



Evaluation of Analgesic activity of *A. corniculatum* L. Blanco. leaves extracts

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Abstract

Aims: the present study aims in evaluating the analgesic activity of *Aegiceras corniculatum* L. Blanco.

Materials and Methods: In the present investigation, the analgesic activity of the methanolic extract of *Aegiceras corniculatum* L. Blanco. was performed using acetic acid writhing method and tail immersion method. The plant material was collected and shade dried. The leaf material of *A. corniculatum* was subjected to soxhlet extraction using methanol. The dried extract was used for the analgesic activity. Preliminary phytochemical analysis was performed using standard procedures.

Results: The methanolic extract showed the presence of flavonoids, terpenoids, glycosides, lignins, sterols and tannins. The extract reveals dose dependent analgesic activity with acetic acid writhing test wherein the number of writhes reduced gradually with increase in the concentration and tail immersion methods which showed longer capability of withstanding the pain when kept in water bath. The results of both the methods were compared with standard analgesic drugs.

Conclusion: This may be due to the presence of various phytochemicals and their synergistic activity. Thus the methanol extract of *A. corniculatum* can be used as an analgesic agent.

Key Words: *Aegiceras corniculatum*, Analgesic activity, Methanolic extract, Soxhlet apparatus, tale immersion

INTRODUCTION

In the current days due to high stress and fast moving lifestyle we come across a lot of pressure on our body. It may be joint pain, body pain or headache etc... to get rid of this condition we take medicines called analgesics.[1,2] Pain is a condition caused due to the inflammatory reactions at the site of injury or tissue damage. Pain may be localized or mild. Any type of the pain starts with the inflammation reactions. During the inflammatory reactions, large number of pro inflammatory mediators are released such as interleukins (IL-6 and IL-12), Interferons (INF- γ) Tumor necrosis factor (TNF). [1,3,4]. Presently, non-steroidal anti-inflammatory drugs NSAIDS are being administered to reduce the inflammation and in turn relieve the pain [5]. These drugs has got adverse side effects in the form of gastrointestinal irritation and acidity. [4] Due these effects of the drugs, there is a need to search for alternative medicines to manage pain and inflammation. Plants are the major resources of bioactive compounds which have tremendous potential to manage vast number of diseases and conditions [6]. Plants are the rich sources of various phytochemicals which are essential for the defense mechanism against many plant parasites and predators. The secondary metabolites of plants are very useful in treatment of large number of human ailments without having any major side effects. Most of the plants are still unexplored for their medicinal properties, so there is an immediate need to take up the study on the same. The term mangrove is also used to describe halophytic and salt resistant marine forests consisting of trees, shrubs, palms, epiphytes, ground ferns and grasses [7]. Many mangrove plants have shown potential medicinal properties in the treatment of many human ailments. *Aegiceras corniculatum* (L.) Blanco. belongs to family *Myrsinaceae*, is a mangrove shrub which grows in highly alkaline conditions near the coastal areas of India. The plant is used in various traditional

medicinal systems for the treatment of rheumatism, painful arthritis, analgesic, bacterial infection, inflammation and antioxidant. The present study aims to evaluate the analgesic activity of the extracts of the *A. corniculatum* leaves.

MATERIALS AND METHODS:

Plant collection

The *A.corniculatum* plant material (leaves) were collected from Karwar, Karnataka (14° 48' N, 74° 11' E) in the month of April 2017. The plant was authenticated by taxonomist, Dr. K. Kotresha, Department of Botany Karnatak Science College, Dharwad. The herbarium was prepared and voucher specimen deposited in the Department of Botany Karnatak Science College, Dharwad. The plant material was washed under running tap water to remove the dust particles and dried under the shade for 15-20 days, powdered coarsely and stored at air tight containers in a cool and dry place.

Extraction of phytochemicals

Around 100g of leaves material was subjected to sequential soxhlet extraction using different solvents in the order of their increasing polarity. The extracts were then concentrated by rotary flash evaporator (BUCHI) to concentrate the extracts. The concentrated extracts were kept in air tight containers and kept at 4 °C till further use.

Preliminary Phytochemical Analysis:

Qualitative phytochemical analysis was carried out by following standard methodology [17]

Animals

Swiss albino mice were used for the experiment. The animals were procured from the animal house of the Department of Zoology, Karnatak University Dharwad. They were kept in polypropylene cages and were given

free access of food and water *Ad libitum*. The animals were acclimatized 15 days before performing the experiment. The current investigation was performed with CPCSEA guidelines with ethical clearance from the institutional ethical committee, Department of Zoology, Karnatak University Dharwad. (639/GO/Re/S/02/CPCSEA)

Acetic acid-induced writhing test in mice

The test was conducted employing Koster et al (1959) method (14). Swiss albino mice were divided into 5 groups of 5 mice each. The first group served as control and was given normal saline orally to act as negative control. Groups II received 10mg/kg Aspirin, acts as positive control for the current investigation. Group III, IV and V received 125, 250, and 500mg extract per kg body weight orally respectively, Thirty minutes later, each mouse was injected with (0.06% acetic acid of 1ml per 100g i.p). The number of abdominal constriction for each mouse was counted five minutes after injection of acetic acid for a period of ten minutes.

Tale Immersion Test:

The tale immersion test was performed using the method described by Turner 1971. The animals were given dose of methanolic extract of *A. corniculatum* orally.

In this method, swiss albino mice weighing about 15-20g were pretreated with drugs 60 minutes before tail immersion. The animals received the standard drug Diclofenac sodium (45 mg/kg, p.o) which served as reference standard. The distal 2-3 cm portion of mouse-tail was immersed in hot water maintained at $55 \pm 1^\circ\text{C}$. The time taken by the mice to withdraw the tail from hot water was noted as reaction time. The cut off time was considered 10-12 sec [18].

Statistical analysis:

The results are expressed as mean \pm SEM. One way analysis of variance is carried to test the significance level. 0.05% was found to be significant.

Table 2- Effect of methanol extract of *A. corniculatum* on response time by Tale Immersion method

Groups	Time interval					
	0	15	30	45	60	90
I	1.08 \pm 0.048	1.02 \pm 0.08	1.1 \pm 0.070	1.2 \pm 0.070	1.12 \pm 0.086	1.08 \pm 0.115
II	1.94 \pm 0.050	8.16 \pm 0.050	8.72 \pm 0.058	8.9 \pm 0.094	9.24 \pm 0.074	9.68 \pm 0.058
III	1.28 \pm 0.066	4.32 \pm 0.101	4.66 \pm 0.050	4.92 \pm 0.037	5.16 \pm 0.050	5.66 \pm 0.050
IV	1.42 \pm 0.066	6.4 \pm 0.114	6.86 \pm 0.050	6.96 \pm 0.04	7.28 \pm 0.058	7.64 \pm 0.050
V	1.46 \pm 0.0812	7.1 \pm 0.070	7.58 \pm 0.165	8.04 \pm 0.050	8.38 \pm 0.073	8.76 \pm 0.050

Group I- Control, Group II- standard drug, Group III- 125mg/kg, Group IV- 250mg/kg, Group V- 500mg/kg
The results are expressed as Mean \pm SEM (n=5)

Table 3- Acetic Acid Induced Writhing Method in Mice

Groups	Number of Writhes (n=5)
	Mean \pm SEM
I (Control)	35 \pm 0.316
II (Standard drug)	18 \pm 0.707
III (125mg/kg)	28 \pm 0.836
IV(250mg/kg)	23 \pm 0.707
V(500mg/kg)	21 \pm 1.048

RESULTS :

The plant extraction was performed using methanol solvent using soxhlet apparatus. This extract was used for the current study. Preliminary phytochemical analysis was performed which showed the presence of flavonoids, glycosides, lignins, terpenoids, sterols and tannins. *In Vivo* analgesic activity was performed using acetic acid writhing method and tale immersion method.

In writhing method, the animals were injected with 1% acetic acid i.p. the number of writhes was counted for the period of 10 minutes. The number of writhes were reduced in a dose dependent manner. The mean number of writhes observed were 28, 23 and 21 for 125 mg/kg, 250 mg/kg and 500 mg/kg respectively which were compared with standard aspirin (10 mg/kg) with 18 writhes. As the concentration of the extract increased the number of writhes reduced gradually indicating the effect of the methanol extract on analgesic activity. The results are depicted in table 2.

The tale immersion test revealed that the central analgesic activity was dose dependent manner at different time intervals which was comparable to standard drug. In this method, the analgesic effect of the methanolic extract started to show within 15 min and best results were obtained at 90 min with 8.76 \pm 0.337 at 500 mg/kg which was compared with standard Diclofenac (45 mg/kg) The results were depicted in table 3.

Table 1- Qualitative phytochemical analysis of methanol extract of *A. corniculatum* leaves extracts

Test	Result
Flavonoids	++
Tannins	+
Sterols	+
Terpenoids	++
Glycosides	+
Lignins	+

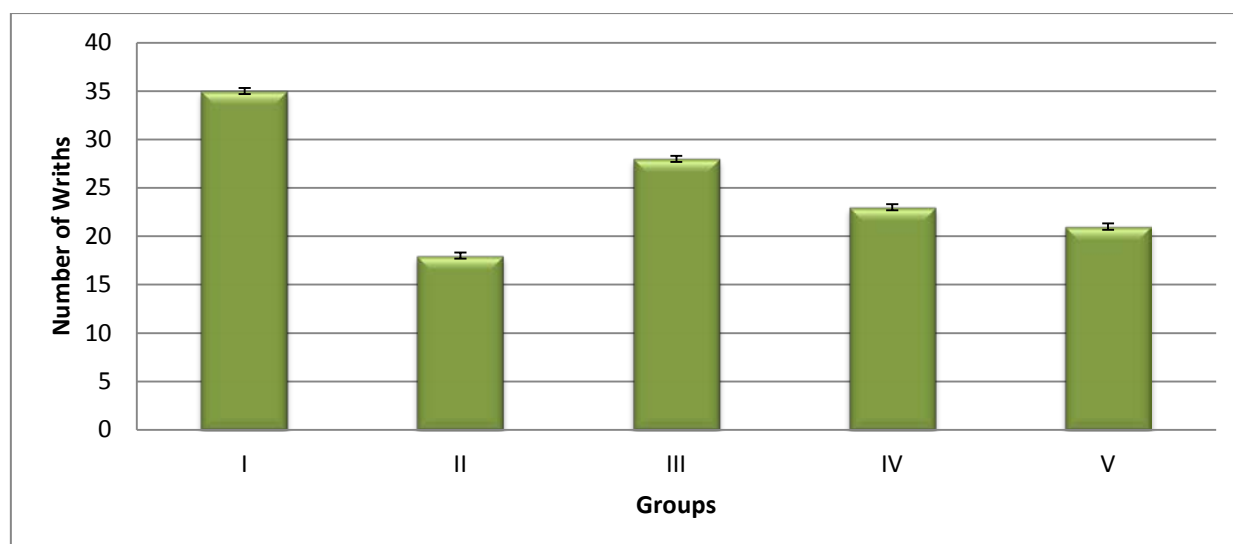


Fig. 1- Acetic Acid Induced Writhing Method in Mice

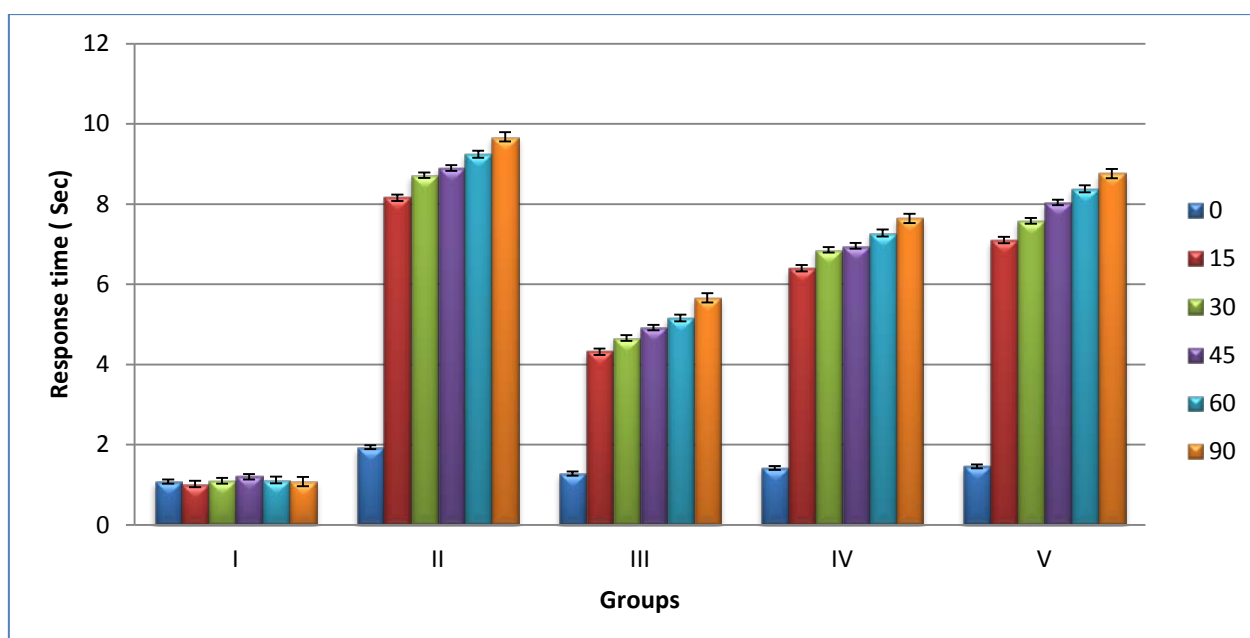


Fig. 2 - Effect of methanol extract of *A. corniculatum* on response time by Tail Immersion method

DISCUSSION

The present investigation aimed at evaluating the analgesic activity of the methanolic extract of the *A. corniculatum* leaves. The preliminary phytochemical analysis revealed the presence of flavonoids, glycosides, lignins, terpenoids, sterols and tannins. These phytochemicals play a major role in eliciting a pharmacological response.

The mangrove plant *Aegiceras corniculatum* is used in the treatment of various diseases by traditional practitioners and it is due to these phytochemical principles role in controlling the disease condition. [8] Flavonoids are involved in the free radical scavenging, anticancer and antimicrobial properties. These flavonoids are also involved in improving the blood circulation to the brain in Alzheimer's disease. Tannins may be employed medicinally in antidiarrheal, haemostatic, and anti

hemorrhoidal compounds. The anti-inflammatory effects of tannins help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. [9] Terpenoids are having various medicinal properties such as anticarcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial or diuretic activity and anticancer activity. [10] Glycosides are used as cardio tonic, purgative, analgesic, anti-rheumatic, demulcent. [11] Sterols are the biological molecules which are mainly used in the treatment of cardiovascular disease, in lowering the LDL-cholesterol levels, in the treatment of breast cancer and prostate cancer.

The analgesic effect of Methanolic extract (ME) of *A. corniculatum* in two models of pain was found to be effective. Two types of stimuli were given such as thermal (tail immersion) and chemical (writhing tests). [12] Acetic

acid-induced abdominal writhing is a effective test to screen peripheral analgesic effect of compounds. It increases the concentration of prostaglandins PGE2 and PGF2 α in the peritoneal fluid.[13,14] the prostaglandins may be involved in perception of pain. The flavonoids present in the methanolic extract of the *A. corniculatum* may inhibit the enzyme prostaglandin synthetase to minimize the pain [15] All the concentrations of the ME were effective against acetic acid induced writhing test. The ME of *A. corniculatum* inhibited the acetic acid-induced pain with effectiveness which was compared with standard aspirin. The standard aspirin which inhibit the peripheral pain induced by direct action of acetic acid in the abdomen by inhibiting the prostaglandin secretion.[5] The central analgesic activity was evaluated by using tail immersion method. This involves the central nociceptive response. [4] The phytochemicals present in the methanolic extract (ME) of *A. corniculatum* may reduce the nociceptive response in turn reducing the pain and inflammation.

In the tale immersion method, the results reveal dose dependent activity with 125mg/kg, 250 mg/kg and 500 mg/ kg body weight. The studies by Singh et al. 2010 have shown that the analgesic activity with this method has shown at around the concentration of 300 mg / kg (16) which was compared with the present study where in the analgesic activity was shown from 125 mg/kg body weight up to 500 mg/kg at different time intervals comparable with the standard diclofenac.

CONCLUSION:

In the present investigation, *A. corniculatum* leaves were shade dried and phytochemicals were extracted with methanol. The methanol extract showed the presence of various phytochemicals which elicited analgesic activity when subjected for writhing and tale immersion tests. The results reveal that the methanol extract showed significant analgesic activity which was comparable with standard drug. The current study can be extrapolated with purification of phytochemicals and their activities can lead to identification of novel pharmacologically effective molecules to treat various human ailments.

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