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Preliminary Evaluation of Antidiarrheal, Hypoglycemic, Analgesic and Antibacterial Activities of *Diospyros blancoi* Barks and Leaves

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Abstract

Diospyros blancoi, a member of the Ebenaceae family, has been broadly used in traditional medicine. This study was carried out to assess the antidiarrheal, hypoglycemic, analgesic and antibacterial activities of methanol extract of barks and leaves of *D. blancoi*. The methanolic extracts of *D. blancoi* barks and leaves at a dose of 400 mg/kg inhibited 29.22% ($p < 0.01$) and 19.13% ($p < 0.05$) diarrheal defecation, respectively. The methanolic extract of barks did not show significant analgesic activity in the acetic acid-induced writhing method. However, the extract of leaves exhibited notable activity with 40.88% ($p < 0.001$) pain-mediated writhing inhibitory effect. Both the extracts of barks and leaves demonstrated very mild antibacterial activity. In the hypoglycemic activity evaluation, both the extracts of barks and leaves significantly reduced the plasma glucose level in the experimental mice at the 2nd and 3rd hours of sample administration. The study outcomes validated the traditional applications of *D. blancoi* leaves and barks, as well as indicated potential medicinal benefits. More comprehensive phytochemical research on *D. blancoi* barks and leaves would be desirable to find bioactive substances.

Key words: *D. blancoi*, antidiarrheal, analgesic, hypoglycemic, antibacterial.

Introduction

Traditional medical practices have relied heavily on medicinal plants, which are described as those that produce therapeutic secondary metabolites or drug precursors (Gurib-Fakim, 2006). The majority of developing nations are promoting herbal medications, which are frequently less expensive than synthetic ones and they have begun to identify and patent therapeutic plants and their derivatives (Dias *et al.*, 2012). Numerous clinical research has shown that a variety of chemicals originating from plants in different classes of chemical groups have a range of potential biological effects (Chaachouay and Zidane, 2024). New and significant leads against a variety of pharmacological targets, such as cancer, HIV/AIDS, Alzheimer's, malaria and pain, are still being searched through medicinal plant drug discovery (Balunas and Kinghorn, 2005; Najmi *et al.*, 2022; Tiwari *et al.*, 2018).

There are more than 240 species of *Diospyros* genus. A well-known plant belonging to the genus, *Diospyros blancoi*, thrives in monsoon climates and on nearly any type of soil, including Bangladesh, Philippines, India, Taiwan, Pakistan and other countries (Howlader *et al.*, 2012). This is an evergreen tree species, widely known as 'Bilati Gub' in Bangladesh. According to ethnopharmacological study, bark, leaves and roots of *D. blancoi* are used to cure various clinical conditions like dermatitis, while the unripe fruit is used as a natural remedy for

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diarrhea and as first aid for wounds. Its seeds are reported to be used in dysentery, whereas fruits infusion is utilized as a gargle for stomatitis (Khan *et al.,* 2016). The leaves and bark are used to relieve skin irritation. The bark is also traditionally used to cure fevers, dysentery and coughs. Recently, anti-asthmatic properties of *D. blancoi* barks were reported (Lee *et al.,* 2012). Several spectrophotometric assay techniques provide evidence of antioxidant and free radical scavenging capabilities of leaves. The plant *D. blancoi* was found to possess phytochemicals, including alkaloids, flavonoids, tannins, terpenoids, saponins, anthraquinones, steroids and glycosides (Ragasa *et al.,* 2009). Given its widespread traditional use, this study was designed and carried out to assess the antidiarrheal, analgesic, hypoglycemic and antibacterial activities of the methanolic extracts of barks and leaves of *D. blancoi*.

Materials and Methods

Collection of plant materials and preparation of crude extract: The barks and leaves of *D. blancoi* were collected from the Manikganj district of Dhaka, Bangladesh. The collected parts were sequentially cut down, dried and ground to get the desired powder content. About 800 g of stem bark and 650 g of leaves (the powdered material) were taken in separate, clean, round-bottomed flasks (5 liters) and dissolved with 2 liters of methanol each and kept for 7 days with occasional stirring. The whole mixture was then filtered through cotton followed by Whatman No. 1 filter paper, and the filtrate thus obtained was concentrated at 40°C with a Helidolph rotary evaporator. The concentrated extract was then air dried to solid residue.

Chemicals and reagents: All the experimental chemicals and reagents were of analytical grade. Normal saline solution (0.9% NaCl) was procured from Beximco Pharmaceuticals Limited, Dhaka; DMSO and tween-80 were from Merck, Germany. Castor oil and acetic acid were procured from local market. Standard glibenclamide, loperamide and diclofenac sodium were obtained from Square Pharmaceuticals Limited, Bangladesh.

Experimental animal: In vivo experiments were executed utilizing both male and female *Swiss albino* mice. The mean weight of the mice was nearly 30 g, and their age during the test period was between 6 and 8 weeks. The animals were housed before the experiments following standard conditions of the temperature at 24 to 27°C and relative humidity of 55 to 65%. An alternate light and dark cycle of equal period was maintained during the housing period. Rodent food and water were provided to the animals, except for the night before the assays (for 12 hours).

Evaluation of antidiarrheal activity: The antidiarrheal activity of leaves and barks of *D. blancoi* was assessed by means of the castor oil-induced diarrhea model (Degu *et al.,* 2016). Diarrhea was brought on by castor oil, a hydrolytic metabolite, ricinoleic acid, which increases peristalsis, produces nitric oxide (NO), and promotes prostaglandin formation (Mascolo *et al.,* 1994). The animals were divided into four groups, with each group consisting of five mice. One group was fed with negative control (0.9% NaCl solution), another group was fed with standard loperamide at a dose of 3 mg/kg and methanolic extracts of barks and leaves were given to remaining two groups at a dose of 400 mg/kg body weight. In the assay, diarrhea was recognized by observing the number of fecal pellets. The test extract, standard loperamide and the saline solution as a negative control, were orally administered to the experimental mice. After 1 hour, all the sample-treated animals were administered 0.5 ml of castor oil orally. White, clean papers were in every animal holding cage for better fecal observation. After 4 hours, total fecal contents were counted. Antidiarrheal activity was interpreted with the percent defecation inhibitory values of the test samples with the standard group and negative control group. The percentage of defecation was enumerated by the following formula:

% Inhibition of defecation = (1-Fs/Fn) x 100

Where Fn = mean number of fecal pellets in the control group and Fs = mean number of fecal pellets in the sample-treated groups.

Evaluation of analgesic *activity:* The analgesic activities of test samples were evaluated by acetic acid induced pain sensation method (Subedi *et al.,* 2016). Due to the pain generation, unusual body movement termed as writhing is observed. The animals were divided into four groups, with each group consisting of five mice. Two groups were treated with methanolic extracts of leaves and barks of *D. blancoi*. One group received diclofenac at a dose of 50 mg/kg as a standard, and the remaining group was provided with only 0.9% saline solution as a negative control. All the test mice experienced intraperitoneal administration of 0.7% acetic acid after 40 minutes. This duration was given for adequate absorption of previously administered samples. The number of squirms or writhing was counted for each mouse for a 15-min time period, starting after 5 min of the administration of acetic acid. Compared to the negative control group, writhing inhibition percentage by the test and standard treated groups represented the test's outcomes. The percentage inhibition of writhing was determined utilizing the following equation:

% Inhibition of writhing = (1 – Ws/Wc) x 100

Where Wc = Mean writhing number (control) and Ws = Mean writhing number (sample)

Evaluation of hypoglycemic activity: The hypoglycemic potential of the methanolic extracts of *D. blancoi* barks and leaves was assessed by monitoring the blood glucose level using the glucose tolerance test (Bartoli *et al.,* 2011). Test samples and the standard antidiabetic drug glibenclamide were prepared using DMSO and tween-80. Test samples at 400 mg/kg and standard glibenclamide at 5 mg/kg doses were orally administered to the test mice. At 60, 120 and 180 min after the test samples were administered, blood droplets were extracted from the tail vein. Immediately after collecting, blood glucose level was measured by means of a glucometer.

Evaluation of antibacterial activity: The disc diffusion method was utilized to assess the antibacterial activity of methanolic extracts of *D. blancoi* barks and leaves (Bauer *et al.,* 1966). In the assay, the agar slants were used to hold pure cultures of the various bacterial strains (Gram positive: *Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Staphylococcus aureus, Sarcina lutea* and Gram negative: *Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Shigella boydii, Shigella dysenteriae, Vibrio mimicus, Vibrio parahemolyticus).* Following that, the subcultures were moved to the sterile Petri dishes. Discs containing 400 µg methanolic extracts of *D. blancoi* leaves and barks along with the discs containing 30 µg of standard kanamycin were placed on the solid agar containing the bacterial species. After that, the plates were kept in a refrigerator to ensure adequate dispersion. To eliminate any last traces of moisture from the agar medium, the plates were maintained inverted. A clear, clean scale was used to measure the zone of inhibition (mm) value.

Statistical analysis: The results obtained from all biological investigations were included for statistical analysis. In the study, one-way analysis of variance (ANOVA), followed by Dunnett's test, was used to conclude the statistical significance of the collected data. The values of $p < 0.001$, $p < 0.01$ and $p < 0.05$ were considered statistically significant. Microsoft Excel (version 2010) and the Statistical Package for the Social Sciences (SPSS) version 25 (IBM Corp., Armonk, NY) software were used to conduct the statistical analyses.

Results and Discussion

Antidiarrheal activity: The methanolic extracts of barks and leaves of *D. blancoi* exhibited significant antidiarrheal activity in the castor oil-induced diarrhea model with the inhibition of 29.22%

($p < 0.01$) and 19.13% ($p < 0.05$) defecation, respectively, at a dose of 400 mg/kg body (Figure 1). The standard loperamide inhibited 43.65% ($p < 0.001$) of defecation compared to the control group.

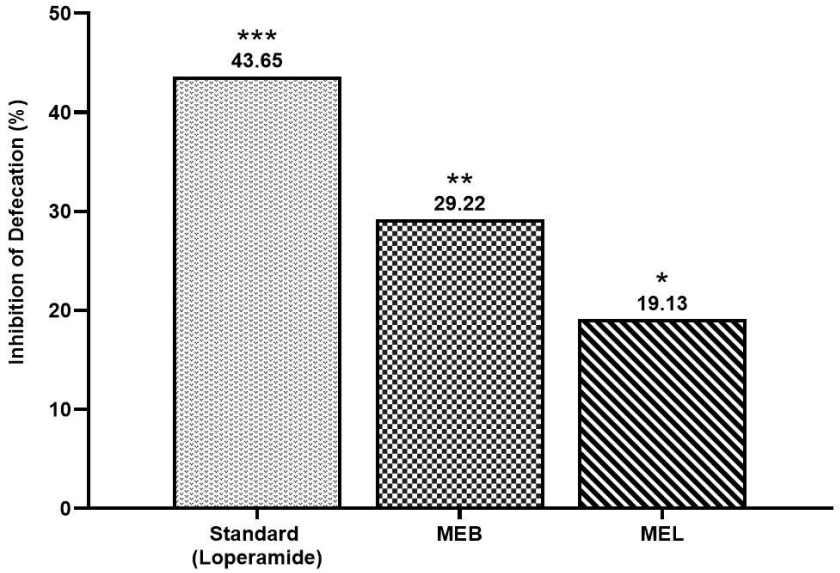

Figure 1. Antidiarrheal effect of methanolic extracts of the barks and leaves of *D. blancoi*. The percentage of defecation inhibition of standard and test samples were presented as compared to the control group. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; MEB, Methanol extract of barks; MEL, Methanol extract of leaves.

The pathophysiological mechanism associated with diarrhea involves the imbalance between secretory and absorptive processes in the gastrointestinal tract along with the alteration in motility leading to the fluid loss with the fecal content (Thiagarajah *et al.*, 2015). The liberated ricinoleic acid from castor oil triggers local inflammation, thus causing prostaglandin secretions. These events are associated with the stimulation of motility and secretion (Tunaru *et al.*, 2012). Any phytochemicals with the ability to stop castor oil-mediated pathways are supposed to inhibit diarrheal occurrence. Previously *D. blancoi* was reported to possess phytocompounds like flavonoids, tannins, alkaloids, terpenoids and so on (Das *et al.*, 2024; Ragasa *et al.*, 2009). Due to their ability to lower fluid activity, alter intestinal flora, and impede gastrointestinal motility, tannins and flavonoids are well recognized to have antidiarrheal properties (Palombo, 2006). To find potential lead compounds associated with antidiarrheal properties, further research might be conducted using the extracts of barks and leaves of *D. blancoi.*

Analgesic activity: In the acetic acid-induced writhing method, the methanolic extract of leaves of *D. blancoi* exhibited significant ($p < 0.001$) analgesic properties with 40.88% writhing inhibitory effect (Figure 2). The standard diclofenac sodium at a 50 mg/kg dose showed 57.23% ($p < 0.001$) writhing inhibition. But no significant analgesic effect was observed in the case of methanolic extract of bark of *D. blancoi*.

From the test outcome, it is obvious that the leaves of *D. blancoi* contain phytocompounds with significant analgesic property. According to the previous report, the acetic acid was found to be associated with the release of prostaglandins, prostacyclins and so on; consequently, pain-generating mediators, including substance P and neurokinins, were released (Gupta *et al.*, 2015). Any substance with writhing-inhibiting ability might be predicted to interfere with the events caused after acetic acid administration (Jahanabadi *et al.*, 2022).

As the methanolic extract of barks of *D. blancoi* showed negligible activity, further phytochemical works with the *D. blancoi* leaves might lead us to find potential analgesic lead-substances capable of interfering with the pain-inducing pathways.

Hypoglycemic activity: The hypoglycemic activity of the test samples was monitored by their blood glucose-lowering ability in the experimental animals at different time intervals. The drug glibenclamide at a 5 mg/kg dose was used as the standard in this assay. The recorded mean blood glucose levels obtained from the test sample-treated mice at 60, 120 and 180 min following administration are presented in Figure 3. The standard glibenclamide reduced the blood glucose level from 6.08 mmol/L to 2.08 mmol/L after 60 min.

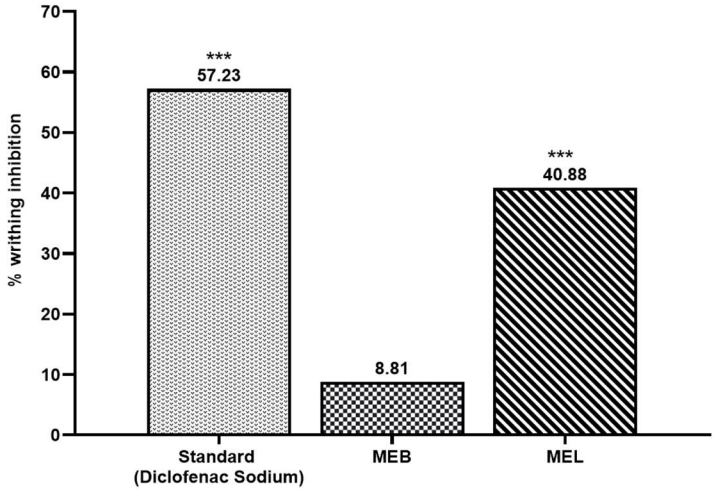

Figure 2. Analgesic effect of methanolic extracts of the barks and leaves of *D. blancoi*. The percentage of writhing inhibition of standard and test samples were presented as compared to the control group; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; MEB, Methanol extract of barks; MEL, Methanol extract of leaves.

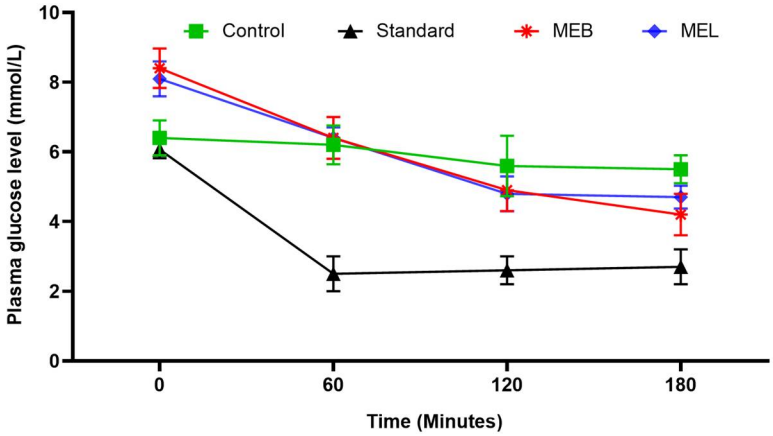

Figure 3. Blood glucose levels in mice treated with methanolic extracts of barks and leaves of *D. blancoi* at different time interval. Values are expressed as Mean ± SEM (N = 5); MEB, Methanol extract of barks; MEL, Methanol extract of leaves.

The reduction percentage of blood glucose level over time is presented in Figure 4. The methanolic extract of barks reduced the glucose level by 23.81%, 41.67% ($p < 0.05$), and 50.0% ($p < 0.01$) after 1, 2, and 3 hours (Figure 4). The methanolic extract of leaf reduced the plasma glucose level by 20.99%, 40.74% ($p < 0.05$), and 41.98% ($p < 0.05$) after that time interval. The standard glibenclamide reduced the blood glucose by 65.4% ($p < 0.001$), 57.15% ($p <$

0.001), and 55.52% ($p < 0.001$) at the 0, 1st, 2nd and 3rd hours, respectively.

The extracts of barks and leaves of *D. blancoi* significantly reduced the blood glucose level after the second and third hour of administration in the experimental mice. Overall, the findings of the oral glucose tolerance test claimed that a mild to moderate hypoglycemic effect can be attained from *D. blancoi* barks and leaves. The presence of several phytochemicals that may work independently or in concert to lower blood sugar levels might be responsible for the hypoglycemic action of the extracts. Different phytochemicals, including flavonoids, alkaloids, terpenoids, glycosides and so on which have glucose-lowering ability (Singh *et al.*, 2022) were found in *D. blancoi* (Ragasa *et al.*, 2009). However, further phytochemical works might lead us to find potential glucose-lowering compounds in *D. blancoi* leaves and barks.

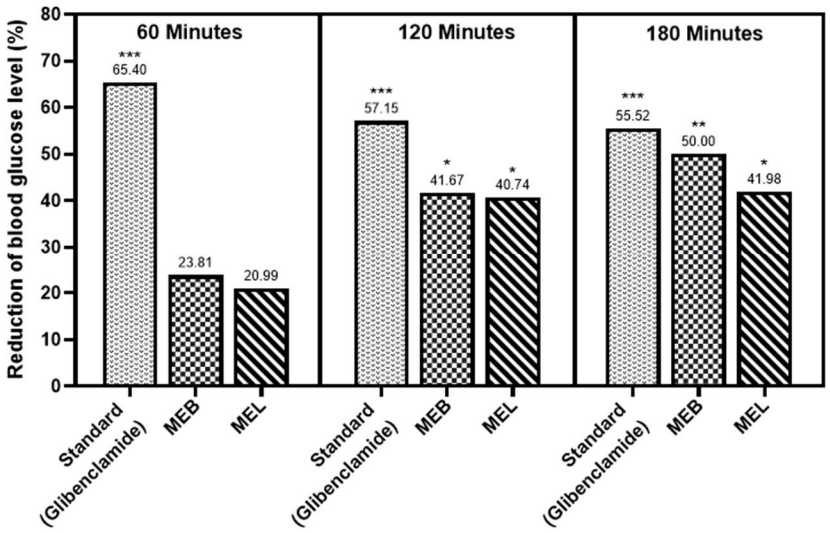

Figure 4. Hypoglycemic effect of methanolic extracts of the barks and leaves of *D. blancoi*. The percentage of blood glucose level reduction by standard and test samples were presented as compared to the control group; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; MEB, Methanol extract of barks; MEL, Methanol extract of leaves.

Antibacterial activity: In the antibacterial potentiality evaluation assay (disc diffusion method), both the methanolic extracts obtained from *D. blancoi* leaves and barks revealed very mild antibacterial activity. Against the thirteen selected bacterial species, bark extract at 400 µg/disc produced a zone of inhibition of between 9-10 mm, and leaves extract at the same dose showed 8 mm zone of inhibition against all the bacterial species. However, the standard kanamycin at 30 µg/disc dose gave zone of inhibition values between 39-42 mm.

Conclusions

Antidiarrheal, hypoglycemic, analgesic and antibacterial properties of the methanolic extracts of the leaves and barks of *D. blancoi* were evaluated in the study. Both the barks and leaf extracts possessed significant antidiarrheal properties. Both the extracts had very mild antibacterial properties. The moderate glucose-lowering ability was shown by both of the extracts. It is apparent from the study results that the barks and leaves of *D. blancoi* contain potent bioactive agents. Further phytochemical investigations with the *D. blancoi* barks and leaves might lead to find bioactive substances.

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