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# Evaluation of Analgesic, antioxidant, and antibacterial activities of methanolic extract of *Litsea glutinosa* bark from Chuadanga, Bangladesh

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## ABSTRACT

From the ancient time plants have been used to treat several ailments and act as a source of bioactive potential compounds in recent time. Nowadays it is important to search for bioactive phytochemicals because of emerging of new lethal diseases such as cancers and Covid-19 and development of antibiotic resistance. In this study, we selected bark of *Litsea glutinosa* for exploring its medicinal values. The methanolic extract from *L. glutinosa* bark was subjected to phytochemical screening, and in-vitro and in-vivo pharmacological assays. It was found that the crude methanolic extract contains several classes of compounds in higher concertation including tannin, flavonoids, and phenols. The methanolic extract was employed for testing analgesic, antimicrobial, and anti-oxidant by standard reported methods. This plant crude extracts showed 60.38% inhibition of acetic acid induced writhing and 68.09% increase in elongation of latency time during tail flick test in mice. In case of antioxidant assay, the plant extract exhibited free radical scavenging activities with $IC_{50}$ value 91.15 mg/ml. Moreover, the methanolic extract of *L. glutinosa* bark showed moderate antibacterial activity against only B. subtilis. This study suggests that *L. glutinosa* has strong central and peripheral analgesic, and anti-oxidant activity, and moderate antibacterial activity. This study also suggests that potent analgesic and anti-oxidant activity of *L. glutinosa* might be due to the presence of bioactive phenolic, flavonoid and tannin compounds. Interestingly, this study might support this plant's traditional uses. Future investigation is needed to explore the others medicinal activities and isolation of bioactive compounds.

**Keywords:** *Litsea glutinosa*, Methanolic extract, Analgesic, Antimicrobial, Antioxidant.

## INTRODUCTION

Medicinal plants contain a huge number of chemical compounds that showed specific physiological effects on human body as a result they have been utilized for thousands of years to cure different types of human diseases. The most significant types of these substances are phenolic, flavonoid, tannin Alkaloids, terpenoids, and saponins, compounds. These substances' medicinal potential and little toxicity have drawn the attention of pharmacists [1]. Many such compounds with potentially unique mechanism of action with less toxicity to the host cell have been discovered from plants. These compounds could be employed in the creation of novel medications to quench ROS and suppress the growth of bacterial and fungal pathogens [2].

Bangladesh is home to a wide diversity of medicinal plants because of its subtropical climate and lush deltaic terrain. In Bangladesh more than 500 species of medicinally important plants may be found, and the country's rural residents have long relied on these plants for their primary medical requirements [3]. Bangladesh's traditional medical system has evolved over many centuries as a result of trial and error. Approximately 250 medicinal plants are being employed in Bangladesh to prepare herbal formulations, and their estimated market worth is about 3.3 billion taka (4.1 million dollar) [4]. These plant-based medications are used by Bangladesh's traditional healers to treat a wide range of illnesses, including skin ailments, gastrointestinal disorders, heart issues, hypertension, diabetes, asthma, and inflammation in diverse communities and locales [3, 5-7].

*Litsea glutinosa* (Lour.) C.B. Rob is belonging to the laurel family, Lauraceae. The medium-sized, scented, evergreen *Litsea glutinosa* tree, is native to India's Western Ghats. Different plant parts are utilized to make extracts that are used to treat rheumatism, diarrhea, dysentery, wound healing, respiratory problems, and sexual disorders [3, 8]. Essential oils possess antipyretic, analgesic, and anti-inflammatory properties in addition to acting as antibacterial, antispasmodic, carminative, and antiviral agents. β-sitosterol, actinodaphnine, boldine, norboldine, tannin, laurotetanine, n-methyllaurotetanine, n-methylactinodaphnine, quercetin, sebiferine, litseferine, etc. were found in the stem bark according to phytochemical investigation [9]. Because the essential oils of Litsea species include several structural

forms of phenolic chemicals and monoterpenoids, they are used in traditional herbal remedies.

As a result, research interests are currently primarily concentrated on assessing the bioactivities of natural compounds derived from herbal sources. The main purpose of this study was to explore the bioactive elements in a methanolic extract of *Litsea glutinosa* bark. Additionally, the extract's analgesic, antioxidant, and antibacterial properties were examined.

## METHODS AND MATERIALS

### Plant materials

The fresh stem bark of *Litsea glutinosa* was collected from Chuadanga, Bangladesh. The bark was collected at starting of the January. It was green and full of life. National herbarium Bangladesh verified the taxonomic identity of it (accession number DACB 91112). The samples were cleaned, rinsed, and shaded air dried for seven days at room temperature (24-26 ºC) with adequate ventilation. Using an electric machine the dried plant material was processed to create a fine powder. The ground sample was stored in a vacuum storage bag for further processing.

### Extract preparation

The powder samples were utilized for solvent extraction with methanol at room temperature. In 2.5 L glass bottle, the sample was macerated in the solvent and swirled three to four times each day for 10 days. With cotton, methanol extract of *L. glutinosa* bark (ME-LG-B) was separated. Utilizing a rotating evaporator set at 40°C, filtrates from the crude extractions were concentrated. The concentrated filtrates were taken in a beaker that had already been measured out and completely dry the solvent. Before usage, dried crude extracts were stored in the freezer after being wrapped in foil.

### Determination of Total Phenolic Content (TPC)

A slightly modified Folin-Ciocalteu method was used to determine the total phenolic content of the methanol extract of *L. glutinosa* bark [10]. Briefly, 5 ml (1:10 v/v in distilled water) of Folin-Ciocalteu reagent and 4 ml of 7.5% sodium carbonate were combined with 0.5 ml of extract (1 mg/mL). After being vortexed for 15 seconds, the mixture was left to stand for 30 minutes at 40ºC to develop its color. Using a spectrophotometer, the absorbance was determined at 765 nm. Gallic acid solutions at concentrations of 0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL were used to create the standard curve. Without using extract or gallic acid, Blank was made by using the same procedures as before. The following equation was used to calculate total phenolic content that is generated from the gallic acid calibration curve (y = 1.578x+0.0487; $R^2$= 0.9857) in Figure 1.

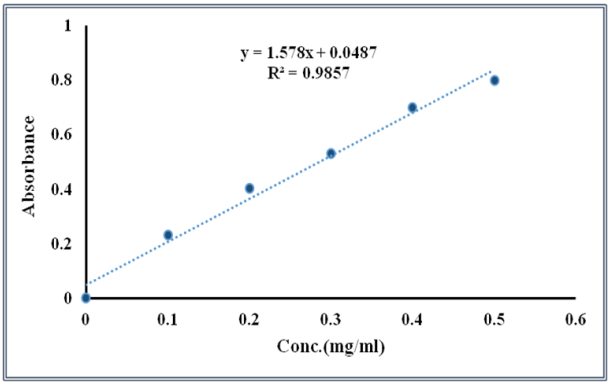


**Figure 1:** Total phenolic content determination from gallic acid standard calibration curve

Total phenolic content values are expressed in terms of mg of gallic acid equivalent (GAE) per gram of dry extract, which is a common reference compound.

To measure the total flavonoid content of the methanolic extract of *L. glutinosa* bark, the $AlCl_3$ colorimetric method was used [11]. One milliliter of an examined plant extract solution (1 mg/mL) was taken. Then 400 mg crystalline sodium acetate and 2.5 mL of $AlCl_3$ reagent (133 mg crystalline $AlCl_3$ and were dissolved in 100 mL of extracted solvent). At room temperature, the combinations were let to stand for 30 minutes. A 510 nm, the absorbance was measured against a blank surface. A blank was created using 5 mL of methanol as the extracting solvent and 2.5 mL of the $AlCl_3$ reagent. The concentration of quercetin in µg/ml was plotted on the x-axis and the reaction complex's absorbance was plotted on the y-axis to create the standard calibration curve using different concentrated solution of quercetin in methanol (0.1, 0.2, 0.3, 0.4, and 0.5 mg/mL). The calibration curve's regression equation (y = 1.0734x+0.0161; $R^2$= 0.9972) was used to calculate the total flavonoid concentration from Figure 2 which represented as milligrams of quercetin equivalent (QE) per gram of dry extract.

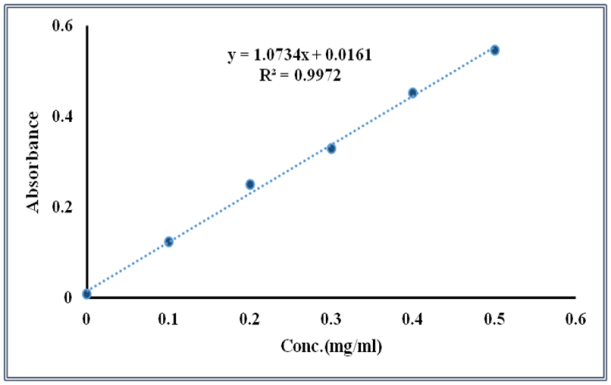


**Figure 2:** Total flavonoid content determination from Quercetin standard calibration curve

### Determination of Total Tannin Content (TTC)

The total amount of tannin was determined by using modified Folin-Ciocalteu method [12]. 0.1 mL of the sample extract was added along with 7.5 mL of distilled water. After that, 0.5 mL of the Folin-Ciocalteu Phenol reagent and 1 mL of a solution containing 35% sodium carbonate was added to each sample. Then, 10 mL of distilled water were added to the mixture for dilution. The mixture was properly shaken, and then 30 minutes were spent at room temperature. After that, absorbance was calculated at 725 nm. The blank was prepared instead of the sample using water. The standard gallic acid solutions (0.1, 0.2, 0.3, 0.4, and 0.5u g/mL) are compared with a control was measured in mg/g of dry extract and used to express the results of tannins. The total tannin concentration was calculated as mg of tannic acid equivalent per gram of dried extract by using the equation deduced from a standard gallic calibration curve (y = 1.7769x+0.0623; $R^2$= 0.9583) in Figure 3.

### DPPH free radical scavenging assay (quantitative analysis of antioxidant activity)

The ability of the methanolic extract of barks to scavenge the stable DPPH (2, 2-diphenyl-1-picryl hydrazyl) free radical allowed researchers to assess its antioxidant [13]. Serial dilution was used to create the aliquots of the extract at the various concentrations (6.25-200 µg/mL). A 0.004% w/v solution of DPPH was added to 3 mL of 1 mL of each concentration. After 30 minutes, 517nm absorbance was measured to calculate the Inhibitory conc. 50% ($IC_{50}$). The $IC_{50}$ value is the sample concentration necessary to remove 50% of the DPPH free radicals. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

The percentage of scavenging effect (DPPH) = {(Absorbance of control - Absorbance of sample) / Absorbance of control} ×100

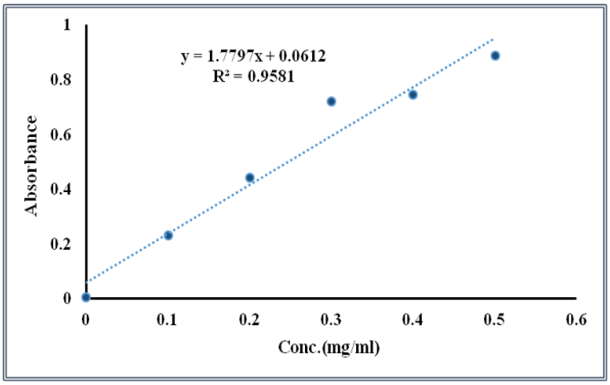


**Figure 3:** Total tannin content determination from gallic acid standard calibration curve

**Analgesic activity**

Acetic acid-induced writhing test was used to assess antinociceptive property [14]. Totally five groups of six mice each were created. The first group acted as the control and received 0.7% acetic acid intraperitonealy (dosage 10 ml/kg). Total number of writhes was counted for 20 minutes following the injection of acetic acid for 5 minutes. The second group received acetyl salicylic acid (100 mg/kg), a common medication. The water-dissolved methanol extract was given orally to the animals in the third to fifth groups at doses of 200, 400 and 600 mg/kg body weight, respectively. All of the groups, with the exception of group I, received acetic acid administration after an hour of incubation. After 5 minutes, the mice in each group were watched for the first signs of writhing, and the number of writhing reactions over the course of 20 minutes was counted. Each group's mean value was computed, and its results were compared to the control.

Utilizing Sewell and Spencer's (1976) tail flick method, antinociceptive activity was assessed. Five groups were created and each group contains six mice. As a control, the first group received merely normal saline (10 ml/kg), whereas the second received the conventional medication acetyl salicylic acid (100 mg/kg,). Three different dose of methanol extract 200, 400 and 600 mg/kg body weight were administered to the animals in the third to fifth groups, respectively. Experimental mice had their tails submerged for one to two centimeters in warm water that was constantly held at 50°C. The time it took the mice to redirect their tails was the pain reaction time. The first reading was thrown out, and the reaction time was calculated as the average of the following two readings. In order to protect the animal's tissue, a cutoff time of 10 seconds was observed.

**Antibacterial assay by disc diffusion method**

The modified agar disc diffusion method was used to conduct an in vitro antibacterial activity test [15]. Firstly, the Agar plates were injected with a standardized inoculum of the test microorganism throughout this method. Following that, 6 mm-diameter filter paper discs containing the test substance at concentrations of 250 and 500 µg /disc each were placed on the agar surface. Positive control such as antibiotic discs containing 30 µg/ disc of kanamycin, were used to confirm that the standard antibiotic showed its activity against the test organisms and to compare the known antibacterial agent's response to that of the test samples. Filter paper discs made of sterile paper were used as samples, and to prepare control disc 10 µL of methanol was poured to the blank discs. In the proper environments the petri plates were incubated and two gram-positive namely *B. subtilis*, *Staphylococcus aureus* and two gram-negative bacteria *Escherichia coli*, and *P. aeroginosa* were taken.

## RESULT

**Total Phenolic Content (TPC)**

A huge number of different physiologically active chemicals make up the primary secondary metabolites known as phenolic compounds. Phenolic compounds have redox characteristics that make them antioxidants and reducing agents [16].

The TPC for methanol bark extract *L. glutinosa* (LG-B-ME) was 60 mg Gallic acid equivalent (GAE)/g extract (Table 1). The ability of phenolics compounds to act as antioxidant and combat free radicals has been the focus of numerous investigations in recent years [10, 17]. The ability of the phenolics to operate as reducing agents by donating hydrogen and scavenging singlet oxygen in compounds is what gives them their antioxidant action [18] additionally, it has been found that phenolic compounds can inhibit the action of a number of oxidizing enzymes, including lipoxygenases, in order to prevent the oxidation of lipids typically, as higher phenolic content is linked to better bioactivity, the ethyl acetate extract was anticipated to have potent antioxidant and antibacterial effects.

**Total Flavonoid Content (TFC)**

One of the most significant biological molecules, flavonoids has numerous biological effects, including anticancer antioxidant, anti-inflammatory, anti-allergic, and anti-angiogenic properties [19].

The TFC for methanol bark extract was 91 mg quercetin equivalent (QE) /g of dry extract (Table 1). Flavonoid is one of the most researched subclasses of polyphenols, which are present in a range of plants. According to some researches special features including anti-inflammatory, antiviral, restraining pathogenic microorganisms, and protecting against UV radiation damage were discovered [20-22].

**Total Tannin Content (TTC)**

The TTC for methanol bark extract is 102 mg Gallic acid equivalent (GAE) /g of dry extract (Table 1). The significance of tannin and its variety have been investigated in numerous studies and reported. They are able to interact with extracellular and soluble proteins as well as join forces with fungi's cell walls, which enables their function. Fungal membranes may be damaged by these chemicals' characteristics [23].

**DPPH free radical scavenging assay (quantitative analysis of antioxidant activity)**

The results in figure 5 show the DPPH inhibition activity by the *L. glutinosa* bark extracts and standard at various concentrations. Ascorbic acid's $IC_{50}$ was 19.23 µg/ml while methanol extract's $IC_{50}$ was 93.25 µg/ml.

This study's antioxidative property had a linear relationship with the extracts' phenolic content ($R^2$ = 0.9857), with more phenolic content resulting in greater antioxidant capacity (Fig. 4). The outcome is consistent with Pereira et al. (2015) study, which discovered that the quantity of phenolic compounds present in the Byrsonima spp. showed antioxidant potential [24].

The spectrophotometric determination of antioxidant activity in plant extracts commonly uses the stable free radical DPPH, which has a strong purple intensity. It discolors and takes on a yellowish hue when chemicals reduce it by transferring electrons or hydrogen. The main reason why DPPH is favoured is because of how quickly it can analyze samples and how well it can detect active chemicals even at low quantities [10]. Hexane's low free radical inhibition capacity may be attributable to the extract's dearth of polyphenolic components. Due to their reducing nature, which gives them the ability to operate as singlet oxygen scavengers and donate hydrogen, phenolics in plants

are known to have considerable antioxidant activity against damaging free radicals [25].

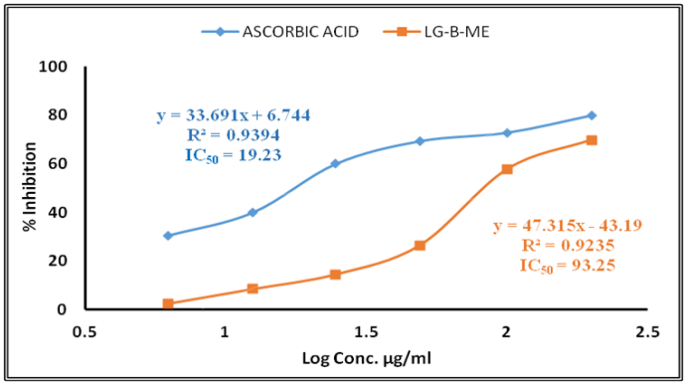


**Figure 4:** DPPH scavenging activity of methanolic extract of *L. glutinosa* bark

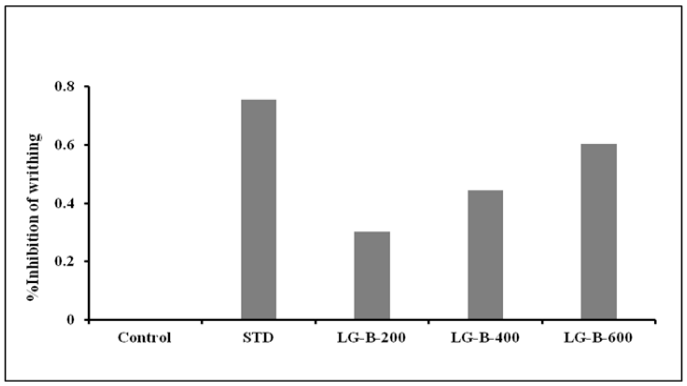


**Figure 5:** Percentage of inhibition of writhing of control, standard and plant extract (200,400,600 mg/kg) using acetic acid induced writhing method. STD = Standard, LG-B = *Litsea glutinosa* bark

### Evaluation of Analgesic activity

### Acetic acid induced writhing test

The finding shows that *L. glutinosa* bark extract significantly reduces acetic acid-induced writhing. Table 2 shows the results of the analgesic effects. It was demonstrated that the plant extract decreased the average number of writing or percentage of inhibition of writing in a dose-dependent manner. As the dose was increased from 200 mg/kg to 600 mg/kg, the percentage of inhibition rose from 30.18% to 60.38%. Acetyl salicylic acid was employed as a conventional medication at a dose of 100 mg/kg, which revealed a 75.47% inhibition rate. The group that received 600 mg/kg and the group that received the reference medication, acetyl salicylic acid (100 mg/kg), did not significantly vary from one another. Figure 5 depicts a comparison between the doses and the reference medication.

### Tail flick test

The outcome of the tail immersion response shown that, as doses were raised the mean pain reaction time (PRT) grew as well. Furthermore, it showed that PRT considerably increased at the 30- and 90-minutes mark following the administration of the extract, as shown in table 3. When the dosage was 200 mg/kg, the PRT at 30 minute was 3.412±0.106. PRT rose with both the passage of time and the concentration of plant extract, reaching a maximum of 8.70±0.30 at 90 minutes and 600 mg/kg. Acetyl salicylic acid (ASA) was employed in this study as a standard reference drug at a dose of 100 mg/kg, and its PRT was increased from 5.38±0.11 to 15.99 ± 0.46 at 60- and 90-minute intervals, respectively. Both natural and conventional plant extracts' effects.

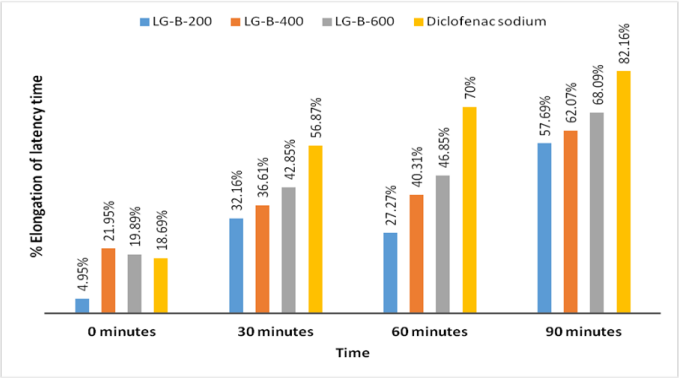


**Figure 6:** Effect of *L. glutinosa* on Percentage elongation of reaction time in tail flick test compared to the standard group

### Antibacterial activity

The methanolic extract of *L. glutinosa* bark exhibited moderate antibacterial activity against *Staphylococcus aureus* but did not show activity against *B. subtilis*, *Escherichia coli*, and *P. aeroginosa*. It supports the plant's traditional usage in wound infection because the plant portion demonstrated activity against *Staphylococcus aureus*.

## DISCUSSION

For natural defence, plants produce and store secondary metabolites. Plants have been employed as therapeutic tools since ancient times because these bioactive substances have various life-saving properties [26, 27]. This study's phytochemical screening of *Litsea glutinosa* verified the existence of phenolic, flavonoid, and tannin compound board spectrum bioactive metabolites, endorsing the plant as a possible therapeutic entity. Numerous important biological processes, including immunological response, signal transmission, and gene transcription, are linked to reactive oxygen species (ROS). superoxide (O2•–), hydrogen peroxide ($H_2O_2$), and hydroxyl radical (•OH) are the most common ROS. As a result of regular physiological processes these free radicals are created, but an excess of ROS can cause oxidative damage to biomolecules like proteins, lipids, and DNA, which can result in a number of diseases like respiratory, inflammatory, cardiovascular, cancer, neurological, and digestive disorders. Cellular death is the ultimate result of this oxidative stress (OS) [28, 29]. Antioxidant intake from exogenous sources or cellular processes can counteract the deleterious effects of OS. Therefore, antioxidants—whether they come from internal or external sources—can scavenge reactive oxygen species (ROS), which lowers biomolecule oxidation and restores cellular activity [30]. *Litsea glutinosa's* antioxidant activity was assessed by quantifying the extract's phenol, flavonoid, and tannin concentrations and its ability to scavenge free radicals using DPPH. The ability of delocalization of the spare electron over the molecule give rise to the DPPH's deep violet colour, which is characterised by an absorption band at around 517 nm [31]. By adding hydrogen atoms or electrons, an antioxidant chemical can decolorize DPPH's visible deep violet colour. This can be quantified from the change in absorbance, which is inversely proportional to the concentration of the respective component [32, 33]. Therefore, the gradual decrease in solution mixture absorbance induced by the steady concentration increases of both crude extracts and ascorbic acid supported the antioxidant activity of the crude extract (Figure 4). The ability of phenolic chemicals to donate hydrogen to neutralise free radicals makes them vital for antioxidant activity in many plants, including tannins and flavonoids [34].

Due to their numerous side effects, currently available analgesic medications cannot be utilised in all situations. As a result, there is an urgent need for novel analgesic medications with reduced side effects and improved pain control capabilities. In order to assess the crude extract's analgesic potential, mice were given acetic acid to elicit writhing. When acetic acid is administered intraperitoneally to mice, it triggers physiological reactions that increase the level of endogenous substances synthesised by the cyclooxygenase pathway, such as the

lipoxygenase pathway and prostaglandin E2 and F2α which increases the level of eicosanoids in the peritoneal fluid. These eicosanoids stimulate nociceptive neurons, which have been linked to pain perception. Peritoneal mast cells, ion channels, and prostaglandin pathways mediate writhing, an evident reaction to pain through nociceptors, characterized by repeated extension of the hind limbs and sustained contraction of the abdominal musculature [35, 36]. As per the current study's findings, *Litsea glutinosa* demonstrated a noteworthy reduction of writhing through the interference with a peripheral pain inhibitory mechanism [37]. It has been noted that reactive oxygen species from phagocytes that invades the inflammation site is the cause of arachidonic acid, the precursor of endogenous chemicals that cause pain and are generated from inflammatory tissue damage [38]. Consequently, the plant extract's analgesic properties were supported by its antioxidant properties. Therefore, there may be a correlation between antioxidant and analgesic properties and pain treatment [39, 40]. Plant materials with phenolic components, such as a potent antioxidant flavonoids are said to primarily target prostaglandins in order to exert their analgesic effects [41]. Moreover, the presence of alkaloids, which were found by phytochemical screening in this investigation, is also accountable for the analgesic impact. The test is sensitive to non-steroidal anti-inflammatory drugs, alkaloids may have analgesic effects through inhibition of the lipooxygenase and/or cyclooxygenase pathway, or through resident peritoneal macrophages and mast cells releasing cytokines like TNF-α, interleukin-1β, and interleukin-8 [42, 43]. A model for detecting opiate analgesics has been verified by measuring the reaction of experimental mice to thermal stimuli using the hot plate test [39, 43]. By acting centrally, the extracts had an antiociceptive effect, as evidenced by the hot plate method's notable increase in latency time. In conjunction with the outcomes of the writhing method caused by acetic acid, we can conclude that the extract exhibited its analgesic properties through both central and peripheral mechanisms The current study examines the antibacterial activity of crude methanolic extracts made from *Litsea glutinosa* bark using the disc diffusion assay. Two gram positive namely *Staphylococcus aureus, B. subtilis* and two-gram negative bacteria *Escherichia coli*, and *P. aeroginosa* the microbial strains examined in this investigation. According to our findings crude methanolic extract shown growth inhibition at both concentrations and demonstrated efficacy against only *B. subtilis.* With increasing concentrations, the crude extract's activity against the microorganism under study increased. Our findings confirm the ethno-medical use of *Litsea glutinosa*, further research is needed to identify the active ingredients and understand how they work.

**Table 1:** TPC, TFC and TTC of bark extract of *L. glutinosa*

| Extract | TPC (mg GAE/g extract) | TFC (mg QE /g extract) | TTC (mg GAE/g extract) |
|---|---|---|---|
| LG-B**ME** | 60 | 91 | 102 |

**Table 2:** Effect of methanolic extract of *L. glutinosa* on acetic acid-induced writhing in mice

| Treatment Groups (n = 5) | Dose (mg/kg b.w.) | Number of Writhing | %Inhibition |
|---|---|---|---|
| **Control** | 1% Tween-80 in water | 21.22±2.28 | 0 |
| **Diclofenac sodium** | 25 | 5.20±2.29*** | 75.47% |
| ***L. glutinosa* extract** | 200 | 14.82±2.30* | 30.18% |
| | 400 | 11.82±2.31*** | 44.34% |
| | 600 | 8.43±2.32*** | 60.38% |

Values are expressed as mean ± SEM, SEM: Standard error for mean, n: Number of mice (5), ***p<0.001, **p<0.01, *p<0.05 is statistically significant with compared to control.

**Table 3:** Effects of methanolic extracts of *L. glutinosa* bark on tail flick test in mice

| Treatment Groups (n = 5) | Dose (mg/kg) | Reaction time (sec) | | | |
|---|---|---|---|---|---|
| | | 0 minutes | 30 minutes | 60 minutes | 90 minutes |
| **Control** | 1% Tween-80 in water | 1.92±0.06 | 2.32±0.17 | 3.36±0.32 | 2.78±0.22 |
| **Diclofenac -Na** | 25 | 2.36±0.13* | 5.38±0.11*** | 11.20±0.18*** | 15.99±0.46*** |
| ***L. glutinosa* extract** | 200 | 2.02±0.15 | 3.41±0.11** | 4.62±0.14* | 6.51±0.09*** |
| | 400 | 2.46±0.06*** | 3.66±0.18* | 5.63±0.05*** | 7.33±0.19*** |
| | 600 | 2.37±0.07** | 4.06±0.05** | 6.32±0.13*** | 8.70±0.30 |

Values are expressed as mean ± SEM, SEM: Standard error for mean, n: Number of mice (5), ***p<0.001, **p<0.01, *p<0.05 is statistically significant with compared to control.

## CONCLUSION

It is possible to draw the conclusion that *Litsea glutinosa* stem extract offers solid proof of its positive health effects. The moderate amounts of phenolic, flavonoid, and tannin content in the methanolic extract of *Litsea glutinosa* bark were found to have analgesic, antioxidant, and antibacterial activities. The methanolic bark extract of *Litsea glutinosa* was discovered to be biologically active in terms of having a good analgesic effect by the secondary bioactive metabolites. These findings may provide scientific support for the traditional medicine's use of *Litsea glutinosa* bark in the treatment of algesia.

**Conflict of interest**

The authors declared no conflict of interest.

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None declared.

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