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Research Article

PHYTOCHEMICAL INVESTIGATION AND EVALUATION OF ANALGESIC ACTIVITY OF *WILLUGHBEIA EDULIS* ROXB AVAILABLE IN BANGLADESH

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ABSTRACT

Purpose: The present study was an attempt to investigate the phytochemical constituent and to explore the analgesic activity of n- butanol fraction of ethanol extract of *Willughbeia edulis* (Family: Apocynaceae). Methods: The n- butanol fraction of ethanol extract of *Willughbeia edulis* was investigated by hot plate and acetic acid induced writhing methods to assess its analgesic activity. Result: In the hot plate method, the extract caused significant ($p<0.001$) inhibition of pain response (64.65% and 77.32% at the doses of 250 and 500 mg/kg body weight). It was found that the extract caused an inhibition of writhing response induced by acetic acid in a dose dependent manner. The extract of *Willughbeia edulis* and Diclofenac sodium produced a significant ($P< 0.01$) inhibition of the second phase response in the acetic acid pain model while the two doses of the extract showed an analgesic effect in the first phase. Conclusion: The result indicates significant analgesic activity of *Willughbeia edulis* on animal models which are comparable with those of standard drugs.

Keywords: *Willughbeia edulis,* analgesic activity, hot plate test**,** acetic acid induced writhing method.

INTRODUCTION

Plants are one of the most important sources of medicines. The medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice[1]. The high cost of acquiring synthetic drugs, their inadequate supplies, the side effects associated with their uses and on other hand, plants cure many diseases including pain and inflammation have led to reawaken of interest in the utilization of plants and plant products in recent years. There is a need to intensify research into medicinal flora especially on those which have beneficial effects in serious disorders[2].

There is no doubt that pain is an unpleasant sensation. Pain which is also a protective mechanism for the body occurs whenever any tissue is actually or potentially damaged and it causes the individual to react to remove the pain stimulus[3]. With many pathological conditions, tissue injury is the immediate cause of the pain and results in the local release of a variety of chemical agents which are assumed to act on the nerve terminals either activating them directly or enhancing their sensitivity to other forms of stimulation[4].

Willughbeia edulis is a tropical fruit of the genus Willughbeia. It is a yellow sour edible fruit. It is known by several names, among them are kuy in Cambodia, gedraphol, laleng-tenga and bel-tata in India, dton-kuy, kuiton, kreua and katong-katiew in Thai, talaing-no in Myanmar and guồi in Vietnamese. *Willughbeia edulis* is a large climbing shrub. Its leaves are coriaceous, glabrous, oblong or ovate-oblong, acuminate and reddish brown beneath. Cymes are auxiliary. Its flowers are fragrant and yellowish. Its fruits are pyriform. Flowering and fruiting occur in May-December. The species sporadically occurs in the greater districts of Chittagong and Chittagong Hill Tracts in Bangladesh[5]. The plant is used in the treatment of snake bite. Ripe fruit is also edible.

MATERIALS AND METHOD

Collection and identification of the plant

Willughbeia edulis was collected from the Kaptai Upazila at Rangamati District in Chittagong Division. It was taxonomically identified by experts at Mirpur Herbarium.

Preparation of the plant extract

The collected plant's leaves and bark were washed with water and separated from undesirable materials or other plant parts. They were aerated by fan aeration to be partially dried and were next heated in an oven at below 40ºC for two days to be fully dried. The fully dried leaves and barks are then grinded separately to make them powder by the help of a suitable grinder. Then powder was dissolved in ethanol (95%) and kept for 7 days with occasional shaking and stirring. Then the whole mixture filtrated by a piece of clean and white cotton following again filtration through Whatman filter paper. The filtrate (ethanol extract) obtained was evaporated by rotary evaporator (Bibby RE-200, Sterilin Ltd., UK) at 5 to 6 rpm and at 68ºC temperature. It rendered a gummy concentrate of chocolate black color which was designated as crude extract or ethanolic extract.

Experimental animals

Young Swiss-albino mice of either sex, 4-5 weeks aged and average weight 20-25 gm were used for the experiment. The mice were purchased from the animal Research Branch of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR, B). They were kept in standard environmental condition (at 24.0±0°C temperature & 55-65% relative humidity and 12 hour light/12 hour dark cycle) for one week for acclimation after their purchase and fed formulated rodent food and water. The set of rules followed for

animal experiment were approved by the institutional animal ethical committee[6-7].

Method for Phytochemical Analysis

The freshly prepared extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Wagner's Reagent in order to observe brownish-red or orange precipitate, flavonoids with the use of dilute ammonia and concentrated H_2SO_4 to form yellow coloration; tannins with 0.1% ferric chloride to form brownish green or a blue–black coloration, terpenoids with dichloride methane to observe formation of layer with a reddish- brown color and saponins with olive oil ability to produce stable foam and Gum and Carbohydrates with a Molish reagent and few drops of sulphuric acid forming a red violet ring at the junction of two liquids .These were identified by characteristic color changes using standard procedures[8].

ANALGESIC STUDIES

Hot-plate test

In this study, the hot-plate test was carried out as previously described by Lanhers et al. (1992) and modified by Mahomed and Ojewole (2004)[9-10]. A 600 ml test beaker was placed on thermostat hot plate (Gallenkamp thermostat Cat No: HL054). The temperature was regulated to 54° ± 1°C. Mice were divided into four groups consisting of five animals in each group. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat induced nociceptive pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken (reaction time in second). Each mouse serves as its own control. Before treatment, its reaction time was taken once. The mean of this one determination constituted initial reaction time before treatment of the mouse. Each of the test mouse was thereafter treated with either Distilled water (DW), Diclofenac sodium (2.5 mg/kg of body wt) and finally prepared extract of leaves (250 mg/kg, 500 mg/kg group individually) body wt. orally. 30 min after treatment, the reaction time of each mouse was again evaluated five times individually in one hour interval on this occasion. This was pooled for the mice in each treatment group and the final test mean value (Ta) for each treatment group was calculated. This final test mean (Ta) value represented the after treatment reaction time (Ta) and was subsequently used to determine the percentage thermal pain stimulus or protection by applying following formula.

$$PAS = Tb\text{-}Ta/Tb \times 100$$

Where, Tb= Reaction time (in second) before drug administration;
Ta = Reaction time (in second) after drug administration.

Acetic acid induced writhing method

To evaluate the analgesic effects of the plant extract, the method described by Dharmasiri JR *et al*, was used with slight modifications[11].Experimental animals were randomly selected and divided into four groups denoted as Group I, Group II, Group III and Group IV consisting of five mice in each group. Each group received a particular treatment i.e. control, standard (Positive control) and two doses of extract. Group I served as the control group and received distilled water. Group II received diclofenac sodium 25mg/Kg body weight per oral (po) the standard drug used to compare the analgesic activity of the extracts. The last two group i.e. Group III and Group IV were treated with the extract (leaves) suspension 250mg/kg and 500mg/kg body weight per oral respectively. For proper absorption, after thirty minutes of drug treatment, each group was treated intraperitoneally (ip) with 0.2 ml 0.7% acetic acid. After five minutes of acetic acid administration, the number of writhes (Abnormal contraction or stretches) was counted for the next ten minutes and recorded. The recorded number of acetic acid induced writhes that occurred in the standard and test group compared with those in the control group[12].

Statistical analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Dunnett's *t*-test was used as the test of significance. P value <0.05 was considered as the minimum level of significance. All statistical tests were carried out using SPSS statistical software.

RESULTS

Phytochemical Analysis

Preliminary phytochemical screening of the ethanol extract of *Willughbeia edulis* revealed the presence of various bioactive components of which flavonoids, alkaloids, terpenoids, tannins, gums and carbohydrates were the most prominent and the result of phytochemical test is summarized in the Table 1.

Table 1: Phytochemical analysis of the *W.edulis* extracts (Ethanol and Butanol)

Extract	Tannin	Flavonoid	Saponin	Gum & Carbohydrate	Alkaloid	Reducing Sugar	Terpenoid
Ethanol Extract (Bark)	+++	+++	++	+++	++	+++	+++
Ethanol Extract (Leaf)	+++	-	+++	+++	+++	+++	+++
Butanol Extract (Bark)	+++	-	++	+++	+++	+++	+++
Butanol Extract (leaf)	++	+++	++	+++	++	+++	+++

Symbols '+++' indicates presence in high concentration; '++' indicates presence in moderate concentration; '+' indicates presence in trace concentration and '-'indicates absence of phytochemicals

Analgesic Activity

Hot Plate Method: Results of hot plate test are presented in Table 2 and Figure 1 for the crude extract of Buanol fraction of *Willughbeia edulis*. The extract of the plant significantly increased the reaction time of heat sensation in mice at the doses of 250 and 500 mg/kg BW and the percentage protection is almost equivalent to the respective doses. In the 3rd hour of study, the extract increased the reaction time of heat sensation to 54.23% and 81.12% at the doses of 250 and 500 mg/kg BW respectively while that of the standard drug was 91.21% and the results were found to be highly statistically significant (P<0.001). The extract exhibited a dose dependent increase in latency time when compared with control.

Table2: Effect of the Butanol fraction of *Willughbeia edulis* on latency to hot plate test

Group	Reaction time at different time intervals (in sec)					
	0 Hr	½ Hr	1 Hr	2 Hr	3 Hr	4 Hr
Control (n=5)	7.3800 ±3.61206	9.8800 ±2.63572	10.0600 ±2.40271	10.0000 ±2.38956	10.0400 ±2.08038	10.5800 ±2.23875
Positive Control (n=5)	7.7200 ±3.12122	13.8800 ±2.97439	14.7200 ±2.55186*	14.5200 ±2.59268*	14.4800 ±2.85867*	14.5200 ±1.77539*
W. edulis (250mg/kg)(n=5)	7.9800 ±2.01792	11.4200 ±3.22289	12.8400 ±3.42170	13.4400 ±3.32836*	15.8000 ±2.13776**	17.4200 ±2.12885***
W. edulis (500mg/kg)(n=5)	9.2600 ±4.22054	15.8000 ±2.85307*	16.8400 ±2.87280**	17.6800 ±2.01792***	18.2800 ±2.18449***	18.7600 ±1.95525***

Data are represented as the mean ± SEM, (n=5); *P < 0.05, **P < 0.01, ***P < 0.001 were considered significantly different in comparison with control.

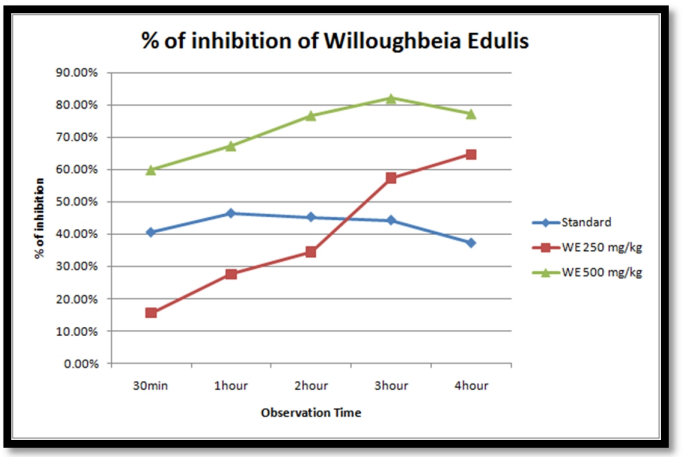

Figure 1: % of inhibition of *Willughbeia edulis* found in hot plate method

Acetic acid-induced writhing test: Inhibition of licking response in mice due to the administration of the test drugs during acetic acid-induced writhing test is shown in Table 3. The oral administration of both doses of Butanol extracts of *Willughbeia edulis* significantly ($p<0.001$) attenuated the acetic acid-induced abdominal writhes in mice in a dose dependent fashion. The percent inhibition of writhing response by the extract was 62.78% and 74.45% at 250 and 500 mg/kg doses respectively while the standard diclofenac sodium (25 mg/kg) showed 66.25% inhibition in comparison with the control.

Table 3: Effect of the Butanol extract of *Willughbeia edulis* on the acetic acid-induced writhing in mice

Group	Dose	Route	No of response	% Inhibition
Control(n=5)	25 mg/kg	p.o	63.4000 ±6.24179	-
Positive Control(n=5)	25 mg/kg	p.o	21.4000 ±5.04579***	66.25%
***Willughbeia edulis* (n=5)**	250mg/kg	p.o	23.6000 ±8.22557***	62.78%
***Willughbeia edulis* (n=5)**	500mg/kg	p.o	16.2000 ±3.42637***	74.45%

Data are represented as the mean ± SEM, (n=5); * p<0.05, **p<0.01, *** p<0.001 were considered significantly different in comparison with control.

DISCUSSION

High Concentration of tannin in extract has been shown to possess potent anti-inflammatory properties. There are also reports on the role of tannins in anti-nociceptive activity[13]. Flavonoids, also known as nature's tender drugs, possess abundant biological and pharmacological activities. Analgesic and anti-inflammatory effects have been observed in flavonoids[14]. It is also reported that flavonoids such as rutin, quercetin and luteolin produced significant antinociceptive and anti-inflammatory activities. Certain flavonoids possess strong inhibitory activity against a wide range of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A2, phosphodiesterases and others[15]. Studies have also demonstrated that terpenoids produced significant analgesic and anti-inflammatory activities[16].

The hot plat test is used to measures the complex response to a non-inflammatory and acute nociceptive input and is also normally used for studying central nociceptive activity. Any agent that causes a prolongation of the hot plate latency using this test must be acting centrally[17]. The fractions of the *Willughbeia edulis* plants and Diclofenac sodium (10 mg/kg) also presented a longer latency time than the control group in the hot plate test in a dose related manner. In the 3rd hour, oral administration of the 250mg/kg and 500mg/kg of butanol fractions of *Willughbeia edulis* was found to have percent of inhibition 57.37% and 82.07% respectively and also in the 4th hour 64.65% and 77.32% respectively.

The fact that *Willughbeia edulis* produced analgesia in all nociceptive models at the doses tested is indicative that it possesses both central and peripheral antinociceptive effects and the mechanism of action of the extracts could be related to lipooxygenase and/or cyclooxygenase of the arachidonic acid cascade in part. The primary mechanism of action responsible for Diclofenac Sodium's anti-inflammatory, antipyretic and analgesic effects is the inhibition of prostaglandin synthesis by competitive blocking of the enzyme cyclooxygenase (COX). Diclofenac sodium is a nonselective COX inhibitor. Results show a comparable effect of the *Willughbeia edulis* and Diclofenac sodium.

Narcotic analgesics inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain[18-19]. Butanol extract of *Willughbeia edulis* exhibited both types of pain inhibition. So we can conclude that the cause of the analgesic effect may be blocking of prostaglandin synthesis.

The acetic acid-induced writhing is a sensitive method to evaluate peripherally acting analgesics. In acetic acid-induced abdominal writhing, the visceral pain model, released arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis played a role in the nociceptive mechanism. This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway.

The writhing response of the mice to the injection of noxious chemical was used to screen for both peripheral and central acting analgesic activity. Acetic acid causes pain by liberating endogenous substances and many others that excite pain at nerve endings. It was found that the intensity of the analgesic effect of the butanolic fractions of *Willughbeia edulis* was similar to that of Diclofenac Sodium and probably be due to blockade of the effect or release of endogenous substances that excite pain nerve endings.

The butanolic extract of *Willughbeia edulis* at dose levels of 250 and 500 mg/kg, produced resistance to mechanical pain after 30 minutes. The weight that indicates pain after treatment was significantly increased ($P<0.001$). The butanolic fractions produced a significant reduction in writhing episodes induced by acetic acid (0.7 %) and the percentage protection at 250 and 500 mg/kg were 62.78% & 74.45% respectively.

CONCLUSION

On the basis of our findings, it can be inferred that the butanol extract of *Willughbeia edulis* possess significant analgesic activities and this is the first report of the investigations. The results support the basis for the traditional use of the plant in folk medicine. However further investigations are required to identify the active components responsible for the activities.

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