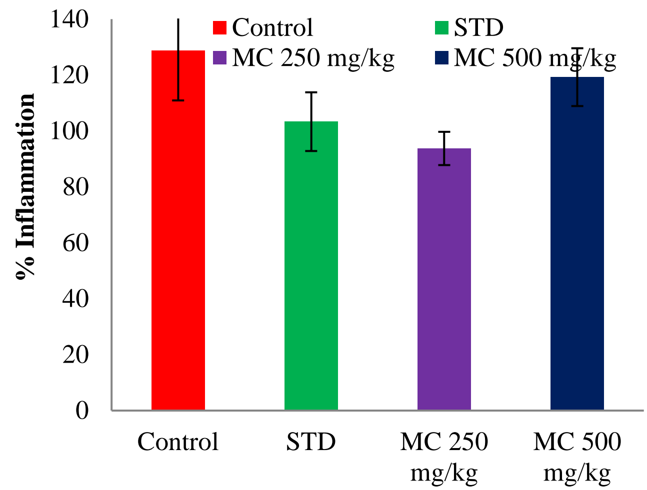


**Figure 4:The effect of MC 250 mg/kg and MC 500 mg/kg in cotton pellet pleurisy test.**

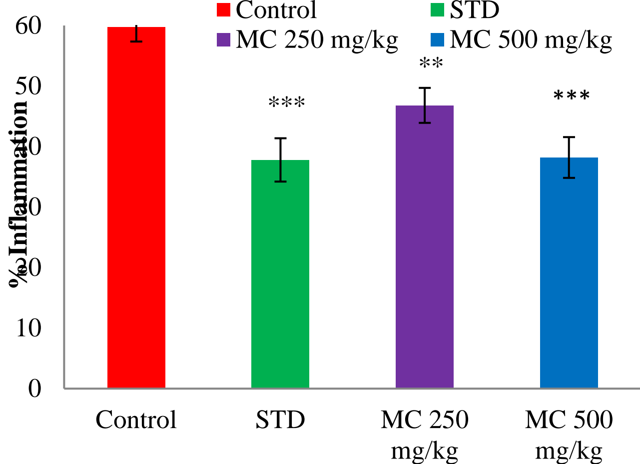


**Figure 5: Graphical representation of the effect of standard, MC 250 mg/kg and MC 500 mg/kg in the xylene inflammation test.**

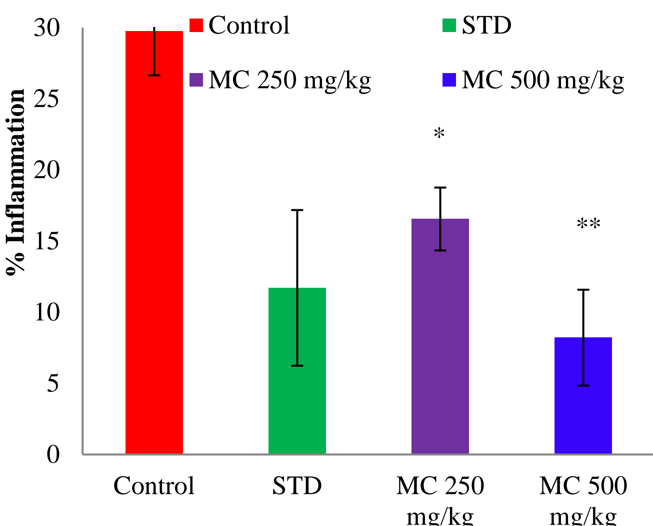


**Figure 6: Graphical representation of the effect of standard, MC 250 mg/kg, and MC 500 mg/kg in the croton oil inflammation test.**

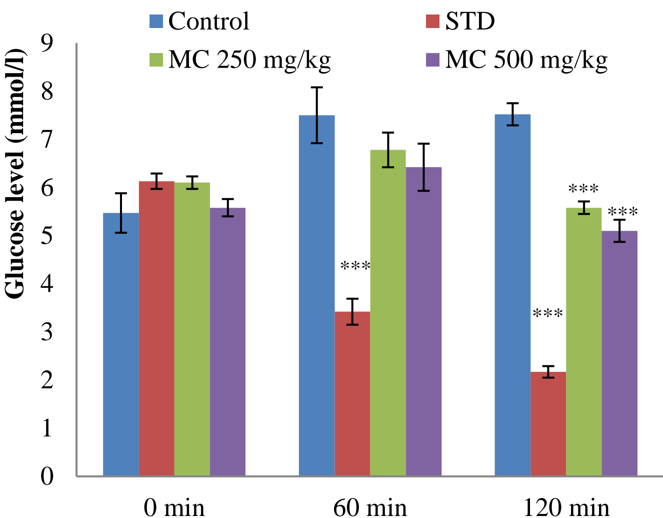


**Figure 7: Impact of *Melochia cochorifolia* on serum glucose level in OGTT in normal rats.**

Several animal models were employed to evaluate the analgesic and anti-inflammatory activities of *Melochia corchorifolia* extract, each addressing an individual aspect of pain and inflammation. The ethanolic extract at 250 mg/kg and 500 mg/kg significantly reduced the number of writhes in the acetic acid-induced abdominal writhing test compared to the control group by 51% ($p<0.05$) and 64% ($p<0.01$), respectively, suggesting a potent anti-nociceptive action (Figure 2). These results were reinforced by the formalin-mediated licking of paw test, which revealed a dose-responsive decrease in paw licking duration following extract administration. At 500 mg/kg, a notable lowering ($p<0.01$) in paw licking was evident in the first five minutes (acute pain phase), while the reduction in the second phase was not statistically significant (Figure 3). In the cotton pellet-induced granuloma model, both doses of the extract reduced granuloma dry weight, with the 250 mg/kg dose producing greater suppression than the 500 mg/kg dose (Figure 4). Interestingly, the anti-proliferative effect at 250 mg/kg was even stronger than that of ibuprofen, a standard medication, although the difference was not statistically significant. The extract effectively reduced inflammation in the xylene-induced ear edema test; both doses inhibited swelling by 21% ($p < 0.01$) and 33% ($p < 0.001$), respectively (Figure 5). These results were similar to the 36% reduction observed with diclofenac (100 mg/kg). Comparing the extract to the control group, the extract significantly decreased ear swelling in the croton oil-induced ear edema model by 44% ($p < 0.05$) at 250 mg/kg and by 72% ($p < 0.01$) at 500 mg/kg (Figure 6).

The glucose-lowering potential of the sample was assessed using the oral glucose tolerance test (OGTT) in non-diabetic subjects. After glucose administration, all groups showed elevated blood glucose levels at 60, 90 & 120 mins. Although the extract had no significant effect at the earlier time points, both doses (250 mg/kg and 500 mg/kg) produced a highly significant suppression of blood glucose ($p < 0.001$) at 120 minutes compared with untreated animals (Figure 7). Overall, the outcomes underscore the analgesic, anti-inflammatory & anti-hyperglycemic capacities of Melochia corchorifolia extract, lending scientific support to its traditional use in treating pain, inflammation, and metabolic disorders.

## *DISCUSSION*

The application of advanced analytical methods, including GC-MS and HPLC, has been pivotal in elucidating the phytochemical composition of *Melochia corchorifolia* (MC) extracts. GC-MS analysis identified several bioactive compounds, including hexadecanoic acid, phytol, and palmitoleamide, while HPLC quantification isolated polyphenolic markers such as catechin hydrate, rosmarinic acid, and rutin hydrate. These bioactive compounds, consistent with the plant's folk medicinal use, reinforce its importance as a source of new therapeutic agents. Further work to isolate and characterize these molecules may elucidate their specific biological roles. Many important phytochemicals, such as polyphenols, alkaloids, glycosides, saponins, and other compounds derived from diverse plant sources, have been reported and discussed as potential agents against various diseases [27]. Rutin hydrate, Vanillic acid, catechin hydrate, β-

sitosterol, and rosmarinic acid are biologically active compounds widely familiar for their diverse potential [28–33]. The antioxidant potential of the MC extract was systematically evaluated using multiple assays, reflecting the complex interplay among its phytochemicals. The DPPH radical scavenging assay demonstrated dose-dependent activity ($IC_{50}$ = 287.35 µg/mL), attributed to the extract's ability to donate hydrogen atoms or electrons. The total phenolic (98.70 mg GAE/g) and flavonoid (154.67 mg QE/g) contents further corroborated its antioxidative capacity, as polyphenols and flavonoids neutralize reactive oxygen species (ROS) through radical scavenging, metal chelation, and enzyme inhibition [34,35].

The phosphomolybdenum assay quantified total antioxidant capacity (50.00 mg AAE/g), reinforcing the extract's ability to mitigate oxidative stress [36]. In vivo evaluations of analgesic activity revealed dose-dependent inhibition of nociception. The writhing response to acetic acid is a model of visceral pain, initiated by prostaglandins and inflammatory mediators, and it showed a 51–64% reduction in constrictions, suggesting peripheral modulation of pain pathways [37,38]. Similarly, the formalin test highlighted the extract's efficacy in the tonic phase (20–25 minutes), implicating inhibition of prostaglandin synthesis. Anti-inflammatory assessments in xylene- and croton oil-induced edema models demonstrated significant suppression of vascular permeability (21–72%) and inflammatory mediators, such as substance P and phorbol esters. The cotton pellet granuloma assay revealed antiproliferative effects, potentially through modulation of fibroblast activity [25]. The hypoglycemic study revealed delayed but significant glucose-lowering effects in normoglycemic rats, with marked reductions at 120 minutes post-glucose challenge. This is consistent with the control of postprandial hyperglycemia, a crucial target in type 2 diabetes mellitus, which is characterized by endothelial dysfunction and insulin resistance [39]. The extract's ability to attenuate glucose spikes without early-phase effects suggests a mechanism distinct from rapid insulin secretion, possibly involving enhanced peripheral glucose uptake or delayed carbohydrate absorption [26]. Collectively, these findings validate *M. corchorifolia* as a multifaceted medicinal plant with analgesic, anti-inflammatory, and glucose-modulating properties, likely mediated by its diverse phytochemical repertoire. Further studies isolating specific bioactive compounds could advance their therapeutic application in chronic inflammatory and metabolic disorders.

## *CONCLUSION*

The findings highlight that the ethanolic extract of *Melochia corchorifolia* contains a range of bioactive compounds and exhibits significant antioxidant, analgesic, anti-inflammatory, and hypoglycemic effects. Its ability to scavenge free radicals, mitigate nociceptive responses, suppress inflammatory mediators, and modulate postprandial glucose levels underscores its therapeutic potential in addressing oxidative stress, pain-related, and metabolic disorders. While these preclinical results align with traditional uses of the plant, further mechanistic studies are essential to elucidate the molecular pathways involved in its bioactivity. Additionally, clinical validation is warranted to assess its efficacy and safety in human populations, potentially positioning *M. corchorifolia* as a valuable candidate for developing natural therapeutics targeting chronic inflammation, pain management, and diabetes-related complications.

## *FINANCIAL ASSISTANCE*

NIL

## *CONFLICT OF INTEREST*

The authors declare no conflict of interest.

## *AUTHOR CONTRIBUTION*

The research, encompassing study design, data collection, analysis, and manuscript preparation, was a collaborative effort by all authors. Md. Mizanur Rahman handled study design, data collection, writing, review and editing, and visualization. Md. Jahir Alam contributed to data collection, data processing, and formal analysis. M. Salahuddin Bhuiya was involved in the investigation and provided essential resources. Ruhul Amin was responsible for conceptualization and drafting the manuscript. Masum Shahriar oversaw the work and managed project administration.

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