PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (8) 1881-1888 August 2023

Evaluation of Antitussive, Expectorant and Analgesic Activities of Aqueous Extracts of Di-herbal Formulation of Whole Plant of *Euphobia hirta* and *Lactuca virosa* Leaf on Rodents

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ABSTRACT; *Euphorbia hirta* and *lactuca virosa* are used to treat various ailment traditionally. This study aims at evaluating the antitussive, expectorant and analgesic effects of aqueous extracts of di-herbal formulation of *Euphobia hirta* and *Lactuca virosa* leaf. Citric acid induced, ammonia induced cough and phenol red dye secretion models were used. Hot plate and acetic acid induced writhing were used for analgesia. Combined 100mg/kg *Euphorbia hirta* and 50mg/kg *lactuca virosa*, 100mg/kg *Euphorbia hirta* and 100mg/kg *lactuca virosa*, 100mg/kg *lactuca virosa* only and 25 mg/kg of codeine phosphate reduces the number of cough bouts in the citric acid induced cough (P<0.05) compared to control. Combined 50mg/kg *Euphorbia hirta* and 100mg/kg *lactuca virosa*,100mg/kg *Euphorbia hirta*, 100mg/kg *lactuca virosa* and codeine phosphate reduces the number of cough bouts in the ammonia induced cough (P<0.001; P<0.0001) compared to the control. Combined 50mg/kg *Euphorbia hirta* and 100mg/kg *lactuca virosa*, 100mg/kg *Euphorbia hirta* and 50mg/kg *lactuca virosa*, 100mg/kg *Euphorbia* hirta and 100mg/kg *lactuca virosa* and 15mg/kg of Bromo-hexane increases the secretion of dye (P<0.0001; p<0.05) compared to control in phenol red dye secretion in mice. 100mg/kg *Euphorbia hirta* and 50mg/kg *lactuca virosa*,100mg/kg of *Euphorbia hirta* and 100mg/kg *lactuca virosa* and 3mg/kg Pentazocin increases the latency of pain threshold of mice (P<0.05). All the doses of the extract including 3mg/kg pentazocin increases the latency to pain threshold in the mice (P<0.001) after 2 hours. All the doses of the extract including aspirin reduces number of writhing (P<0.01). *Euphorbia hirta* and *lactuca virosa* has antitussive, expectorant and analgesic properties.

DOI: https://dx.doi.org/10.4314/jasem.v27i8.35

Cite this paper as: UWAYA, DO; BELLO, AK; AIKPITANYI, I. (2023). Evaluation of Antitussive, Expectorant and Analgesic Activities of Aqueous Extracts of Di-herbal Formulation of Whole Plant of *Euphobia hirta* and *Lactuca virosa* Leaf on Rodents. *J. Appl. Sci. Environ. Manage.* 27 (8) 1881-1888

Dates: Received: 10 July 2023; Revised: 25 July 2023; Accepted: 14 August 2023 Published: 30 August 2023

Keywords: *Euphorbia hirta*; *lactuca virosa*; anti-tussive; analgesic; inflammation.

Plants have at all times remained connected in our life both as a source of vegetables or medicine (Dafam *et al.*, 2016). Studies have shown that vegetables offer a noble source of remedy against numerous diseases and illnesses (Dansi *et al.*, 2008). These medicinal plants are rich sources of ingredients which can be used in drug development and synthesis (Chekole, 2012). Numerous plants, including *Euphobia hirta* and *Lactuca virosa* have been used in herbalism because of their medicinal activities. *Euphorbia hirta* Linn is usually called asthma weed plant and spurge plant. It belongs to family Euphorbiaceae. In Nigeria, the yorubas called it "Emi-ile or Egele", the Igbos called it "Odane inenemii" and Hausas called it "Nononkurchiya". *Euphorbia hirta* is a small annual herb seen occupying open waste spaces, roadsides, grasslands, pathways and rice field as a weed (Burkill, 1994). The plant is used in traditional medicine to treat a variety of diseases such as respiratory diseases (cough and asthma), virus diseases, and gastrointestinal disorders, wound healing, pain, inflammation, pulmonary disorders, urogenital

disorders, tumors and lactation in women (Johnson *et al.,* 1999; Lanhers *et al.,* 1990). Researched have shown the plant possesses anti-inflammatory, antifungal, anti- bacterial, antidiarrheal, sedative, anxiolytic, analgesic, antipyretic, antioxidant, anti-asthmatic, anti-tumor, anti-diabetic, antiviral, anti-helmentic anti-allergic, anti-anaphylactic, anti-arthritic, spasmogenic, anti-thrombocytopenic anti-malarial, larvicidal, diuretic, and increases electrolytes (Basma *et al.,* 2011; Kumar *et al.,* 2010; Shil *et al.,* 2011; Martinez *et al.,* 1999; Sharma *et al.,* 2007; Brindha *et al.,* 2010; Chandrakant, 2011). *Lactuca virosa* commonly called wild lettuce and opium lettuce belongs to family Asteraceae. It is called "Yanri" by the Yorubas, "Ugu" by the Igbos and "Nonan-Barya" by the Hausas in Nigeria. (Darkwa and Darkwa, 2013). The plant is used for treating coughs, asthma, insomnia, nervousness, muscles spasm or joint pains, colic pains, painful menstruation, painful digestion, fevers and used as a mild sedative in traditional medicine (Salau, 2015; Arawande *et al.,* 2013; Koukoui, 2015). Research has shown that leaf of *Lactuca virosa* possesses antioxidant activities, brain protective effect, anticancer effects, anti-malaria activity, cardio-protective effect, anti-microbial activity, DNA protective ability, hypo-lipidaemic action, anti-lipidperoxidation, neuro-protective, anti-cancer, anti-arthritic and anti-inflammatory properties (Tayman *et al.,* 2013; Koukoui *et al.,* 2015; Salau, 2015; Salisu *et al.,* 2014; Sanoussi *et al.,* 2015; Koukoui *et al.,* 2017; Owoeye and Onwuka,2016; Thomford *et al.,* 2016; Owoeye and Arinola, 2017; Bello *et al.,* 2017; Ololade *et al.,* 2017; Adinortey *et al.,* 2018). The aim of this study was to evaluate the anti-tussive and analgesic activities of the aqueous extract of di-herbal formulation of whole plant of *Euphobia hirta* and *Lactuca virosa* leaf.

MATERIALS AND METHODS

Plant Collection: Euphobia hirta plant was collected from the neighborhood of the University of Benin in Ovia North East Local Government Area, Edo State, Nigeria. *Lactuca virosa* plant was collected from Ife-East Local Government Area, Ile-ife, osun state, Nigeria. The two plants were identified and authenticated by Dr. H. A. Akinnibosun of the Department of Plant biology and Biotechnology, Faculty of life Sciences, University of Benin City, Edo State, Nigeria.

Plant Preparation: Euphobia hirta plant was washed and air dried for 14 days in the Department of Science Laboratory Technology, University of Benin, Benin City. The plant was grinded into powder using an impact mill. The powdered plant material was macerated for 24hr after which filtration was done. *Lactuca virosa* leaves were washed and chopped. The chopped leaves were blended with distilled water and filtration was done. The filtrate of both plants were then freeze dried using a freeze dryer with model no LI- LYFO-55 at the Energy Centre, University of Benin.

Experimental Animals: Mice of either sex weighing 20-35 g were purchased from a commercial animal house in Ibadan. Guinea pigs of either sex weighing 200-500g were purchase from the animal house, Department of Pharmacology, Ambrose Ali University, Ekpoma, Edo State, Nigeria. All the animals were acclimatized for two weeks in the animal facility of the Department of animal and environmental biology, Faculty of Life Sciences, University of Benin, Benin City. They were allowed to have free access to pellets and tap water and were exposed to natural light-dark cycle and room temperature. All animals were handled according to standard protocols for the use of Laboratory animals.

Experimental Procedure

Antitussive Effect: The anti-tussive studies were carried out using Citric acid induced cough model in guinea pigs and Mucus expectorant model according to (Ozolua *et al.,* 2012; Salami *et al.,* 2013), Ammonia induced cough model on mice according to (Xu *et al.,* 2005).

*Citric Acid-Induced Cough in Guinea Pigs***:** The guinea-pigs were pre-screened for the experiment and this was done by putting them in a Perspex box (24 × 12 × 24 cm) and exposed with 7.5% citric acid aerosol through an ultrasonic nebulizer for 5 minutes. Cough was detected with a characteristic sound and by stretching of limbs accompanied by inspiration and then expiration. The guinea pigs were observed for cough reflexes and the numbers of coughs were counted. Animals that gave 10 cough bouts and above were used for the test. Selected animals were then fasted overnight with water. After overnight fasting, the selected guinea pigs were divided into 7 groups with 4 animals each in a group and were treated as follows: Group 1 was the normal control and was administered 2 ml/kg of distilled water. Groups 2 was administered 50 mg/kg of EH and 100 mg/kg of WL, Group 3 was administered 100 mg/kg of EH and 50 mg/kg of WL, Group 4 was administered 100 mg/kg of EH and 100 mg/kg of WL, Group 5 was administered 100 mg/kg of EH, Group 6 was administered 100 mg/kg of WL and Group 7 was the positive control and was administered 25 mg/kg of codeine phosphate (CP). All administrations were done orally. 1hour after the administration of the extract, distilled water and the standard drug, all the

animals were re-exposed to the citric acid aerosols and the number of cough bouts were counted again. The percentage cough suppression was calculated for each animal as:

Percentage Cough Suppression (C1-C2)/C1) × 100 (where, C1 = number of cough bouts before drugs administration and C2 = number of coughs after drugs administration

Ammonia induce cough in mice: Twenty-eight mice weighing 20-30g were divided into seven groups with 4 mice in each group and were treated as follows:
Group 1 received distilled water (10 ml/kg), Group 2 received standard (25 mg/kg of codeine phosphate),
Group 3 received 50 mg/kg of WL and 100 mg/kg of EH, Group 4 received 50 mg/kg of EH and 100 mg/kg of WL, Group 5 received 100 mg/kg of EH and 100 mg/kg of WL, Group 6 received 100 mg/kg of EH and Group 7 received 100 mg/kg of WL.

All administration was given orally. After 1hour of administration, each mouse was placed in a 1000 ml diameter special chamber embedded with cotton wool and exposed to 25% NH_3OH for 45s. Mice was taken out and put in a chamber with an opening at the top and cough frequency was counted for 5 minutes and the antitussive activity was assessed as the percentage of inhibition of the number of coughs (Xu *et al.,* 2005).

Phenol Red Mucus Expectorant: Thirty-two mice of either sexes weighing 20-30g were grouped into 8 groups with 4 mice in each group and were treated as follows:

Group 1 received distilled water (2 ml/kg), Group 2 received 50 mg/kg of EH and 100 mg/kg of WL, Group 3 received 100 mg/kg of EH and 50 mg/kg of WL, Group 4 received 100 mg/kg of EH and 100 mg/kg of WL; Group 5 received 100 mg/kg of EH,
Group 6 received 100 mg/kg of WL, Group 7 received 15 mg/kg of bromo-hexane hydrochloride and Group 8 received 50 mg/kg of sodium cromoglycate.

All treatment was administered orally for seven days except for sodium cromoglycate that was administered intra-peritoneally only on the 8th day. After an overnight fasting, on the 8th day treatment was done as usual and the animals in group 8 were given 50mg/kg (IP) of sodium cromoglycate 30 minutes before the oral administration of the secretagogue, ammonium chloride (5 mg/kg). 30minutes later, each mouse was injected with phenol red (500 mg/kg) intra-peritoneally. All the mice were sacrificed by cervical dislocation 30 minutes after phenol red injection and their trachea was removed. Each trachea was kept for 30 minutes in 2 ml normal saline. 0.1ml of 1M Sodium hydroxide was added to the fluid to stabilize the pH. The absorbance of phenol red released from the trachea was read at 460 nm using a spectrophotometer. A standard curve (graph of absorbance against concentration) was plotted from which the concentrations of phenol red were extrapolated, r^2= 0.999 (Ozolua *et al.,* 2012).

Analgesic Activity of the Aqueous Extract of Euphobia hirta and Lactuca virosa Plants: The analgesic studies were carried out using hot plate method in mice according to (Badilla *et al.,* 2003) and Acetic acid writhing in mice according to (Akor *et al.,* 2015).

Hot Plate Method: Twenty-eight mice weighing 20-35g of both sexes were screened for suitable reaction time, 24 hours before the experiment by maintaining the hot plate temperature at 55± 1^{o}C. Licking, biting of the hind paw or jumping was taken as a sign of pain perception. The animals were divided into 7 groups of 4 mice each and were treated as follows: Group 1 was the control and was administered distilled water (2 ml/kg), Group 2 was treated with 50 mg/kg of EH and 100 mg/kg of WL, Group 3 was treated with 100 mg/kg of EH and 50 mg/kg of WL, Group 4 was treated 100 mg/kg of EH and 100 mg/kg of WL, Group 5 was treated with 100 mg/kg of EH, Group 6 was treated with 100 mg/kg of WL and Group 7 treated with the standard drug and was administered Pentazocine (3 mg/kg).

All administration was done orally except for the pentazocine which was administered intraperitoneally. 30minute later, each animal was placed on the hot plate and the index of the response latency (time between placement and licking, biting the hind paws or jumping) was recorded. Response latencies were taken at 30, 60 and 90 and 120 minutes after treatment and the reaction time was recorded (Badilla *et al.,* 2003)

Acetic Acid-Induced Writhing in mice Twenty-eight mice weighing 20-35g of both sexes were divided into 7 groups with 4mice per group were treated as follows: Group 1 was the control and was administered distilled water (10 ml/kg orally), Groups 2 was treated with 50 mg/kg of EH and 100 mg/kg of WL, Group 3 was treated with 100 mg/kg of EH and 50 mg/kg of WL, Group 4 was treated with 100 mg/kg of EH and 100 mg/kg of WL, Group 5 was treated with 100 mg/kg of EH, Group 6 was treated with 100 mg/kg of WL, Group 7 was treated with the standard and was administered aspirin (100 mg/kg). All administration was done orally. 1hour later after the administration, 0.1ml of 0.6% acetic acid was injected intra-

peritoneally to each mouse. Number of writhing, which comprised constriction of the abdominal muscle together with a stretching of the hind limbs was counted for 30 minutes following acetic acid injection. Inhibition of pain was expressed as a percentage of protection:

$$\% IP = \frac{MW_{Control} - MW_{treated}}{MW_{control}} x\ 100$$

IP = Inhibition of pain; ME $_{(control);}$ = Mean writhing control; $_{MW\ (control)\ =}$ Mean writhing for treatment
KEY: EH: *Euphorbia hirta:* WL: *Lactuca virosa*

Where Mean writhing $_{(control)}$ is the mean writing of the distilled water treated animals and Mean writhing $_{(treated)}$ is the mean writhing of the animal given the standard drug or each dose of leaf extract (Akor *et al.*, 2015).

Statistical Analysis: Data were expressed as mean ± standard error of mean (SEM) and 'n' represents the number of guniea pigs or mice per experimental group. One-way analysis of Variance (ANOVA) were performed with Newman Keuls' post hoc test. All data were analyzed using Graph Pad Prism (UK) software version 6. P<0.05 shows a significant difference between compared data.

RESULTS AND DISCUSSION

Antitussive effects: Citric acid induced cough in guinea pigs result: Euphobia hirta (EH) and *Lactuca virosa* (WL) at doses (100mg/kg of EH and 50mg/kg of WL, 100mg/kg of EH and 100mg/kg of WL, 100mg/kg of EH alone and 100mg/kg of WL alone) significantly reduces cough bouts (**P<0.01) and codeine phosphate (25mg/kg) significantly reduces cough bouts (*P<0.05) when compared to control (Table 1).

Table 1: The effect of aqueous extract of *Euphobia hirta* and *Lactuca virosa* on citric acid induced cough in guinea pigs.

Groups (mg\kg)	Coughbouts Before	Coughbouts After	%Cough Suppression
CONTROL	16.50±2.40	17.50±3.86	-3.42±12.87
50EH+100WL	16.75±2.78	11.00±2.74	35.77±11.46
100EH+50WL	16.50±2.50	6.50±1.32*	60.09±7.64**
100EH+100WL	16.50±2.63	7.25±1.34*	55.87±5.98**
100EH	18.00±3.63	8.50±1.04	48.01±9.44**
100WL	6.00±2.16	7.50±1.26*	55.66±6.90**
CP (25mg/kg)	16.00±2.08	8.50±0.96*	45.21±7.12*

*Combine 100mg/kg of EH and 50mg/kg of WL, 100mg/kg of EH and 100mg/kg of WL, 100mg/kg of EH alone and 100mg/kg of WL alone increase percentage cough suppression (**P<0.01) and Codeine Phosphate also increase percentage cough suppression (*P<0.05) compared to control. EH: Euphobia hirta, WL: Lactuca virosa, CP: Codeine Phosphate. Data are represented as Mean ± SEM, n = 4.*

Ammonia induced cough in mice result: Combine 50mg/kg of EH and 100mg/kg of WL, 100mg/kg of EH and 100mg/kg of WL protected the animals against cough ($^{\$}$P<0.001), combined 100mg/kg of EH and 100mg/kg of WL also protected the animals against cough (**P<0.01), 100mg/kg of EH + 50mg/kg of WL also gives protection against cough (*P<0.05) likewise Codeine Phosphate protected the animals against cough bouts ($^{\#}$P<0.0001) when compared to the control (figure 3)

*Phenol Red Mucus Expectorant in Mice result***:** The result showed that *Euphobia hirta* and *Lactuca virosa* extracts at doses (50mg/kg of EH and 100mg/kg of WL) and Bromo-hexane (15mg/kg) increases phenol red dye secretion ($^{\#}$P<0.0001), 100mg/kg of EH+ 50mg/kg of WL, 100mg/kg of EH+ 50mg/kg of WL increases phenol red dye secretion (*P<0.05) when compared to control (figure 4).

Analgesic Effects: Hot Plate Method Result: The result shows that the extracts at doses (50mg/kg of EH and 100mg/kg of WL) significantly increases the latency of pain threshold in the mice (P<0.001), also at doses (100mg/kg of EH+ 50mg/kg of WL, 100mg/kg of EH, 100mg/kg of WL) and the standard drug Pentazocine (3mg/kg) significantly increases the latency of pain threshold in the mice (P<0.01) when compared to control (Table 2).

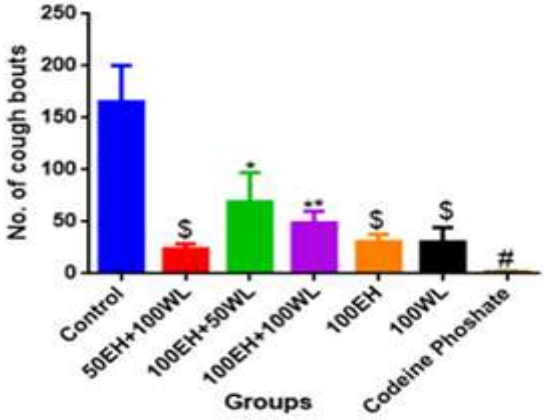

Fig 3: The effect of aqueous extract of *Euphobia hirta* and *Lactuca virosa* on ammonia induced cough in mice. Combine 50mg/kg of EH and 100mg/kg of WL, 100mg/kg of EH and 100mg/kg of WL reduced the number of cough bouts($^{\$}$P<0.001) combined 100mg/kg of EH and 100mg/kg of WL reduced the number of cough bouts(**P<0.01), 100mg/kg of EH+ 50mg/kg of WL reduced the number of cough bouts(*P<0.05) and Codeine Phosphate reduced the number of cough bouts($^{\#}$P<0.0001) compared to control. Data are represented as Mean ± SEM, n = 4.

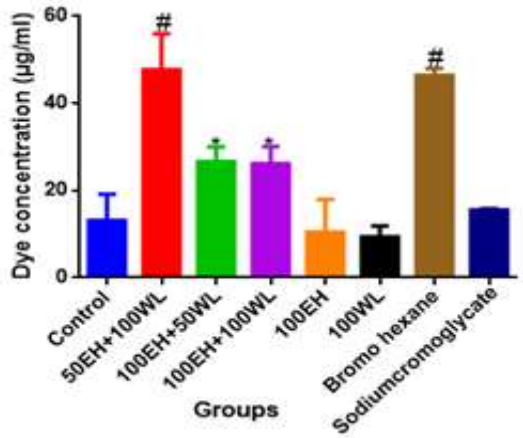

Fig 4: The effect of aqueous extract of *Euphobia hirta* and *Lactuca virosa* on phenol red Mucus expectorant model in mice. Combine 50mg/kg of EH and 100mg/kg of WL and Bromo-hexane increases phenol red dye secretion ($^{\#}P<0.0001$), 100mg/kg of EH+ 50mg/kg of WL, 100mg/kg of EH+ 50mg/kg of WL increases phenol red dye secretion (*P<0.05) compared to control. Data are represented as Mean ± SEM, n = 4

Acetic acid induced writing in mice result: The result showed that the extract at doses (50mg/kg of EH and 100mg/kg of WL) significantly reduces the number of writhing in the mice (P<0.001). Also at doses (100mg/kg of EH+ 50mg/kg of WL, 100mg/kg of EH, 100mg/kg of WL) and the standard drug Aspirin (100mg/kg) significantly reduces the number of writhing (P<0.01) compared to control (Table 3).

Euphobia hirta and *Lactuca virosa* plants have been used traditionally for years in the treatment of cough, asthma, inflammation, pain and respiratory disorders (Salau, 2015). In citric acid induced cough, the aqueous extract of *Euphobia hirta* and *Lactuca virosa* plants reduced the cough bouts in the guinea pigs (Table 1). As a tussigenic agent when inhaled, citric acid stimulates transient receptor potential on the C-fibers, this then causes the release of tachykinnins to mediate bronchoconstriction and mucus secretion which stimulates rapidly adapting receptors (RAR), a widely studied cough receptor (Myers *et al.*, 2002; Canning *et al.*, 2001). Combined 100mg/kg of *Euphobia hirta* and 50mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta* and 100mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta* alone and 100mg/kg of *Lactuca virosa* alone and the standard drug, codeine phosphate increases percentage cough suppression (Table 1). The extracts exhibited antitussive effect by increasing the percentage of cough suppression and reduces the number of cough bouts similar to the standard drug codeine phosphate (Table 1).

Table 2: The effect of aqueous extract of *Euphobia hirta* and *Lactuca virosa* on Hot plate induced pain in mice.

Groups (mg\kg)	Reacting Time (Min.)				%Pain Inhibition
	30	60	90	120	
CONTROL	7.50±0.65	8.25±0.85	6.75±0.75	9.75±0.48	___
50EH+100WL	18.75±1.65	16.25±1.89	16.75±1.11	19.25±1.49*	49.35
100EH+50WL	17.50±2.22	20.50±1.56**	20.50±3.00**	19.25±2.78*	49.35
100EH+100WL	17.75±0.85	18.00±1.47	21.00±1.47**	21.75±0.48**	55.17
100EH	19.25±1.80	22.25±3.71**	24.25±2.43***	24.00±2.68***	59.38
100WL	20.00±1.00	18.00±2.35	20.50±2.72**	22.50±2.18***	56.67
PENTAZOCIN	18.50±2.02	18.00±1.87	22.25±3.04**	24.00±1.47***	59.38

Combine 50mg/kg of EH and 100mg/kg of WL increases the latency of pain threshold in the mice (P<0.001), 100mg/kg of EH+ 50mg/kg of WL, 100mg/kg of EH, 100mg/kg of WL and Pentazocine (P<0.01) increases the latency threshold in the mice compared to control. Data are represented as Mean ± SEM, n = 4.

Table 3: The effect of aqueous extract of *Euphobia hirta* and *Lactuca virosa* on acetic acid induced writhing in mice.

Groups (Mg\kg)	Number Of Writhing	% Pain Inhibition
CONTROL	102.80±2.40	__
50EH+100WL	43.50±11.84***	57.68
100EH+50WL	55.25±5.84**	46.25
100EH+100WL	66.50±4.56*	35.31
100EH	51.75±3.57**	49.66
100WL	50.00±5.58**	51.36
ASPIRIN	53.25±6.54**	48.20

Combine 50mg/kg of EH and 100mg/kg of WL reduces the number of writhing in the mice (P<0.001), 100mg/kg of EH+ 50mg/kg of WL, 100mg/kg of EH, 100mg/kg of WL and Aspirin (P<0.01) reduces the number of writhing compared to control. Data are represented as Mean ± SEM, n = 4. ***KEY:*** *EH is Euphorbia hirta; WL is Lactuca virosa*

In ammonia induced cough model in mice, both the aqueous extract of *Euphobia hirta* and *Lactuca virosa* plants and the standard drug reduced the cough bouts in the mice. Combined 50mg/kg of *Euphobia hirta* and 100mg/kg of *Lactuca virosa*, combined 100mg/kg of *Euphobia hirta* and 100mg/kg of *Lactuca virosa*, combined 100mg/kg of *Euphobia hirta* + 50mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta* alone, 100mg/kg of *Lactuca virosa* alone and the standard drug Codeine Phosphate, reduced the number of cough bouts. The extracts of the two plants reduces the number of cough bouts and increases the percentage

cough suppression in the mice at all doses (table 1). Codeine phosphate is an opioid drug that belongs to the morphine family. It shows its antitussive effects by binding to the μ-receptors in the central nervous system and thus suppresses the cough reflex through a direct effect on the cough center in the medulla (Saraswathy *et al.,* 2008). To determine the expectorant activity of the aqueous extracts of *Euphobia hirta* and *Lactuca virosa,* the trachea phenol red secretion assay was used. This model is developed on a principle that when phenol red is injected after an expectorant is given for seven consecutive days. There will be enhancement of phenol red secretion from the trachea. Both the aqueous extract of *Euphobia hirta* and *Lactuca virosa* plants and the standard drug bromo hexane increase the phenol red secretion in mice and this was enhanced by the ammonium chloride. This implies that it helps in the secretion of more mucus from the airway. Bromo-hexane is an expectorant that acts on the gastric mucosa to stimulate the vagus nerve and thus making mucus less thick, sticky and easier to cough up. It also breaks down the chemical bonds between the molecules in the mucus, this in turns lowers the viscosity by altering the mucin containing components (Donaldson *et al.,* 2006; Daviskas *et al.,*2005). The antitussive and expectorant effects of the aqueous extract of *Euphobia hirta* and *Lactuca virosa* plants has been traced back to their traditional uses in the treatment of cough (Johnson *et al.,* 1999). Researchers have shown that both *Euphobia hirta* and *Lactuca virosa* plants contains alkaloids, flavonoids, tannins, saponins, triterpenoids, glycosides and phenols which are the secondary metabolites present in plants that give plants their medicinal and therapeutic effects (Xu *et al.,* 2006). These secondary metabolites also possess antioxidant properties. Antioxidant are substances that helps to scavenge and mop up free radicals which are caused by oxidative stress and in turns causes several deadly diseases including ageing, cardiovascular diseases cancer and neurodegenerative disease (Soforowa, 1982). Alkaloids have been reported to possess a marked antitussive and expectorant activities for the treatment of cough (Wang *et al.,* 2012). Inflammation also plays an important role in a cough. Flavonoids, triterpenoid also helps in the antitussive and expectorant property of plants extracts because of their anti-inflammatory action (Brightling *et al.,* 2000). The mechanism of action of the extracts may be due to the presence of alkaloids, flavonoids and triterpenoid in the plants (Hazimi *et al.,* 2008; Salau, 2015). The analgesic effect of the aqueous extracts of *Euphobia hirta* and *Lactuca virosa* were investigated using hot plate method and acetic acid induced writhing in mice. In hot plate method, both the aqueous extract of *Euphobia hirta* and *Lactuca virosa a*nd the standard drug pentazocine increases the latency of pain in the mice (Table 2). Combined 50mg/kg of *Euphobia hirta* and 100mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta* + 50mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta* alone, 100mg/kg of *Lactuca virosa* alone the standard pentazocine increases the latency of pain threshold in the mice. The extracts of the two plants increases the latency of the pain in the mice as the standard drug pentazocine (Table 2). The increase in the pain threshold produced by the extracts suggest the extracts involvement in central pain pathway which might have involved several complex processes including opiate, dopaminergic descending noradrenergic and serotonin system in the central nervous system (Cena *et al.,* 2003). Pentazocine is an opoid drug that produces analgesic effect by binding to the k- receptors in the central nervous system (DeHaven-Hudkins and Dolle, 2004). In the acetic acid induced writhing in mice, both the aqueous extract of *Euphobia hirta* and *Lactuca virosa a*nd the standard drug aspirin reduces the number of writhing in the mice. Combined 50mg/kg of *Euphobia hirta* and 100mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta* + 50mg/kg of *Lactuca virosa*, 100mg/kg of *Euphobia hirta*, 100mg/kg of *Lactuca virosa* and the standard drug aspirin reduces the number of writhing in the mice and thus increases the percentage pain inhibition in the mice. The effects of the extracts of the two plants at all doses were found to be similar as that of the standard drug aspirin (Table 3). Acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins, bradykinnins and substance P, all of which are pain mediators (Cena *et al.,* 2003). Aspirin is a non-steroidal anti-inflammatory drug that acts by inhibiting the cyclooxygenase enzymes. This enzyme is needed in the arachidonic pathway for the production of prostaglandins and bradykinnins which are pain and inflammatory mediators (Camuesco *et al.,* 2004). The ability of the plants extracts to increase the pain threshold and inhibit number of writhing shows that *Euphobia hirta* and *Lactuca virosa* are both centrally and periperally acting pain reliever. In anti-tussive studies, the plants extracts seem to suppress cough as codeine phosphate which is an opioid receptor agonist. Also, in analgesic studies, the plants extracts seem to also behave like pentazocine which is also an opioid agonist. This suggest that the mechanism of action of the plants extracts may be due to their interaction with the opioid receptors or by inhibiting the cyclooxygenase enzymes. In addition, *Euphobia hirta* and *Lactuca virosa* are good antitussive since they suppress cough, possess expectorant ability and analgesic activities. This study supports the use of these plants in the treatment of cough and pain in ethno-medicine.

Conclusion: This study shows that aqueous extract of *Euphobia hirta* and *Lactuca virosa* has antitussive, expectorant and analgesic activities. However, the bioactive components of these plants should be explored and also more research is needed to further prove other probable mechanism of action by which these plants carry out their effects.

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