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Antioxidant, Antibacterial and Analgesic Activities of *Buddleja asiatica* Extract

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ABSTRACT

Introduction: *Buddleja asiatica* is an upright, evergreen shrub of the Loganiaceae family that can reach up to 1 to 8 meters tall and found between 300- 2000 meters height in Nepal.

Objectives: The objectives of this study are to assess the antibacterial, antioxidant, and analgesic properties of *Buddleja asiatica* aerial component.

Methods: A typical chemical test was outlined to conduct the phytochemical analysis. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging experiment was used to quantitatively quantify the antioxidant activity of the methanolic extract. By using the well diffusion method, the antibacterial activity was assessed against *Escherichia coli* and *Staphylococcus aureus*. Swiss albino mice were utilized to test the analgesic efficacy of the extracts using the acetic acid-induced writhing method and the tail flick method.

Results: The phytochemical screening identified the presence of reducing sugars, tannin, phenol, alkaloid, glycoside, and flavonoids. With a zone of inhibition of 19 mm, antibacterial efficacy against *Staphylococcus aureus* was demonstrated. The extract demonstrated scavenging potential in the DPPH free radical scavenging experiment, with an IC50 value of 123.68 *µg/mL*. At 600, 800, and 1000 mg/kg, the acetic acid-induced writhing method showed a significant ($P < 0.05$) analgesic effect. The tail flick approach, which evaluates the pain reaction time, also produced similar results.

Conclusion: Different secondary metabolites contained in *Buddleja asiatica* were found in the methanolic extract, and these secondary metabolites were determined to be physiologically active in terms of good antioxidant, analgesic, and antibacterial activities.

Keywords: Analgesic affect; antimicrobial screening; antioxidant activity; *Buddleja asiatica*; DPPH scavenging; phytochemical screening.

INTRODUCTION

The genus Buddleja contains over 100 species found in warmer portions of Southern Asia, Africa, and America. The genus has a wide range of therapeutic benefits, and numerous of its species have been employed in a variety of health issues around the world. *Buddleja asiatica* is one of the most common and widely grown Loganiaceae plants.[1] It is 1-8 m tall shrub, undershrub, or small tree. Branchlets are terete or nearly so, heavily stellate-pubescent or -wooly, and coated with white, grey, or fulvous hairs and leaves are opposite.[2] This plant's ethnomedical applications include curing colds and cystitis with its flowers, acting as an abortifacient, treating head tumors, weight loss, and treating malaria with a fusion of its roots. Phytochemical research on the genus Buddleja have revealed the separation of a variety of natural compounds, including as sterols, aryl esters, triterpenoid glycosides, phenylethanoids,

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flavonoids, phenolic fatty acid esters, and diterpenes . There have been antimicrobial, anti-inflammatory, anti-rheumatic, anti-hyperglycemic, and antioxidant benefits identified.[3]

Owing to the diverse medicinal uses of the genus Buddleja and the phytochemical diversity of the genus, the present study was undertaken to evaluate the biological processes like antioxidant, antibacterial, and analgesic actions of *B. asiatica* aerial parts.

METHODS

Plant material

The research was conducted for a period of six month from 2016 April to October in laboratory of Manmohan Memorial Institute of Health Sciences (MMIHS), Kathmandu, Nepal (Affiliated to Tribhuvan University). The aerial parts of *B. asiatica* were collected in the Balkot area of the Bhaktapur district of Nepal. The collected plant material was identified and authenticated as *B. asiatica* (Voucher specimen No. 925/072/073) at the National Herbarium and Plant Laboratory Godawari in Lalitpur, Nepal and was housed in MMIHS. The plant material was thoroughly washed with water and shade dried at room temperature. Foreign organic matter was removed from the dried plant material. The dried plant material was then ground into a coarse powder and preserved in air tight container for further use.

Extraction

Powdered sample was taken and extracted with methanol using a soxhlet apparatus. Thus, obtained extract was concentrated on rotary evaporator and dried to obtain the solid mass. A dried extract was kept in refrigerator at 4^{o}C for further analysis.[4]

Qualitative Phytochemical Screening

The qualitative phytochemical screening of methanol extract was performed based on Hossain M et. al.[5] Determination of alkaloid, flavonoid, phenol, tannin, steroids and sterols, carbohydrate, saponin, protein and amino acid and glycosides were performed.[6]

Antioxidant Activity

DPPH free radical scavenging activity was used to perform antioxidant activity. Ascorbic acid reference samples and plant extract sample solutions were prepared in ethanol at various concentrations (1000, 500, 100, and 50 g/ml). A 0.1 mM DPPH solution in ethanol was prepared, and 4 ml of it was added to 1 ml of each concentration of sample plant extracts and ascorbic acid solutions. The mixture was kept in the dark for 30 minutes. Similarly, 4 ml of 0.1mM DPPH was mixed with 1 ml of ethanol (solvent) and left in the dark for 30 minutes as a control. The absorbance was measured at 517 nm thirty minutes later. The following equation was used to calculate the ability to scavenge the DPPH radical:

$$\text{Percentage of scavenging} = \frac{(AO\text{-}AT)}{AO} X100\%$$

Where, AO = Absorbance of DPPH solution and AT = Absorbance of test or reference sample.

The percentage scavenging was then plotted against concentration and regression equation was obtained from which IC_{50} values were calculated for each concentration.[6]

Antibacterial Activity

The well diffusion method was used to test antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. As a standard reference drug, cefixime was used. Three wells were made in each agar plate using a sterile cork-borer with a diameter of 8 mm and labeled appropriately as test, blank, and standard. In different cups, 100 µl of 100 mg/ml extract was mixed with the same volume of standard sample, i.e. 0.6% cefixime. Plates were left for a short period of time to allow the extract to diffuse in the medium before being incubated at 37°C for 24 hours.[7]

Acute Toxicity Test

Twenty-five mice were randomly divided into five groups of five mice each (A-E). Groups A, B, C, D, and E mice were given doses of 100, 500, 1000, 2000, and 3000 mg/kg orally via gastric gavage. The animals were allowed unlimited access to food and water. They were monitored for signs of toxicity and mortality for 24 hours as per Organization for Economic Co-operation and Development (OECD) guidelines 423. The ethical clearance was taken from institutional review committee of Manmohan Memorial Institute of Health Sciences and Manmohan Memorial Medical College.[8]

Analgesic activity

The analgesic activity of extract was determined through acetic acid induced writhing method using acetic acid induced abdominal writhing reflex pain model and tail flick method by measuring tail withdrawal time from hot water.

a. Acetic Acid Induced Writhing Method

The acetic acid-induced abdominal writhing reflex pain model was used in this study. Twenty-five mature mice were randomly divided into five groups (A-E) of five mice each, fasted for 12 hours, and then treated as follows: The negative control group received 10ml/kg normal saline, the positive control group received 400mg/kg Aspirin, and the groups C, D, and E received 600, 800, and 1000mg/kg *B. asiatica* methanol extract via gastric gavage, respectively. One hour after the drug and extract administration, all mice received 0.1ml 1% glacial acetic acid intraperitoneally (I.P) to induce abdominal contortions or writhing. Each mice analgesic effect was measured and recorded for 30 minutes. The following formula was used to calculate the degree of analgesia. [8,9]

$$\text{Degree of analgeisa} = \frac{\text{mean of control group} - \text{mean of treated group}}{\text{mean of control group}} X100\%$$

b. Tail Flick Method

The tail withdrawal time from hot water was measured during the experiment. Mice were divided into five groups (A-E) of five mice each and fasted for 12 hours. For group A the mice were pretreated with 10 ml/kg normal saline one hour before the experiment; group B (positive control) received 400 mg/kg aspirin and groups C, D, and E (treatment groups) received 600, 800, and 1000 mg/kg of extract via gastric gavage. Each mice tail was dipped into a water bath containing warm water kept at 50±10ºC, and the time it took for the mice to flick the tail, known as the pain reaction time (PRT), was recorded for all mice.[9]

RESULTS

Acute Toxicity Studies

The acute toxicity of the different extracts of *B. asiatica* aerial parts were determined according to the OECD guidelines no. 423 (Acute toxicity class method). This study revealed the non-toxic nature of the entire extract even at the maximum starting dose of 2000 mg / kg of animal body weight.

Phytochemical Screening

Methanol extract extractive value was found to be 23.85%. The phytochemical analysis of extracts reveals the presence of alkaloids, glycosides, flavonoids, phenol, tannin, reducing sugar, and the absence of steroids and sterols, saponins, protein, and amino acid. Table 1 shows the details of phytochemical screening.

Table 1: Phytochemical screening of methanol extract of *Buddleja asiatica*.

S.No.	Class of compound	Name of test	Methanol Extract
1.	Alkaloids	Wagner test	+
2.	Glycosides	Glycosides test	+
3.	Flavonoids	Shinoda test and lead acetate test	+
4.	Phenol and tannin	Lead acetate test	+
5.	Steroids and sterols	Salkowski's test	-
6.	Reducing sugars	Fehling's test	+
7.	Saponins	Honey comb test	-
8.	Proteins and amino acids.	Biuret test and ninhydrin test	-

(Note: In above table, (+) indicates presence of respective class of compounds and (-) indicates the absence of respective class of compounds.)

Table 2: IC_{50} values of ascorbic acid, n-hexane and methanolic extract of *Buddleja asiatica*.

Plant extract	IC_{50} (µg/ml)
Ascorbic acid	16.34
Methanol	123.68

Antioxidant Activity

DPPH free radical scavenging activity of extracts showed that Ascorbic acid, and methanolic extract has IC_{50} value of 16.34 μg/ml, and 123.68 μg/ml respectively. It shows that methanolic extract has significant antioxidant activity. The details of IC_{50} values are as shown in table 2.

Antibacterial screening

The antibacterial activity of methanolic extract of *B. asiatica* was examined against gram (+) and gram (-) organisms; *Staphylococcus aureus* and Escherichia coli. Cefixime 0.6% w/v was used as standard. It shows that ZOI for standard was found to be 35 mm for both bacteria's while extract exhibits its antibacterial activity against S. aureus only with ZOI of 19 mm.

Analgesic activity

The effect of B. asiatica extract on acetic acid induce writhing effects in mice is presented on table 3. The results shows that the extract (600, 800, 1000 mg/kg) and the reference drug aspirin (400 mg/kg) significantly ($P<0.05$) reduced abdominal writhing in mice when compared to the negative control group reducing the mean number of writhing from 40.2 ± 2.223 in the negative group to 26.8 ± 4.271 at the dose of 1000 mg/kg. The reduction was in a dose dependent manner. Also the extract caused a

Table 3: Effect of *Buddleja asiatica* on acetic acid induced writhing effect in mice.

Groups	Treatment	Dose(mg/kg, PO)	Mean no. of writhing after 60min±SEM	% Protection
1	Water	10 ml/kg	40.20±2.223	0
2	Aspirin	400	30.00±2.366	25.37
3	*B. asiatica*	600	32.80±4.872	18.408
4	*B. asiatica*	800	31.00±1.581	21.393
5	*B. asiatica*	1000	26.80±4.271*	33.333

*P<0.05 when compared to negative control

able 4: Effect of *Buddleja asiatica* on tail flick response in mice.

Groups	Treatment	Dose(mg/kg, PO)	Mean PRT±SEM in sec		
			At 0 min	At 60 min	At 120min
1	Water	10ml/kg	4.50±0.658	3.44±0.343	3.6±0.600
2	Aspirin	400	5.80±0.374	7.32±0.450*	8.2±0.583*
3	*B. asiatica*	600	4.4±0.812	4.90±0.781	7.0±0.707*
4	*B. asiatica*	800	4.4±0.510	4.5±0.224	7.4±0.748*
5	*B. asiatica*	1000	4.2±0.663	6.8±1.594*	7.6±0.51*

*P<0.05 when compared to negative control

dose dependent increase in inhibition of abdominal writhing, increasing it from 0% in negative control group to 33 % at the dose 1000 mg/kg.

The results of orally administered *B. asiatica* extract on tail flick response in mice are summarized in table 4. All Extracts (600, 800 and 1000 mg/kg), at 1 and 2 hours after its administration, significantly ($P < 0.05$, respectively) increased the tail flick latency, when compared with the control group. The reference drug aspirin (400 mg/kg p.o) significantly ($P < 0.05$) increased the tail flick latency at 1 and 2 hours, as compared to the control group.

DISCUSSION

The purpose of this study was to determine the phytochemicals, antibacterial, antioxidant, and analgesic activity of *B. asiatica* extract. The presence of alkaloids, glycosides, flavonoids, phenols, tannins, and reducing sugar was confirmed by phytochemical screening in this study, which is similar to the finding of Nafees et al 2022[10] who credited the plant as a potential medicinal entity.

The DPPH assay is widely used for screening antioxidant activity because it is sensitive enough to detect active ingredients at low concentrations. The highest DPPH radical scavenging activity was found in 1 mg/ml ascorbic acid, followed by *B. asiatica* extracts. The IC_{50} value was used to determine the potency of antioxidants in the extracts; a low IC_{50} value indicates strong antioxidant activity. The IC_{50} value of ascorbic acid was determined to be 16.34 mg/ml, while the IC_{50} value of plant extract was determined to be 123.68 mg/ml.

The methanolic extract of *B. asiatica* produced no death or signs of toxicity even at the dose of 3000 mg/kg which suggests that the extract was well tolerated by the mice and that the doses used were safe.

Farman Ali et al (2011) found that whole plants of *B. asiatica* were studied for its antibacterial activity. The crude extract and different plant fractions were tested against 11 human pathogens. The results showed that it was most effective against both Gram (+) and Gram (-) bacteria.[11] In this study, however, antimicrobial activity was observed against S.aureus but not against E.coli. It could be because of the collection time, extraction method, or geographical variation.

Because currently available analgesic drugs are not used in all cases due to a variety of side effects, new analgesic drugs with improved pain management capacity and minimal side effects are urgently needed. Thus, the analgesic activity of the crude extract was assessed in mice using the acetic acid-induced writhing and tail flick model. This protocol is widely thought to investigate crude extract's peripheral analgesic activity.[12] The intraperitoneal administration of acetic acid causes pain in mice via physiological stimuli that stimulate the cyclooxygenase pathway to synthesize increased levels of local endogenous substances, such as

prostaglandin E2 and F2α, as well as the lipoxygenase pathway to synthesize increased levels of eicosanoids in the peritoneal fluid that stimulate nociceptive neurons, as has been previously demonstrated.[10] The current study finding indicate that plant extract inhibited writhing and increased tail flick latency by interfering with the peripheral mechanism of pain inhibition.[13] As a result, the antioxidant activity of plant extract supported its analgesic activity. As a result, analgesic and antioxidant activities may be linked in pain management.[14] Plant materials containing phenolic compounds, such as flavonoids (a potent antioxidant), are said to have analgesic activity by targeting prostaglandins. Furthermore, the alkaloids, which were discovered during phytochemical screening in this study, are responsible for the analgesic effect.[15]

The extraction was carried out in methanol only and few bacteria's were used for antibacterial testing which were the major limitations of this study.

CONCLUSSION

It is possible to conclude that *B. asiatica* extract provides convincing evidence of beneficial health effects. The phenolic, flavonoids, alkaloids, glycosides, tannin, and reducing sugar content of the methanolic extract were discovered. Secondary bioactive metabolites isolated from *B. asiatica* were found to be biologically active in terms of antioxidant, analgesic, and antibacterial activity. The findings of this study support the traditional use of *B. asiatica* to treat a variety of disorders.

Conflict of Interest: None

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