

Phytochemical Screening and *In vivo* Analgesic Activity of *Ampelocissus barbata* (Wall.) Planch.

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Abstract

Ampelocissus barbata (Wall.) Planch. is a native medicinal herb in Bangladesh. The present study was aimed at exploring the phytochemical compositions and analgesic potential of the plant *in vivo*. The methanol extract of *A. barbata* along with its different fractions were subjected to phytochemical evaluation through established chemical tests for ascertaining the available pool of secondary metabolites within the plant. Eventually, the presence of at least eleven classes of phytoconstituents *viz.* alkaloids, flavonoids, phenolics, glycosides, saponins, starch, phytosterols, coumarins, tanins, resins and quinones were demonstrated. Among the four partitions, ethyl acetate soluble fraction of *A. barbata* illustrated the maximum types of secondary metabolites qualitatively. The ability of the crude methanol extract (MEAB) and its ethyl acetate soluble fraction (EAAB) to suppress nociception both centrally and peripherally were investigated in Swiss albino mice by tail immersion method and acetic acid-induced writhing method, respectively. At the 90th minute of the tail immersion study, both MEAB and EAAB administered at the doses of 250 and 500 mg/kg body weight (MEAB1, MEAB2, EAAB1, EAAB2) exhibited 240.41%, 285.34%, 302.83% and 339.60% elongation of pain response, respectively, in comparison to the standard, pentazocine which effectuated 421.36% elongation at the same mark. Moreover, the test samples at the same doses as before exhibited reduction of pain sensation by 66.81%, 74.08%, 76.81%, and 83.52%, respectively, in the acetic acid-induced writhing response in mice. Both the central and peripheral analgesic potentials of EAAB were discernibly higher than those of MEAB at both doses. The findings of the study represents the pharmacological potential of this species in appropriate animal model for the first time and provides valid foundation to warrant future scientific endeavors into the plant.

Key words: *Ampelocissus barbata*, phytochemical screening, phytoconstituents, central analgesic, peripheral analgesic.

Introduction

Plant-derived herbal drugs have always been utilized as sources of medicine to treat various types of ailments throughout the world (Biswas *et al.*, 2010). As natural products illustrated relatively lesser degree of adverse reactions and better safety profiles, development of new therapeutic agents through the isolation of potential bioactive molecules from plant extracts and their subsequent structural modification into lead compounds, became the prime route of drug discovery (Farnsworth *et al.*, 1985). Traditional

medicinal systems, especially the Chinese, Indian and Egyptian folk medicines have experienced extensive development over different time periods aided by the information from ethnic groups about the pharmacological benefits of indigenous traditional herbal products (Hasib *et al.*, 2020; Schultes and Raffauf, 1990; Katewa and Galav, 2005). However, only around 8% of all known plants have been enumerated till date for their medicinal applications and they have also contributed extensively in providing structurally diverse drug molecules and

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novel chemical entities for drug designing and development. So, the preliminary phytochemical investigation is helpful to ascertain the importance of any medicinal plant in order to provide relevant information about the available classes of phytoconstituents as well as structural diversity within the plant (Bari *et al.*, 2021).

Ampelocissus is a large genus consisting of about 100 species classified under the Vitaceae family (grape family). Among them, *A. barbata* (Wall.) Planch. is native to the Indian subcontinent and is utilized extensively for its medicinal values. It was originally identified and described from Sylhet, Bangladesh by Nathaniel Wallich (Roxburgh *et al.*, 1820). The plant is also distributed widely in many regions of Southern India *viz.* West Bengal, Assam, Meghalaya, Tripura, Mizoram, Nagaland, Darjeeling, Sikkim, Uttar Pradesh, Arunachal Pradesh, Bhutan, Myanmar, Vietnam Thailand, Andaman and Nicobar islands (India Biodiversity Portal, 2021). Within Bangladesh, the plant grows widely in the forests of Chittagong, Chittagong Hill Tracts, Tangail and Sylhet (Flora of Bangladesh, 2019). The common Bengali name of the plant is “Amoluka”, “Gowalia-lata” or “Jungli angoor” and it is generally known as “Panibel” in Hindi (Chaudhuri and Ray, 2020; Flora of Bangladesh, 2019). It grows as shrub for as long as 15 meters in the form of herbaceous climber. Roots are tuberous in nature. Simple leaves are formed as entire or lobed or digitately 3-5 foliolate, flowers are numerous, small in size and normally deep reddish in color and fruits are spherical, black and normally seeded (Chaudhuri and Ray, 2020; Pednekar, 2013). In Bangladesh, the local medicinal practitioners as well as the common people use different parts of *A. barbata* for treating various diseases including swellings, pains, and jaundice. Crushed stems and tubers are applied topically and rubbed for attenuating edema and pain sensation. In Khagrachari district, decoction of the leaves mixed with water is used as a bath water in jaundice treatment (Morshed, 2013). Juice of its roots mixed kernel of coconut is employed as a depurative and aperients. Furthermore, root juice is used as blood purifier and diuretic to ensure removal of waste products from body.

Moreover, root paste is utilized in the treatment of fractures, sprains, bruises, injuries, wounds (Morshed, 2013).

Numerous phytochemical as well as *in vitro* and *in vivo* pharmacological studies have been conducted on *A. latifolia* and few other related species, but any study on *A. barbata* is not reported yet. As this plant is native to our country and have been widely recorded for its usefulness in treating pain, this study have endeavored to explore its analgesic potential *in vivo* as well as its phytochemical composition accordingly.

Materials and Methods

Drugs and reagents: Glacial acetic acid, lead acetate, ferric chloride, sulphuric acid, acetic anhydride, hydrochloric acid (dilute), chloroform, iodine, sodium hydroxide, nitric acid, ammonia, ammonium hydroxide, Meyer’s reagent, Dragendoff’s reagent, Fehling’s solution A and B, Ninhydrin reagent, copper sulphate, acetic acid (0.6%), methanol were procured from authorized and trustworthy chemical suppliers from Dhaka, Bangladesh. Authentic samples of diclofenac sodium and pentazocine along with normal saline were obtained from leading pharmaceutical companies of the country. Procurement of all the reagents in analytical grade was strictly ensured.

Preparation of plant material: Fresh whole plants of *A. barbata* were collected from Moulvibazar area of Sylhet district of Bangladesh in September 2019. Adequate plant identification was ensured by experts from the Bangladesh National Herbarium located at Mirpur, Dhaka. For future reference and correspondence, a voucher specimen number (48328) was issued from the same institution. After collection, the whole plants were cleaned for any adhering dirt, followed by shade-drying for adequate time period. Dried plant parts were subjected to extensive grinding to obtain a coarse powder that can enhance extraction efficiency.

Extraction and partitioning: Around 700 gm of powdered plant parts was placed in an amber colored bottle to soak in adequate volume (around 2.4 liters)

of distilled methanol for fourteen days along with occasional agitation to ensure maximum extraction of phytochemicals. After the maceration process, the mixture was initially filtered using sterilized cotton followed by further clarification through the Whatman (No. 1) filter paper. Subsequently, the resultant filtrate was introduced into a rotary evaporator maintained at low temperature and pressure in order to minimize the large volume of solvent. Eventually, the filtrate was reduced to a very concentrated mass that was then transferred to a pre-weighted beaker and the solvent was allowed to evaporate completely in the following days. Weighing the beaker allowed for the determination of the total weight of extract and 5 gm of the extract was subjected to modified Kupchan partitioning protocol as developed by Van Wagenen *et al.* (1993) to acquire four different fractions of varying polarity viz. *n*-hexane (HSF), dichloromethane (DCMSF), ethyl acetate (EASF) and aqueous soluble (AQSF) fractions. All Kupchan partitionates along with crude extract were subjected to phytochemical screening and evaluated for *in vivo* analgesic activity.

Phytochemical screening: The freshly prepared crude methanol extract of *A. barbata* and its subsequent fractions (HSF, DCMSF, EASF, AQSF) were subjected to qualitative chemical methods for determining the presence of alkaloids (Meyer's and Dragendoff's test), flavonoids and tannins (Lead acetate test), anthraquinones (Chloroform layer test), coumarin (Alcoholic test), quinone (Sulfuric acid test), glycosides and phenolic compounds (Ferric-chloride test), cardiac glycosides (Ammonia test), saponins (Frothing test), phytosterol (Liebermann-Burchard test), resins (Acetic anhydride test), amino acids (Ninhydrin test), carbohydrates (Fehling's test), starch (Iodine test) and proteins (Biuret test) (Onwukaeme *et al.*, 2007; Seow *et al.*, 2013).

Experimental animals: Both male and female Swiss albino mice of aged around 4-5 weeks were procured from the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b) Dhaka. Thirty six animals were kept at the animal house of Department of Pharmacy, East West University,

which is constantly maintained at standard laboratory conditions, i.e. 55-65% relative humidity, room temperature at $25.0 \pm 2^\circ\text{C}$, and half day light-half day dark cycle. The animals were routinely supplied with icddr,b formulated standard diet along with unlimited tap water and their weight was maintained in the range of 25-35 g. For each *in vivo* analgesic evaluation, eighteen experimental animals were randomly categorized into six equal groups of three mice in each, representing one positive control, one negative control and four test groups.

Determination of central analgesic activity: As developed by Dewey *et al.* (1970), the tail immersion method, alternatively known as the tail flick method, was employed to evaluate the pain reducing effects of *A. barbata*. As part of the process, four test groups received the extracts i.e. MEAB and EAAB at either 250 or 500 mg/kg body weight doses. Simultaneously, the positive and negative controls were administered with standard drug pentazocine intraperitoneally at the dose of 10 mg/kg body weight and normal saline orally at the dose of 10 ml/kg, respectively. Administration of the standard or sample was followed by a thirty minutes time lapse to ensure adequate absorption into systemic circulation. For the tail immersion phase, warm water was constantly maintained at 55°C and the tip of the tail of each mice (1-2 cm) was submerged in it to observe the response. The time required by each mice to realize the warmth of the water followed by the withdrawal of their tails from it, was recorded as the response time. However, a lack of observed response from the mice for at least twenty seconds was considered as complete suppression of pain response and the process was immediately discontinued to avoid any damage to the mice. Responses from the mice were recorded at 30, 60 and 90 minutes of administration and respective percent (%) elongations of pain responses were determined accordingly.

$$\text{Percent elongation (\%)} = (T_T / T_C - 1) \times 100\%$$

where, T_T and T_C represent the average tail immersion time of the test groups and the control, respectively.

Determination of peripheral analgesic activity: The abilities of both *A. barbata* extract and fraction to effectuate peripheral analgesia were investigated through acetic acid-induced writhing model in mice (Whittle, 1964). In this study, mice of positive control group received standard diclofenac sodium (10 mg/kg body weight) orally whereas negative control group received oral administration of normal saline solution at the dose of 10 ml/kg bw. Among the four test groups, two groups were received methanol extract of *A. barbata* (MEAB1, MEAB2) and another two groups were given ethyl acetate fraction of *A. barbata* (EAAB1, EAAB2) orally at doses of 250 and 500 mg/kg of body weight, respectively. Thirty minutes after the administration of the control or sample, pain was induced in each mouse through intraperitoneal administration of 0.6% (v/v) acetic acid solution at the dose of 10 ml/kg bw. Afterwards, each mouse was observed individually in order to record the number of writhing demonstrated by them for 15 minutes. However, complete writhing was not always exhibited by the mice and each incomplete writhing movement was registered as half-writhing. Accordingly, one count of writhing was registered in place of two incomplete or half-writhing. A significant decrease in writhing counts as demonstrated by the tested animal contrasted with that of the negative control group was considered as successful induction of analgesia. The percent (%) inhibition of writhing response, which represented peripheral analgesic response, was calculated using the following formula:

$$\text{Percent Inhibition (\%)} = (1 - W_T / W_C) \times 100\%$$

where, W_C and W_T represent the mean count of writhing responses exhibited by the mice of control and test groups, respectively.

Results and Discussion

Phytochemical screening: Preliminary phytochemical investigation may assist in the enumeration of the importance the plant medicinally by indicating the available classes of secondary metabolites in the plant parts. The availability of the secondary metabolites such as alkaloids, glycosides,

cardiac glycosides, flavonoids, tannins, carbohydrates, anthraquinones, amino acids, proteins, phenolic compounds, saponins, resins, phytosterols, coumarins and quinones, in varying amount in the different fractions of *A. barbata* crude extract have been enumerated in Table 1.

Analgesic activity: In the tail immersion method, both the MEAB and EAAB demonstrated dose-dependent induction of central analgesia in experimental mice and the results have been presented in Table 2. EAAB exhibited more potent pain suppression compared to that of MEAB at both experimental doses. Furthermore, both of their activities were comparable to that of standard pentazocine which demonstrated pain suppression of 421.36% at 90th minute of intervention. Both 250 and 500 mg/kg body weight doses of MEAB and EAAB attenuated the pain response significantly ($p < 0.01$) by 240.41%, 285.34%, 302.83% and 339.60%, respectively, at the same time interval. Among the samples, the maximum extent of central analgesia (339.60%) was illustrated by EAAB at 500 mg/kg body weight dose.

Inhibitory effect of the methanol extract (MEAB1 and MEAB2) as well as the ethyl acetate fraction (EAAB1 and EAAB2) of *A. barbata* on the acetic acid-induced writhing of mice has been delineated in Table 3. Oral administration of both MEAB and EAAB at the doses of 250 and 500 mg/kg body weight significantly ($p < 0.01$) abated abdominal writhing response in mice following a dose dependent manner. The percent inhibition of pain responses by 66.81%, 74.08%, 76.81%, and 83.52% were demonstrated by MEAB and EAAB at both doses, respectively, whereas the positive control (diclofenac sodium, 10 mg/kg) showed 85.51% peripheral analgesia.

Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolics, glycosides, tannins, saponins, resins, coumarins, quinones, starch, and phytosterols in the methanol extract of *A. barbata*. Among the four partitionates, the ethyl acetate soluble fraction of *A. barbata* expressed the maximum content of phytochemical

classes except coumarins, phytoestrogens and saponins. Rich presence of flavonoids and phenolics within the fractions indicates that this plant may possess potent free radical scavenging properties which may translate into anti-oxidative activity and can prevent oxidative damage of macromolecules (Doss, 2009; Seow et al., 2012). Similarly, such antioxidant

property can also be attributed to anti-cancer potentials of the plant (Doss, 2009). It has been demonstrated previously that tannins exhibits potential cytotoxic, anti-proliferative and anticancer effects (Aguinaldo et al., 2005). Moreover, saponins have been extensively recognized for their potential

Table 1. Phytochemical compositions of *A. barbata* extracts.

Phytochemical class	Chemical test involved	HSF	DCMSF	EASF	AQSF
Alkaloids	Dragendorff's & Meyer's test	++	+++	++	+++
Phenolics	10% Ferric chloride test	-	-	+++	++
Flavonoids	Lead acetate test	-	-	+++	++
Anthraquinones	Chloroform layer test	-	-	-	-
Coumarins	Alcoholic NaOH test	++	+++	-	-
Quinones	Sulfuric acid test	++	++	+++	+++
Glycosides	Fehling's test	-	-	+++	++
Cardiac glycosides	Ammonia test	-	-	-	-
Amino acids	Ninhydrin test	-	-	-	-
Proteins	Biuret test	-	-	-	-
Phytosterols	Liebermann-Burchard test	-	++	-	-
Carbohydrates	Fehling's test	-	-	-	-
Starch	Iodine test	-	-	++	-
Tannins	Lead acetate test	-	-	+++	++
Saponins	Frothing test	-	++	-	-
Resins	Acetic anhydride test	-	-	++	-

*Positive (+) and negative (-) signs denote the presence and absence of specific class of phytoconstituents, respectively. HSF = Hexane soluble fraction, DCMSF = Dichloromethane soluble fraction, EASF = Ethyl acetate soluble fraction, AQSF = Aqueous soluble fraction

Table 2. Central analgesic activity as demonstrated by the methanol extract and ethyl acetate fraction of *A. barbata*.

Test groups	Dose (mg/kg)	Time of tail immersion (Mean \pm SEM)			Elongation of pain inhibition (%)		
		30 min	60 min	90 min	30 min	60 min	90 min
Negative control	10 ml	2.01 \pm 0.07	2.23 \pm 0.18	2.36 \pm 0.09	-	-	-
Positive control (Pentazocine)	10	6.78 \pm 0.32	9.56 \pm 0.10	12.29 \pm 0.16	236.59	329.34	421.36
MEAB1	250	4.51 \pm 0.14	6.63 \pm 0.14	8.02 \pm 0.16	124.17	197.60	240.41
MEAB2	500	5.05 \pm 0.09	7.36 \pm 0.07	9.08 \pm 0.19	150.83	230.39	285.34
EAAB1	250	5.23 \pm 0.027	7.17 \pm 0.4	9.49 \pm 0.16	159.60	222.16	302.83
EAAB2	500	5.65 \pm 0.22	8.25 \pm 0.11	10.36 \pm 0.31	180.79	270.66	339.60

All experimental values have been presented as mean \pm standard error of mean for n = 3 mice. Statistical significance of p < 0.01 was calculated for all responses compared against standard.

Table 3. Peripheral analgesic activity as demonstrated by the methanol extract and ethyl acetate fraction of *A. barbata*.

Test groups	Dose (mg/ kg bw)	Average number of writhing (Mean \pm SEM)	Inhibition of pain response (%)
Negative Control (Normal saline)	10 ml	64.5 \pm 2.08	-
Positive control (Diclofenac sodium)	10	9.33 \pm 0.441	85.54
MEAB1	250	21.33 \pm 1.17	66.81
MEAB2	500	16.67 \pm 0.73	74.08
EAAB1	250	15 \pm 1.26	76.81
EAAB2	500	10.67 \pm 1.30	83.52

All experimental values have been presented as mean \pm standard error of mean for n = 3 mice. Statistical significance of p < 0.01 was calculated for all responses compared against standard.

for antioxidative, anti-inflammatory, hypoglycemic and anticancer activities (Rathore *et al.*, 2012). The presence of such secondary metabolites provides scientific basis for promising biological activities of *A. barbata* extract. Thus, it can be inferred that bioactivity-guided isolation of phytochemicals from this plant is warranted in future to yield potentially lead compounds.

The acetic acid-induced writhing assay in mice have been established as a robust and reliable method for estimating peripheral analgesic activity. Inflammatory responses are often initiated through the liberation of free arachidonic acid secondary to specific stimuli (Ahmed *et al.*, 2006) which then acts as a substrate for cyclooxygenase (COX) enzyme, and undergoes chemical conversion to result in prostaglandin biosynthesis (Duarte *et al.*, 1988). Subsequently, such pro-inflammatory prostaglandin molecules mediates pain sensation in experimental animals. Active phytoconstituents in any plant are capable of attenuating such responses through the suppression of either prostaglandin synthesis or their interaction at receptor sites. Eventually, peripheral analgesia is induced which is illustrated through diminished number of writhing responses (Duarte *et al.*, 1988; Ferdous *et al.*, 2008). The remarkable capacities of both the methanol extract and ethyl acetate soluble fraction of *A. barbata* in suppressing peripheral pain sensation might be due to the

presence of analgesic agents within its phytochemical pool.

The plant also exhibited strong central analgesic action in the tail immersion test. Antagonism of central nociceptors located within the brain and spinal cord, can lead to central analgesic effect (Sharma *et al.*, 2003; Khanna and Bhatia, 2003). Opiates, especially morphine, represent one of the most potent and anciently recognized natural analgesic agent which exert their anti-nociceptive activity by interacting with specific class of central nociceptors known as opioid receptors (Wani *et al.*, 2012). In this assay, both the methanol extract of *A. barbata* and its ethyl acetate soluble fraction effectuated prominent central analgesia as compared to that of pentazocine, a potent analogue of morphine. Hence, the central analgesic activity of *A. barbata* may be attributed to its capacity to interact with opioid receptors and require further investigations in order to validate such estimation.

Therefore, it can be assumed that classes of phytoconstituents that have been confirmed through the qualitative phytochemical screening may be the principal mediators of observed analgesic properties. On that note, noteworthy analgesic properties have been previously attributed to both flavonoids and alkaloids, especially through the suppression of prostaglandin synthesis (Rajnarayana *et al.* 2001; Rao *et al.*, 1998; Uche *et al.*, 2008) and both of these

classes have been positively estimated to be present in *A. barbata*.

Conclusion

Based on the aforementioned projections, it can be rationally enumerated that the methanol extract of whole plants of *A. barbata* and its Kupchan partitionates possess various important classes of secondary metabolites. Dose-dependent analgesic activities were observed in both tail immersion test and writhing test, implying significant potentials for central and peripheral analgesia by both the methanol extract and its ethyl acetate soluble fraction. However, extensive and detailed phytochemical investigations into the plant are required in future in order to identify and characterize potential bioactive components responsible for such central and peripheral analgesic activities.

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