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Phytochemical screening, antioxidant activity and analgesic effect of *Waltheria ovata* Cav. roots in miceOscar Herrera-Calderon^{1*}, Edwin Enciso-Roca², Bertha Pari-Olarte¹, Jorge Arroyo-Acevedo³¹Academic Department of Pharmaceutical Sciences, Faculty of Pharmacy and Biochemistry, Universidad Nacional San Luis Gonzaga de Ica, Ica, Peru²Academic Department of Human Medicine, Faculty of Health Sciences, Universidad Nacional San Cristóbal de Huamanga, Ayacucho, Peru³Pharmacology Laboratory, Faculty of Medicine, National University of San Marcos, Lima, Peru

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ABSTRACT

Objective: To determine the phytochemical screening, antioxidant activity and analgesic effect of crude ethanol extract of *Waltheria ovata* (*W. ovata*) Cav. root in mice.**Methods:** Phytochemical screening was developed by color or the precipitate formation. The evaluation of antioxidant activity and total phenolic content were assessed using 2,2-diphenyl-1-picrylhydrazyl and Folin-Ciocalteu's reagent, respectively. The analgesic effect was determined by acetic acid and formalin test. Different doses of *W. ovata* (50, 150, 300 and 500 mg/kg body weight) were administered *p.o.* to various groups of mice.**Results:** Phytochemical screening confirmed the presence of phenolic compounds, flavonoids, tannins, saponins, terpenoids and steroids. The antioxidant activity showed 25% for 0.1 µg/mL and was significantly higher ($P < 0.01$) than trolox and vitamin C, meanwhile, the total phenolic content (gallic acid equivalent) was 2200 mg/g of dry extract. *W. ovata* demonstrated an independent analgesic effect in different experimental models, like, acetic acid (72.51%, $P < 0.01$) and formalin (first phase: 58.6%, $P < 0.01$; second phase: 91.5%, $P < 0.01$, respectively) at dose of 300 mg/kg, similar to diclofenac (5 mg/kg) and morphine (30 mg/kg), respectively.**Conclusions:** *W. ovata* root's crude ethanol extract showed strong antioxidant activity and high phenolic content. The analgesic effect was demonstrated in two experimental models of pain implying that both peripheral and central mechanisms were involved. This might be due to the presence of various phytochemicals in the extract.

1. Introduction

The International Association for the Study of Pain has defined the pain as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[1]. Pain is the response to inflammatory mediators like prostaglandins, serotonin, histamine, interleukins and others regulating systems that may lead to the sensitization of nociceptors and somatosensory neurons[2]. However, this condition represents a serious problem that limits the quality of life, and more than 1.5 billion people with chronic pain will spend millions of dollars each year in analgesic drug[3]. Furthermore, these drugs are toxic for the liver generating a risk for patients especially with chronic pain[4].

Reactive oxygen species are necessary for the cells to carry out several biochemical functions as cell signaling, apoptosis or metabolism of xenobiotics[5]. However, free radicals are toxic in high quantities during an imbalance of redox state as long as the generation of reactive oxygen species in neuronal diseases plays an important role in the neuronal cell death and neurological dysfunction[6].

Although free radicals are linked to a transition of acute to chronic pain[7,8], recent studies have demonstrated the relationship between free radicals and chronic pain with two oxidants like superoxide and peroxynitrite which are involved in the development of chronic pain and the resistance of some opioid analgesics that are linked to tyrosine nitration proteins in the dorsal horn of the spinal cord[9,10].

Waltheria ovata (*W. ovata*) Cav. (Family: Sterculiaceae) is known as "lucraco" by the population of South Peru (Region, Ica), which is used as a folkloric remedy for the treatment of prostate inflammation, gastrointestinal disorders, diarrhea and headaches[11]. The use of traditional medicine in Peru is one of the most extended activities through out history[12]. *W. ovata* is used as an alternative medicine, but no scientific evidence exists to support its use. Therefore, it is necessary to validate the pharmacological effects of this plant for assessing the efficacy and safety.

The low incidence of chronic diseases in societies whose diets are rich in fruits, vegetables and derived products has been

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extensively documented[13,14]. Thus, the current study was carried out to evaluate the analgesic effect of crude ethanol extract of *W. ovata* root by using two *in vivo* models of pain. The phytochemical screening, total phenolic content and the antioxidant activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were also studied.

2. Materials and methods

2.1. Animals

A total of 72 BALB/c albino mice (20–30 g) of male sex obtained from the National Institute of Health (Lima, Peru) were used in the study. Mice were kept in plastic cages with access to pelletized food and water *ad libitum* and housed in animal room with controlled temperature (22–24 °C) and 12 h light/dark cycle. All mice were divided into six groups of six animals each and acclimatized to the laboratory 15 days previous to the experiments. All experimental procedures involving animals were conducted in accordance to the guidelines established by the European Union on Animal Care (CCE Council 86/609) and approved by the Institute for Ethics in Health of the National University of San Marcos (01414-R-12-UNMSM).

2.2. Plant material

The roots of *W. ovata* were collected, in April, 2015 from Rosario de Yauca, Ica Region, Peru, and identified by Mario Benavente. A voucher specimen (51-USM-2015) was deposited at the National Herbarium of National University of San Marcos, Lima, Peru.

2.3. Preparation of the plant material

The roots of *W. ovata* (500 g) were dried at room temperature and pulverized at the Chemical Laboratory, Faculty of Pharmacy and Biochemistry, Universidad Nacional San Luis Gonzaga. The powder material was exhaustively soaked with 96% ethanol with intermittent shaking every day for 7 days. The extract was filtered in Whatman No. 1 filter papers. The filtrate was evaporated by using a rotavap. *W. ovata* extract (21 g) was stored at 4 °C and light protected until further studies.

2.4. Phytochemical screening

The extract obtained was screened to determine the presence of phytochemical constituents, such as alkaloids, terpenoids, quinone, flavonoids, tannins, saponins, steroids and phenolic compounds, with the standard qualitative phytochemical methods described by Trease and Evans[15].

2.5 Determination of total phenolic content (TPC)

TPC was estimated by spectrophotometry, for each sample, 100 µL of sample extract was put into three tubes. Then 600 µL distilled water was added to each tube, and 150 µL of eight-time diluted Folin-Ciocalteu's reagent was added and allowed to stand for 5 min at room temperature. After 5 min, 150 µL of 20% sodium carbonate was added to react for 90 min. The absorbance was estimated at 760 nm with UV-vis spectrophotometer. A gallic acid standard curve (1–7.5 µg/mL) was used to measure phenolic content. The TPC was expressed as gallic acid equivalent per gram of dried extract (mg GAE/g DE)[16].

2.6. Antioxidant activity

The antioxidant activity of the ethanol extract of *W. ovata* roots and positive controls (Trolox and vitamin C) were estimated

according to Brand-Williams *et al.*[17]. Aliquots (300 µL) of ethanol extract and standards at various concentrations (0.1, 1.0, and 10.0 µg/mL) were mixed with 2700 µL of DPPH. The mixture was shaken and stored at room temperature for 30 min in the dark. The absorbance (ABS) of the reactive solution was measured by spectrophotometric method at 517 nm. The percentage of antioxidant activity of the samples was calculated according to the equation:
% Antioxidant activity = [(ABS control – ABS sample)/ABS control] × 100

2.7. Analgesic effect

2.7.1. Writhing test

A randomized selection of six animals of six groups each were selected. Acetic acid (0.6% v/v) was administered by intraperitoneal route (*i.p.*) in a volume of 1 mL/100 g body weight. Control group (distilled water), diclofenac (5 mg/kg) and *W. ovata* at the doses of 50, 150, 300 and 500 mg/kg were administered orally 1 h previous to acetic acid injection. After that, each animal was placed in an individual glass box. The number of abdominal writhes was recorded for 30 min[18].

2.7.2. Formalin induced paw licking test

All mice were transported to the acclimatization chamber 10 min previous to the experiment. Then, mice were divided into six groups of six animals each. *W. ovata* (50, 150, 300 and 500 mg/kg; *p.o.*), control group (distilled water; *p.o.*) and morphine (10 mg/kg; *i.p.*) were given 30 min before carrying out the test. Then, each animal was injected with 20 µL of formalin by intraplantar route. Two phases: 0–5 min and 15–30 min post-formalin injection were measured. The time spent in licking/biting the injected paw was counted[19].

2.8. Statistical analysis

All the parameters were given as mean ± SEM. Values were analyzed by One-way ANOVA, followed by Tukey's test. Comparisons were considered significant when $P < 0.05$.

3. Results

3.1. Qualitative phytochemical screening of *W. ovata*

Table 1 shows the phytochemical constituents present in *W. ovata* roots. Preliminary phytochemical screening of *W. ovata* indicated the presence of various classes of secondary metabolites except alkaloids.

Table 1

Phytochemical constituents of the ethanol extract from *W. ovata* roots.

Constituents	Test	Result
Alkaloids	Mayer	–
	Dragendorff	–
	Wagner	–
Flavonoid	Shinoda	+
Quinone	Bornträger	+
Phenolic compounds	Ferric chloride	+
Saponins	Frothing	+
Terpenoids and steroids	Liebermann-Burchard	+
Tannins	Gelatin	+

+: Positive; –: Negative.

3.2. Determination of TPC

The ethanol extract was composed of a high quantity of total phenolic content with (2200 ± 23) mg GAE/g DE. The calibrated curve was: $y = 0.0709x + 0.2104$ and $R^2 = 0.9998$.

3.3. Antioxidant activity

The antioxidant activities of three different concentrations (0.1; 1.0; 10.0 µg/mL) were compared with trolox and vitamin C. The IC₅₀ value of *W. ovata* was (3.50 ± 0.02) µg/mL. The extract was significant ($P < 0.01$; Tukey's test) at concentrations for 0.1 µg/mL and 1.0 µg/mL (Figure 1).

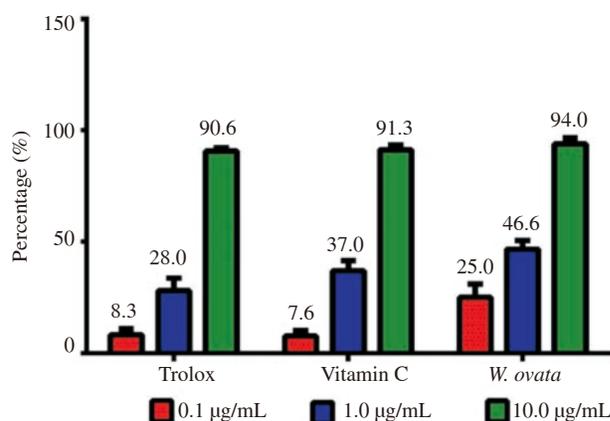


Figure 1. Antioxidant activity of the ethanol extract from *W. ovata* roots. Each bar presents the percentage of inhibition for DPPH radical.

3.4. Acetic acid-induced writhing test

Figure 2 shows that *W. ovata* presented a significant inhibitory effect on the number of abdominal writhes in each group, the highest reduction (34.83 ± 3.54) with an inhibition of 36.8% was observed at the dose 300 mg/kg ($P < 0.01$). The standard drug (diclofenac; 5 mg/kg) produced a reduction of 90.50 ± 2.88 with an inhibition of 72.5% ($P < 0.05$) compared to the control group (distilled water).

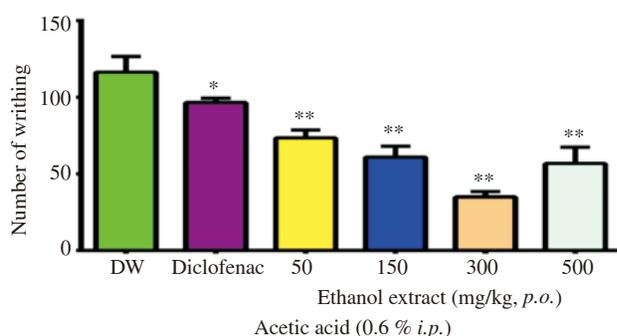


Figure 2. Effect of the oral administration of the ethanol extract from *W. ovata* roots on the acetic acid-induced writhing in mice. Each bar presents the data as mean ± SEM ($n = 6$). *: $P < 0.05$; **: $P < 0.01$, indicating statistically significant difference compared to the control group. DW: Distilled water; Data were analyzed using One-way ANOVA followed by Tukey's test.

3.5. Formalin induced paw licking

The extract reduced significantly the number of licks on the injected paw in the early phase with a significant inhibition (87.33 ± 7.06, $P < 0.01$) and a percentage of reduction of 58.6%, observed at the dose 300 mg/kg. The effect was active significantly (40.0 ± 9.96, $P < 0.01$) with an inhibition of 92.5% at the dose 300 mg/kg in the second phase. While morphine was significant at the first and second phase (29.17 ± 7.36; 31.17 ± 6.68) which was 73.2% and 91.8% respectively (Figure 3A, B).

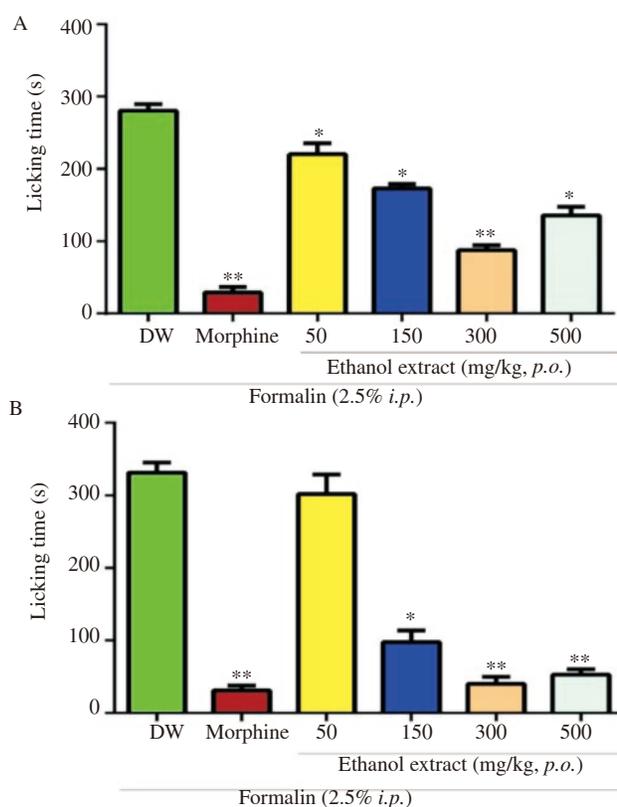


Figure 3. Effects of the oral administration of the ethanol extract from *W. ovata* roots on the first (A) and second (B) phase of the formalin-induced pain in mice. Each bar presents the mean ± SEM ($n = 6$). *: $P < 0.05$; **: $P < 0.01$, indicating statistically significant difference compared to the control group. DW: Distilled water. Data were analyzed using One-way ANOVA followed by Tukey's test.

4. Discussion

Free radicals such as oxygen singlet, superoxide and hydroxyl are produced in normal metabolism but the imbalance of redox system is involved in many diseases like rheumatic joint inflammation, Alzheimer disease, Parkinson, diabetes, hypertension, atherosclerosis and stress[20].

It is well documented that many plants with high contents of phenolic compounds (polyphenols, flavonoids and tannins) evidence a strong antioxidant activity[21]. Even though, the trolox and vitamin C have always shown a strong effect in antioxidants test. *W. ovata* was more effective than those antioxidants standards (Figure 1) and various factors like stereo-selectivity, solubility of the extract, polarity of the solvent, functional groups present in phytochemical compounds could be able to increase the reaction with DPPH radical[22].

Different studies were related to the analgesic, anti-inflammatory and antioxidant activity with flavonoids by interacting with prostaglandins and superoxides[23]. It has been demonstrated that a single dose of acetic acid by intraperitoneal route releases histamine, serotonin, prostaglandins or by direct stimulation of acid sensitive receptors[24]. In regard to the mechanism, the secondary metabolites present in *W. ovata* extract on writhing test may interfere with inflammatory mediators or the central nervous system by blocking the pain transmission signals (Figure 2). Furthermore, it could modulate the production of mediators or enzymes during an inflammatory event. Acetic acid is a chemical agent with a non-specific anti-nociceptive mechanism and several drugs with different pathways could act on this experimental model[25].

Formalin test was used to determine if the ethanol extract could be involved in the first or second phase to relieve pain. An injection of formalin into the dorsal hind paw produces a biphasic nociceptive

response by inhibiting the neurogenic response (central pain) or inflammatory mediators (peripheral pain)[26]. In the early phase (first phase), substance P and bradykinin are involved and the late phase is considered an inflammatory pain because of numerous mediators such as serotonin, histamine, and prostaglandin[27]. In this research, *W. ovata* showed an effect in both phases and might disrupt the neurogenic and inflammatory mediators. Furthermore, the minimum dose that decreased the number of licks was 150 mg/kg in both phases, but at 300 mg/kg was significant compared to control group (Figure 3A, B).

Morphine is a prototype drug that is prescribed for the treatment of strong pain and its mechanism is produced by activation of opioids receptors. On the other hand, diclofenac is a non-steroidal anti-inflammatory that inhibits the synthesis of prostaglandin by producing the inactivation of pain by inflammatory mediators[28]. Although, the mechanism of the extract was not elucidated completely, the phytochemicals constituents like saponins, terpenoids, tannins, flavonoids could be responsible of the analgesic effect. Furthermore, it might interfere with enzymes keys, stabilization of the cell membrane, ionic channels, or others cellular components or by disrupting the oxidative chain in an inflammatory process.

In this study, we demonstrated and validated that crude ethanol extract of *W. ovata* root has antioxidant activity as well as a high phenolic content and its analgesic effect in mice. These effects could be attributed to the synergic effect of the phytochemical components, therefore, it can serve as a coadjutant natural medicine for the pain management and oxidative stress associated with chronic inflammation.

Conflict of interest statement

We declare that we have no conflict of interest.

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