ORIGINAL ARTICLE

IN VIVO ANALGESIC AND ANTIPYRETIC ACTIVITIES OF N-BUTANOL AND WATER FRACTIONS OF *OCIMUM SUAVE* AQUEOUS LEAVES EXTRACT IN MICE

Shibiru Tesema[1], Eyasu Makonnen[2]

ABSTRACT

BACKGROUND: Ocimum suave willd is one of the plants traditionally used for the treatment of inflammation and related disorders in different parts of Ethiopia. The aim of the current study was to evaluate the analgesic and antipyretic activities of the solvent fractions (n-butanol and water) of O. suave aqueous leaves extract.

MATERIALS AND METHODS: Acetic acid writhing and tail flick tests were used to evaluate the analgesic activity, and yeast-induced fever in mice was used to evaluate the antipyretic activity of the solvent fractions.

RESULTS: Both solvent fractions exhibited inhibitory effect against acetic acid induced writhing at all tested dose levels in a dose dependent manner. The water fraction inhibited writhing by 47.69% at a dose of 200 mg/kg which was comparable to that by ASA, the standard drug. In the tail flick test, 200 mg/kg dose of both solvent fractions showed significant activity (P<0.05) after 0.5h, 1h and 3hrs of their administration. Both n- butanol and water fractions produced significant reduction in yeast induced fever at all doses employed.

CONCLUSION: From these findings, it can be concluded that the n-butanol and water fractions of O. suave aqueous leaves extract have potential analgesic and antipyretic activity in mice.

KEYWORDS: Analgesic, antipyretic, writhing, tail flick, O. suave, water fraction, butanol fraction

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INTRODUCTION

Pain and fever are the most common symptoms of injuries and diseases (1, 2). The current analgesic and antipyretic drugs, such as Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), are associated with adverse effects like gastrointestinal irritation, renal dysfunction, liver dysfunction and many others (3). Gastrointestinal side effects of NSAIDs have been overcome to a certain extent with the introduction of selective cyclooxygenase-2 (COX-2) inhibitors (4). However, these agents were later found to be toxic to the hepatic cells, glomeruli, cortex of brain, and heart muscles (5). Opioid analgesics such as morphine have strong addictive potential and other side effects including respiratory depression, drowsiness, decreased gastrointestinal motility and nausea (6).

Due to these and other inherent problems associated with the current analgesics and antipyretics, a continuous search for alternative medications is going on, especially from natural sources. The demand for plant medicines is increasing rapidly as they are generally believed to be safer than synthetic agents. Further as health care costs continue to escalate, the attraction for low cost remedies has stimulated consumers to re-evaluate the potential of alternatives (7).

Ocimum suave willd is one of the plants traditionally used for the treatment of

[1]Department of Pharmacology, College of Medical Sciences, Jimma University, Ethiopia
[2]Department of Pharmacology, School of Medicine, Addis Ababa University, Ethiopia
Corresponding Author: Shibiru Tesema, Email: shibtesema007@yahoo.com

inflammation and related disorders. It belongs to the family lamiaceae, syn.: labiatae (8). Most members of this family such as Hyptis, Thymus, Origanum, Salvia and Mentha species are considered economically useful because of their basic natural characteristics as essential oil producers (9).

O. suave willd is a small aromatic ramified shrub which grows to an average height of 1m. It is found in Tropical Asia and in West and East Africa where its geographical distribution is limited to mountainous areas (8). It is found in different regions of Ethiopia at an altitude between 1600-2200m and is known by its vernacular name "Ancabbi" (oromiffa). It is one of the common medicinal plants traditionally used in Ethiopia for treatment of different disorders such as headache, febrile illness and other inflammatory disorders by sniffing the juice squeezed from fresh leaves or drinking after infusion in water (10).

The leaves extract of *O. suave* has been reported to exhibit the mosquito repellent (11), acaricidal (12), anti-inflammatory (13), antidiabetic (14), wound healing and anti-ulcer activities (8, 15, 16). It has also been shown to possess analgesic and antipyretic activities. Ethanol extract of the plant material was found to produce greater protection against acetic acid induced writhing when compared to the aqueous extract at higher doses, i.e. (600-800 mg/kg) (17). Analgesia measured by tail flick and hot plate test showed more or less equal efficacy of both aqueous and ethanol extracts (10). The aqueous extract of *Ocimum suave* produced greater antipyretic activity than its ethanol extracts (18).

Although the analgesic and antipyretic activities of the aqueous and ethanol extract of the plant have been studied, there is no study on the solvent fractions of the plant material to date. The aim of this study was, therefore, to evaluate the analgesic and antipyretic activities of the solvent fractions (n-butanol and water) of the aqueous leaves extract of the plant material.

MATERIALS AND METHODS

Collection and authentication of plant materials: The leaves of *O. suave* were collected from Jimma (around bedda bunaa) about 324 Km South West of Addis Ababa in October, 2011. The plant was authenticated by a taxonomist and voucher specimen representing *O. suave* (Herbarium No. 001) was deposited at the National Herbarium, Department of Biology, Addis Ababa University, Ethiopia.

Chemicals and drugs: The drugs and chemicals used in this study were: n-butanol (BDH chemicals, England), chloroform (Fisher Scientific UK limited), acetylsalicylic acid (Bayer Schering Pharma AG, Germany), yeast extract (England), acetic acid (BDH chemicals, England).

Experimental animals: The experiments were performed on in-house bred albino male and female mice (weighing 25-35g), which were obtained from the Ethiopian Health and Nutrition Research Institute, Department of Pharmacology, School of Medicine, Addis Ababa University and Biology Department, Addis Ababa University. They were all acclimatized to the animal house prior to use. Mice were kept in cages in animal house with a 12 hr light: 12 hr dark cycle. They fed on pellets and drank tap water *ad libitum*. The care and handling of mice were in accordance with the internationally accepted standard guidelines for use of animals (19). The experiment was conducted after obtaining approval from the Ethical Committee of the Department of Pharmacology, School of Medicine, Addis Ababa University, Ethiopia.

Preparation of crude extract and fractionation: Eight hundred grams of dried undershade and coarsely powdered leaves of *O. suave* were macerated with cold water for 3 days. After filtration with muslin cloth and then with Whatman filter paper no.1, the extract was lyophilized, and 92.4g (11.55%) dry powder was obtained. Eighty grams of the aqueous extract was dissolved in warm distilled water and filtered through Whatman filter paper no.1. The filtrate was then added in a separatory funnel and mixed with 50 ml of chloroform. After shaking, it was allowed to stay for some time until complete formation of two layers. Then, the lower layer, i.e. chloroform layer, was collected. The procedure was repeated three times. After the chloroform layer was collected, 50 ml of butanol was successively added three times in the aqueous residue, and then the upper layer (butanol) was taken. Finally, the chloroform and butanol fractions were concentrated under vacuum in a rotary evaporator to give 0.97% (w/w) and 11.88% (w/w) gummy residue, respectively, and the aqueous residue was lyophilized to give

24.25% (w/w) dried fraction. The butanol and water fractions, which showed good effect during the pilot study, were selected for the experiments.

Acute oral toxicity study: Acute oral toxicity of n-butanol and water fractions of the aqueous extract of *O. suave* leaves was performed as per OECD guideline-423 (20). It was observed that the test extracts were not mortal even at the dose of 2000 mg/kg. This shows that the lethal dose (LD_{50}) is greater than 2000mg/kg body weight.

Analgesic activity evaluation

Writhing test: The mice were divided into eight groups of five mice in each. Group I received distilled water (10ml/kg p.o.) and served as a control. Group II received acetylsalicylic acid (200mg/kg) and served as a standard. Group III, IV and V received water fraction of *O. suave* aqueous leaves extract at doses of 50,100 and 200 mg/kg p.o, respectively. Group VI-VIII received butanol fraction of *O. suave* aqueous leaves extract at doses of 50, 100 and 200 mg/kg p.o, respectively. After one hour, 0.35ml of 0.6% (v/v) acetic acid solution was administered intraperitoneally to each mouse. Abdominal constrictions (writhes) were counted for 20 minutes, starting 10 minutes after administration of acetic acid (17). Inhibition against acetic acid induced pain was expressed as reduction in the mean number of writhes for control mice versus extract or standard drug treated mice (17) using the following relation:

$$\% \text{ inhibition} = \frac{\text{Writhes in control- Writhes in test}}{\text{Writhes in control}} \times 100$$

Tail flick test: The mice were divided into eight groups of six mice in each. Group I received distilled water (10ml/kg p.o.) and served as a control. Group II received acetylsalicylic acid (200mg/kg) and served as a standard. Group III, IV and V received water fraction of *O. suave* aqueous leaves extract at doses of 50,100 and 200 mg/kg p.o, respectively. Group VI-VIII received butanol fraction of *O. suave* aqueous leaves extract at doses of 50, 100 and 200 mg/kg p.o, respectively. The test was carried out with a radiant heat analgesiometer (Mark-1B, SL. NO.720121, India) according to Tadele *et al.* (21).The mice were held in a restrainer with the tail extending out, and the tip of the tail was loosely held on the radiant heat source during the test.

Reaction time was measured with a stop clock to the nearest second. The end point was a flick of the tail to remove it away from the heat and each mouse was tested twice before the extract or the standard drug administration. Results of the second test were employed as baseline responses for subsequent comparison of treatments, which was designated zero concentration. The test was repeated for each mouse 30, 60, 120, and 180 min after administration of the extract, standard drug or distilled water. A 15-s cut-off was imposed as a protection against tissue damage (21).

Antipyretic study: The procedure described by Makonnen, *et al* (18) was adopted for this study. A thermister probe was inserted about 3 cm into the rectum of each mouse, and their basal rectal temperatures were recorded on a digital thermometer (CE 0434, Taiwan). The mice were then injected with 30% (w/v) suspension of yeast in 0.9% NaCl in a dose of 10 ml/kg below the nape of the neck. The rectal temperature of each mouse was again recorded after 18 hrs of yeast administration. Mice that did not show a minimum increase of 0.5°C in temperature 18 hrs after yeast injection were discarded. Forty eight selected mice were grouped into eight groups, 6 mice in each. Group I received distilled water (10 ml/kg p.o.) and served as a control. Group II received acetylsalicylic acid (200 mg/kg) and served as a standard. Group III, IV and V received water fraction of *O suave* aqueous leaves extract at doses of 50,100 and 200 mg/kg p.o, respectively. Group VI-VIII received butanol fraction of Ocimum suave aqueous leaves extract at doses of 50, 100 and 200 mg/kg p.o, respectively**.** Rectal temperature of all the mice was then recorded by inserting digital thermometer into the rectum of each mouse every 30 min for three hours after dosing (22). Percent reduction in rectal temperature was calculated considering the total fall in temperature to normal level as 100% (22).

$$\% \text{ reduction} = \frac{\text{Temp 18hrs after yeast-temp after drugs/extracts at different hours}}{\text{Temp 18hrs after yeast-normal body temperature prior to yeast administration}} \times 100$$

Statistical analysis: Data was analyzed using SPSS version 16 and results were expressed as

mean ± S.E.M. ANOVA followed by Dunnett's test was used to compare results between treatment and control groups. Students paired t-test was used to test significance for the difference between initial and final results within the same group. Results were considered significant when p <0.05.

RESULTS

Acetic acid-induced writhing test: Both water and butanol fractions of *O. suave* aqueous leaves extract showed inhibition against acetic acid induced writhing in a dose dependent manner (Table 1). The water fraction reduced acetic acid induced writhing significantly (P<0.05 at 50 mg/kg dose and p<0.001 at 100 mg/kg and 200 mg/kg dose levels) compared to control (distilled water). The water fraction exhibited higher inhibition of writhing than the butanol fraction at high dose (Table 1). The butanol fraction was found to be more effective than water fraction at low and medium doses, while less effective at the highest dose employed in the present study. The water fraction inhibited writhing by 47.69% at a dose of 200 mg/kg which is comparable to 50.99% by ASA, the standard drug (Table 1).

Table1: Effect of solvent fractions (water and n-butanol) of *O. suave* aqueous leaves extract on Acetic Acid Induced Writhing in mice

Treatment	Dose mg/kg	No. of writhes (mean ± SEM)	% inhibition
Distilled water		60.8±2.56	-
ASA	200	29.8±2.63[a]	50.99
Water fraction	50	48.2±2.69[c]	20.72
	100	39.2±2.85[a]	35.53
	200	31.8±2.75[a]	47.69
Butanol fraction	50	45.0±1.87[b]	25.99
	100	37.0±3.15[a]	39.14
	200	35.4±2.16[a]	41.78

Data were analyzed by using One-way ANOVA followed by Dunnett's multiple comparison tests. *Values are expressed as mean ± S.E.M. (n = 5);* [a] extremely significant (p<.001), [b] moderately significant (p<.01), [c] significant (p<.05) when compared with control (distilled water).

Tail flick test: Both fractions showed analgesic activity in all tested dose levels (Table 2). The water fraction showed significant activity (p<.001) at 2 hrs (2.9±.14[a]) and at 3h (3.3±.19[a]) at a dose of 50 mg/kg. At a dose of 100 mg/kg, water fraction showed significant activity (P<.001) after 2 hrs (3.4±.34[ax]) as compared to baseline latency. The butanol fraction showed significant (p<.001) increase in latent time after 3 hrs (3.1±.15[a]) at a dose of 100 mg/kg as shown in Table 2. A dose of 200 mg/kg of both water and butanol fractions showed significant (P<.05) activity at 0.5 hrs, 1 h and at 3 hrs as co Tail flick test.

Both fractions showed analgesic activity in all tested dose levels (Table 2). The water fraction showed significant activity (p<.001) at 2h (2.9±.14[a]) and at 3h (3.3±.19[a]) at a dose of 50 mg/kg. At a dose of 100 mg/kg, water fraction showed significant activity (P<.001) after 2 hrs (3.4±.34[ax]) as compared to baseline latency. The butanol fraction showed significant (p<.001) increase in latent time after 3 hrs (3.1±.15[a]) at a dose of 100 mg/kg as shown in Table 2. A dose of 200 mg/kg of both water and butanol fractions showed significant (P<.05) activity at 0.5 hrs, 1 hr and at 3 hrs as compared with the corresponding value of the control (distilled water).

Table 2: Effect of solvent fractions (water and n-butanol) of *O. suave* aqueous leaves extract on nociception in mice (tail flick method)

Treatment	Dose(mg/kg)	Latency at different time(hour) in second				
		0h	.5h	1h	2h	3h
Distilled water		1.4±.10	1.4±.11	1.3±.15	1.3±.18	1.3±.19
ASA	200	.1.0±.06	5.8±.34^{ax}	6.5±.44^{ax}	5.5±.49^{ax}	5.0±.47^{ax}
Water fraction	50	1.2±.08	1.4±.05^{c}	2.5±.13^{b}	2.9±.14^{a}	3.3±.19^{a}
	100	1.1±.10	2.2±.19^{a}	2.7±.25^{ax}	3.4±.34^{ax}	3.5±.41^{b}
	200	1.1±.10	2.7±.34^{bx}	3.2±.31^{bx}	2.7±.36^{b}	3.9±.89^{cx}
Butanol fraction	50	1.4±.06	2.0±.23	2.5±.28^{c}	2.4±.19^{b}	2.7±.52
	100	1.5±.10	2.0±.14^{c}	2.8±.31^{bx}	3.1±.41^{b}	3.1±.15^{a}
	200	1.1±.09	3.4±.49^{bx}	3.2±.50^{bx}	5.0±1.19^{cx}	5.0±1.12^{cx}

Data were analyzed by Paired samples T-test and One-way ANOVA followed by Dunnett's multiple comparison tests. Values are expressed as mean ± S.E.M. (n = 6);^{a} extremely significant (p<.001), ^{b}moderately significant (p<.01),^{c} significant (p<.05)when compared with 0h of the same group.
^{x} statistically significant (p<.05) when compared with the corresponding value of the control (distilled water).

Antipyretic effect

The results of the antipyretic effect of the two solvent fractions (n-butanol and water) of O. suave aqueous leaves extract are presented in Table 3. Both fractions reduced yeast induced fever at all doses employed. The water fraction significantly reduced yeast induced fever (P< 0.001 at 1.5 hrs and 2 hrs, P<0.01 at 0.5 hrs, 1 hr, 2.5 hrs and 3hrs) at a dose of 50 mg/kg as compared to 0h rectal temperature of the same group. This fraction showed significant reduction in fever (P<0.05 at all-time intervals after 1 hr) at a dose of 100 mg/kg. It also produced significant decrease in rectal temperature (P<0.01 at 0.5 hrs and 1.5 hrs, P<0.001 at 1 hr, 2 hrs, 2.5 hrs and 3hrs) at 200 mg/kg dose. The butanol fraction produced significant effect (P<0.01 at 2 hrs, 2.5 hrs and 3 hrs) at all doses employed as compared to 0 hr rectal temperature of the same group. It also significantly reduced fever (P<0.05 at 0.5 hrs, 1.5 hrs, 2.5 hrs and 3 hrs) at 100 mg/kg dose level as compared to the corresponding value of the control (distilled water).

Table 3: Effects of solvent fractions (butanol and water) of *O. suave* aqueous leaves extracts on yeast induced pyrexia in mice

Treatment	Dose mg/kg	Rectal temperature in ^{0}c							
		-18h	0h	0.5h	1h	1.5h	2h	2.5h	3h
Distilled water		36.55±.15	37.78±.13	37.62±.12	37.58±.22	37.55±.18	37.58±.10	37.63±.17	37.58±.10
ASA	100	36.97±.17	38.17±.14	37.07±.24^{b}	37.02±.16^{a}	37.02±.08^{b}	36.98±18^{bx}	37.05±.20^{bx}	37.10±.19^{b}
Water fraction	50	36.88±.10	38.17±.21	37.48±.19^{b}	37.25±.16^{b}	37.35±.17^{a}	37.37±.19^{a}	37.35±.10^{b}	37.42±.10^{b}
	100	36.52±.12	37.48±.18	36.93±.16^{c}	36.77±.17^{cx}	36.78±.06^{cx}	36.78±.07^{cx}	36.75±.04^{bx}	36.75±.10^{bx}
	200	37.13±.08	38.37±.19	37.30±.18^{b}	37.25±.13^{a}	37.35±.17^{b}	37.38±.17^{a}	37.37±.15^{a}	37.48±.12^{a}
Butanol fraction	50	36.62±.16	37.63±.18	37.10±.30^{c}	37.10±.23^{c}	37.05±.23^{c}	37.07±.13^{b}	37.02±.12^{bx}	37.12±.16^{b}
	100	36.28±.11	37.95±.14	36.75±.26^{bx}	36.98±.12^{b}	36.97±.10^{bx}	37.10±.10^{b}	36.90±.17^{bx}	36.88±.21^{bx}
	200	37.02±.14	37.78±.09	37.33±.09^{b}	37.37±.11^{c}	37.22±.08^{b}	37.17±.09^{b}	37.10±.13^{b}	37.23±.15^{b}

Percent reduction in rectal temperature by solvent fractions (water and n-butanol) of *O. suave* aqueous leaves extract is shown in Table 4. Two hundred mg/kg water and butanol fractions reduced the elevated rectal temperature by 90.32% (at 1 hr) and 89.47% (at 2.5 hrs), respectively which is comparable to 99.17% (at 2h) by the standard drug, acetyl salicylic acid. Both fractions exhibited significant antipyretic activity in a dose dependent manner, and the water fraction was found to be slightly more potent than the butanol fraction.

Table 4: Percent reduction in rectal temperature by solvent fractions (water and n-butanol) of *O. suave* aqueous leaves extract

Treatment	Dose (mg/kg)	%reduction in rectal temp.					
		0.5hr	**1hr**	**1.5 hrs**	**2hrs**	**2.5hrs**	**3hrs**
Distilled water		13	16.26	18.7	16.26	12.2	16.26
ASA	100	91.67	95.83	95.83	99.17	93.33	89.17
Water Fraction	50	53.49	71.72	63.57	62.02	63.57	58.12
	100	57.29	73.96	72.92	72.92	76.04	76.04
	200	86.29	90.32	82.26	79.83	80.65	71.77
Butanol	50	52.48	52.48	57.43	55.45	60.4	50.5
Fraction	100	71.86	58.08	58.68	50.9	62.87	64.07
	200	59.21	53.95	73.68	80.26	89.47	72.39

DISCUSSION

Acetic acid induced writhing test, which is the visceral pain model, was employed to evaluate the peripheral analgesic activity of the plant material. The abdominal constriction response induced by acetic acid is a sensitive procedure to determine analgesia at peripheral level. This response is thought to involve local peritoneal receptors (23). Acetic acid is known to trigger the production of noxious substances such as prostaglandins specifically PGE_2 and PGF_2 as well as lipoxygenase products (24). These prostaglandins and lipoxygenase products cause inflammation and pain by increasing capillary permeability (25). Acetic acid may also cause release of other algesic mediators such as bradykinin, histamine and 5-hydroxytryptamine (26). The substance inhibiting the writhings will have analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (27). The result of the present study, therefore, hints that the water and butanol fractions of *O. suave* aqueous leaves extract may possess peripheral analgesic activity. Butanol fraction was found to be more effective at low (50 mg/kg) and medium (100 mg/kg) doses whereas the water fraction was observed to be more effective at high (200 mg/kg) dose.

Tail-flick test was used to evaluate the central analgesic activity as it mediates a spinal reflex to nociceptive stimuli (27). Tail flick test is very sensitive to centrally acting analgesics (10). Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems. It is well established that thermal nociceptive tests are more sensitive to opioid μ-agonists (28). Both fractions (water and butanol) of *O. suave* aqueous leaves extract showed significant analgesic activity by this test indicating that the plant may also act by central mechanism. The results of the present study, however, do not confirm the exact mechanism of analgesic action.

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states (7). Antipyretics are agents, which reduce elevated body temperature. Regulation of body temperature requires a delicate balance between the production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. This set point is elevated in fever, and NSAIDs like ASA promote its return to normal by blocking central cyclooxygenase production of prostaglandin E_2(PGE_2) (29, 30). ASA does not influence body temperature when it is elevated by factors such as exercise or in response to ambient temperature (29). Yeast induces pathogenic fever and its etiology could involve production of prostaglandins (31). In this study, both fractions

(water and butanol) of *O. suave* aqueous leaves extract produced very good antipyretic effect in a dose-dependent manner, and the maximum percent reduction in rectal temperature produced by both fractions was almost similar to that of the standard drug, Acetyl Salicylic Acid. The anti-pyretic action may be due to the inhibition of prostaglandin biosynthesis as prostaglandin is believed to be involved in regulation of body temperature. There are also several mediators for pyrexia, and the inhibition of any of these mediators may bring about antipyresis (25). The antipyretic effects of both fractions at all tested doses were observed as early as half an hour and the effect was maintained for three hours after oral administration suggesting that the plant has reasonable kinetic profile. The water fraction was found to be slightly more potent than the butanol fraction. Thus, it can be suggested that the water fraction has more active principle(s) responsible for the antipyretic activity.

The efficacy of most plant remedies is attributed to a combination of various active constituents (26). Previous phytochemical investigation of *O. suave* aqueous and ethanol extracts has revealed the presence of phenolic acids, flavonoids, glycosides and sugars as major chemical constituents (18).The components that are present in abundance in the extracts may, therefore, be responsible for the observed analgesic and antipyretic effects of *O. suave*. For example, flavonoids are known to target Prostaglandins involved in late phase of acute inflammation and pain perception, and have therefore been linked with analgesic, anti-inflammatory and antipyretic activities (28).

In conclusion, the study showed that butanol and water fractions of the *O. suave* aqueous leaves extracts possess both peripheral and central analgesic activity along with marked antipyretic activity in mice. The water fraction was observed to be more effective in reducing yeast induced pyrexia. This finding, thus, supports the traditional use of *O. suave* for the treatment of various ailments like ‘mich’, febrile illness, and headache and further confirms the findings from previous studies conducted on crude extract of the same plant.

Further studies should be carried out by identifying and isolating the possible active phytoconstituents responsible for the analgesic and/or antipyretic properties of the plant. Additional studies are also required to determine the exact mechanism of analgesic and antipyretic action of the plant.

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