

# Hydro-alcoholic extract of *Otostegia integrifolia* Benth (Lamiaceae) produces peripheral antinociception and central analgesia in mice models

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## Research note

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# Abstract

Objective Pain and inflammatory conditions are the commonest health problems reported to this date. The advent of numerous pharmacologic agents cannot still abated the demand due to the associated side effects, the search for satisfactory new molecule particularly from herbal sources is the main endeavor as experience shows. Thus, this study evaluated the analgesic activity of the 80% methanol leaf extract of *Otostegia integrifolia* in mice models of pain.

Results Analgesic effect of various oral doses of the hydro-alcoholic extract (100, 200 and 400 mg/kg) was determined in hot plate and acetic acid induced writhing methods. In all models, the higher doses of the extract (400mg/kg) exhibited significant central and peripheral analgesic activities without significant difference compared to the standard drugs morphine and aspirin respectively. However, the lowest dose of the extract lacks central analgesic activity. The experimental finding from this study corroborates perhaps the presence of similar constituents within the genus *Otostegia* that might be responsible for the analgesic effects observed on other species. Thus, *Otostegia integrifolia* could be potential source for development of new analgesics.

## Introduction

Pain is a universal concept and the most common reason a patient sees a physician [1]. Pain, especially when chronic, markedly decreases individuals' health status and quality of life and can detrimentally affect the families of patients. It often interferes with every day work activities [2]. In addition, the presence of a long lasting pain syndrome is a leading risk factor for suicide. It is thus, a serious and costly public health problem [3].

Even though there are wide range of medicines available, the management of pain is sometimes inadequate, leading to inappropriate pain control and patient suffering [4]. Although there is an increment of knowledge and developments in technological resources regarding pain, many patients still experience pain [5]. Moreover, due to extensive use of analgesic agents, the toxicity and untoward effects do occur especially when therapy of pain involves the use of higher doses of analgesics for prolonged period of time [4, 6]. This results in an increase in the fatigue levels and impairments in daily life activities and social interactions. Finally, the patient ends up living with impaired functional ability and a reduced quality of life [7].

Plants are one of the most important sources of medicines [8]. About 80% of the total population of Ethiopia depends on traditional medicines [9]. Many medicines of plant origin have been used without major severe adverse effects [10]. It is therefore, essential that efforts should be made to introduce new medicinal plants to develop more effective and cheaper drugs. Plants represent a large natural source of useful compounds that might serve as a lead for the development of novel drugs [8, 11]. In addition, this study may serve as baseline information for further investigation and identification of the specific agents responsible for the analgesic activity of the plant in an effort to contribute to the discovery of perhaps new molecule with high activity and low toxicity.

The genus *Otostegia* (Lamiaceae) consists of about 15 species. It is endemic to the northern part of tropical Africa and South-western and Central Asia [12]. It is commonly known with the vernacular name of "Tunjite" in Amharic, and its morphological characteristic is described elsewhere [12, 13]. From the genus *Otostegia* the species *O. persica* and *O. fruticosa* revealed significant analgesic and anti-inflammatory activities [14, 15]. Thus, the purpose of this study was to verify whether the plant of interest possesses similar analgesic activity in mice models.

## Methods

### Drugs and chemicals

All chemicals, drugs and reagents used in the study were analytical grade; methanol (Carlo erba group reagents, Italy), morphine (EPHARM, Ethiopia), acetyl salicylic acid (EPHARM, Ethiopia), distilled water (EPHARM, Ethiopia), and glacial acetic acid and diethyl ether (sigma- Aldrich laborachemikalin, Germany) obtained and used from the respective vendors.

### Plant material

The leaf of *Otostegia integrifolia* was collected in December 2017 from a town called Tulu Dimtu, which is located in North West Shewa, Oromiya, its geographical coordinates are 9° 41' 0" North, 38° 40' 0" East, about 29 km southeast of Addis Ababa, Ethiopia (figure 1). Identification & authentication of the plant specimen was done by a taxonomist and a voucher specimen (AD001) was deposited at the National Herbarium, College of Natural and Computational Sciences, Addis Ababa University, for future reference.

### Extraction

Five hundred gram of air-dried and powdered plant material was extracted with 80% methanol by cold maceration technique for three consecutive days at room temperature to get the crude hydroalcoholic extract. The resulting liquid extract was then filtered with Whatman no 1 filter paper & concentrated using rotavapor (Buchilabortechnik AG, Switzerland) at 40°C under reduced pressure and the concentrated extract was freeze-dried using a lyophilizer (Heto Power Dry LL3000 freeze-dryer,, USA). A yellowish brown, particularly of apricot type, hygroscopic shiny powder with percentage yield of 16.6% (w/w) was obtained.

### Phytochemical analysis

The 80% methanol extract of the seeds of *Otostegia integrifolia* was screened for the possible presence of secondary metabolites, including alkaloids, tannins, flavonoids, terpenoids and saponins, phenols using qualitative phytochemical screening procedures described elsewhere [16]

### Experimental animals

A total of 50 healthy swiss albino mice (25–35 g, 6–8 weeks of age) of either sex were obtained from the animal house of school of pharmacy, Addis Ababa University. They were provided with standard pellet and water *ad libitum* under a controlled environment (12 h light– dark cycle and temperature of 23–25°C). Animals were acclimatized for one week before commencement of the experiment and after completion of the study the animals were anesthetized with diethyl ether and euthanized. The care and handling of animals were in line with international guidelines [17] and the protocol was approved by institutional review board of the School of Pharmacy (Reference no. ERB/SOP/120/11/2017).

# Grouping and dosing of animals

A total of 60 male Swiss albino mice were used for all the experiments. Mice were randomly divided into five groups with each group consisting of 6 mice. Group I served as negative control were administered with distilled water. Group II, Group III and Group IV were given 100mg/kg, 200mg/kg and 400mg/kg of the extract, respectively as per the acute toxicity study result conduct elsewhere. Group V served as positive control received standard drug morphine for hot plate method, 150 mg/kg aspirin for acetic acid induced writhing test. Administration of all agents was performed via an oral route.

## Analgesic activity

### Hot Plate method

In this method the animals were tested for analgesia screening and mice showing greater than 15 s of latency time on a hot plate maintained at  $-50 \pm 0.1$  °C were excluded. The induction of analgesia (time in seconds for which mice-remained on the hot plate without licking or flicking of hind-limb or jumping) was recorded at 0, 30, 60 and 120 min after the administration of the plant samples and drug. To avoid tissue-damage, cut off-time of 15 s was set-for all animals. Percent analgesia-was calculated using the following formula [18].

$$\text{Max. Analgesia} = \frac{\text{Reaction time for the test} - \text{reaction time for DW} \times 100}{15 \text{ sec} - \text{reaction time for DW}}$$

### Acetic acid- induced writhing method

For the writhing test overnight fasted mice were grouped into control and three experimental groups of six mice each and administered with respective doses of the extract and aspirin. To assess analgesic activity of various groups, the number of writhes that was indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb was counted for each mouse for 20 min using a latency period of 5 min, and the percentage was calculated using the formula described below[19].

$$\% \text{ inhibition of writhing} = \frac{\text{Mean no. of writhes (control)} - \text{mean no. of writhes (test)} \times 100}{\text{Mean number of writhes control}}$$

## Statistical Analysis

All data found from the research were expressed as mean  $\pm$  standard error of the mean (SEM). Data was analyzed by one way analysis of variance (ANOVA) followed by Tukey post-hoc test to determine statistical significance

using statistical package for social science (SPSS) version 25. P values less than 0.05 were taken as statistically significant.

## Results

### *Phytochemical analysis*

Preliminary phytochemical screening of the 80% methanol leaf extract of *O. integrifolia* revealed that the extract contains phenolic compounds, saponins, and flavonoids while alkaloids, tannins and steroidal compounds were absent.

### *Hot plate method*

Table 1: Effect of 80% Methanol leaf extract of *Otostegia integrifolia* on hot plate test in mice.

Latency (sec) and Maximum possible protection (%)									
Group	0 min	15 min	%	30 min	%	45 min	%	60 min	%
DW	6.21.77	51.095		5.61.029		40.77		4.40.77	
MO	4.80.37	12.62.18 <sup>a1</sup>	76	14.23.38 <sup>a3c2d2e1</sup>	91.4	14.96.54 <sup>a3c2</sup>	99.6	14.52.2 <sup>a2</sup>	95.2
OI100	5.20.20	8.21.11	32	9.41.50	40.4	8.61.07	40	11.22.2	64.1
OI200	3.80.58	7.80.97	28	9.81.31	44.6	12.81.42 <sup>a1</sup>	80	11.61.53 <sup>a1</sup>	67.9
OI400	5.000.707	11.41.69 <sup>a1</sup>	54	12.24.47	70.2	12.81.42 <sup>a1</sup>	80	13.60.81 <sup>a2</sup>	86.8

Values are expressed as Mean  $\pm$  S.E.M (n=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; **a** against the control, **b** against the standard drug, **c** against AF100, **d** against AF200, **e** against AF400, <sup>1</sup>P<0.05, <sup>2</sup>P<0.01, <sup>3</sup>P<0.001; OI refers to 80% Methanol leaf extract of *Otostegia integrifolia*, MO: Morphine; and DW stands for Distilled Water. Control received distilled water (10ml/kg), whereas standard received Morphine (20mg/kg) orally.

Regarding hot plate method both the extract and morphine prolonged the reaction time as compared to negative control throughout the observation period (table 1). There was no increase in latency observed with the lower dose (OI 100). The latency observed by the higher dose and by the standard drug at 60 minutes were significantly greater (P <0.01) than the negative control. There was also difference in latency among treatment groups. At 15 min only standard drug and OI400 have significantly (P< 0.05) increased latency compared to negative control. At 30min the standard drug resulted in higher effect than all dose of the extract however, in 45min and 60 min middle and high dose of the extract have increased latency. At 60min high dose of the extract and standard drug have significantly (P< 0.01) increased in latency period compared to negative control.

### *Acetic acid induced writhing test*

Table 2: Effect of 80% Methanol leaf extract of *Otostegia integrifolia* on writhing test in mice.

Group	Mean No. of writhing ± S.E.M	% Inhibition
DW	1656.28	-
ASA	49.84.52 <sup>a3c1</sup>	69.1%
OI100	96.213.85 <sup>a3</sup>	41.6%
OI200	54.65.88 <sup>a3c1</sup>	66%
OI400	62.210.05 <sup>a3</sup>	62.3%

Values are expressed as Mean ± S.E.M (n=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; **a** against the control, **b** against the standard drug, **c** against OI100, **d** against OI200, **e** against OI400. <sup>1</sup>P<0.05, <sup>2</sup>P<0.01, <sup>3</sup>P<0.001; ASA: Aspirin, OI : 80% Methanol leaf extract of *Otostegia integrifolia*.

The peripheral effect of the extract using writhing test, revealed that mice treated with all doses of the extract showed a significant protection (P< 0.001) against acetic acid induced writhing compared to negative control group ( table 2). Though ASA produced a significant greater protection than controls and OI100 of the extract, no appreciable change were observed when compared to OI200 and OI400. Percent inhibition of writhing among ASA, OI200 and OI400 groups was found to be comparable.

## Discussion

Pain induced by thermal stimulation using hot plate method is specific model to investigate central mediated analgesic activity. It was selected for this study because of several advantages including sensitivity to strong analgesics, limited tissue damage, accuracy of the result and it is also less time consuming [20, 21].

Profound increase in latency time was observed at 60 min with the high dose (OI400) of the extract and its percentage inhibition was also comparable with the standard drug morphine. The standard drug also showed greater latency at 30 min perhaps this may indicate the duration of time for pick activity of the extract (60min) was longer than the standard (30 min). This time gap may be exhibited due to the lag time between drug entering the central compartment and distribution to the target site or formation of an active metabolite that are endowed with analgesic activity. All doses of the extract's percentage inhibition increased with increasing time throughout the experimental period, such attributes might indicate the consistent pharmacokinetic profile of all experimental groups [22].

The writhing test, also less commonly known as abdominal contraction test is used for screening the peripheral anti nociceptive activity of different compounds [21]. Intra peritoneal administration of acetic acid induces irritation to the peritoneal cavity and provokes a very stereotype behavior in mice. This response is characterized by abdominal contraction and accompanied by stretching of the hind paws particularly. The writhing response is due to sensitization chemosensitive nociceptors by prostaglandins particularly PGE2 and PGF2 as well as lipoxgenase produced leukotrines [20, 23].

In the writhing test, in contrast to the hot plate method 100mg/kg of the extract showed significant peripheral analgesic activity. The higher doses of the extract exhibited comparable activity with ASA, this perhaps indicates as the dose of the extract increases the phytochemical agents responsible for the observed effect have also

increased. All doses of the extract showed significant decrement in writhing compared to the negative control. This effect of the extract might be attributed together to its action on visceral targets which mediate pain pathway and propagation peripheral.

Thus, the finding indicates that hydro-alcoholic leaf extract of *O. integrifolia* possesses analgesic activity which probably mediated through both central and peripheral analgesic mechanisms. Therefore, the experimental finding further corroborates perhaps the presence of similar constituents within the genus *otostegia* that might be responsible for the analgesic effect observed on other species [14, 24].

## Limitations

The limitations of this study include purification and isolation of phytochemical constituents responsible for the observed pharmacological activity were not performed, no mechanistic investigation and chronic toxicity study carried out.

## Abbreviations

ANOVA:analysis of variance; ASA:acetyl salicylic acid; DW:distilled water; MO:morphine; OI:Otostegia integrifolia; PG:prostaglandins; SEM:standard error of the mean; SPSS:statistical package for social science

## Declarations

## Acknowledgements

We would like to thank Addis Ababa University College of Health Sciences School of Pharmacy for allowing the study to be conducted with facility of the institution.

## Author's contributions

AD conceived the idea, conducted the experimental work, did the data analysis, and write up. RT were involved in the experimental work and write up.

## Funding

No specific funding was granted for this project.

## Availability of data and material

The information supporting the conclusions of this article is included in the article.

## Ethics approval and consent to participate

The protocol was approved by institutional review board of the School of Pharmacy with Reference no. ERB/SOP/120/11/2017.

# Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Tables

### Hot plate method |

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DW	6.2 ±1.77	5 ±1.095		5.6 ±1.029		4 ±0.77		4.4 ±0.77	
MO	4.8 ±0.37	12.6 ±2.18 <sup>a1</sup>	76	14.2 ±3.38 <sup>a3c2d2e1</sup>	91.4	14.96.54 <sup>a3c2</sup>	99.6	14.5 ±2.2 <sup>a2</sup>	95.2
OI100	5.2 ±0.20	8.2 ±1.11	32	9.4 ±1.50	40.4	8.6 ±1.07	40	11.2 ±2.2	64.1
OI200	3.8 ±0.58	7.8 ±0.97	28	9.8 ±1.31	44.6	12.8 ±1.42 <sup>a1</sup>	80	11.6 ±1.53 <sup>a1</sup>	67.9
OI400	5.00 ±0.707	11.4 ±1.69 <sup>a1</sup>	54	12.2 ±4.47	70.2	12.8 ±1.42 <sup>a1</sup>	80	13.6 ±0.81 <sup>a2</sup>	86.8

Values are expressed as Mean ± S.E.M (n=6); analysis was performed with One-Way ANOVA followed by Tukey post hoc test; **a** against the control, **b** against the standard drug, **c** against AF100, **d** against AF200, **e** against AF400, <sup>1</sup>P<0.05, <sup>2</sup>P<0.01, <sup>3</sup>P<0.001; OI refers to 80% Methanol leaf extract of *Otostegia integrifolia*. MO: Morphine; and DW stands for Distilled Water. Control received distilled water (10ml/kg), whereas standard received Morphine (20mg/kg) orally.

## Figures



**Figure 1**

Photograph showing *Otostegia integrifolia* Benth shrub during collection.

## Supplementary Files

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