Nigerian Journal of Pharmaceutical and Applied Science Research, 9(2): 40-46, May 2020 (ISSN 1485-8059. Available at www.nijophasr.com)

Comparative study on anti-inflammatory and analgesic effects of the leaf, stem and root of *Dracaena arborea* (Wild) Linn. (Asparagaceae)

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ABSTRACT

Dracaena arborea is one of the plants used in Akwa Ibom State ethnomedicine to treat pain and inflammatory related diseases. This research was structured to study the anti-inflammatory and analgesic abilities of the leaf, stem and root of *D. arborea.* The leaf, stem and root of *D. arborea* plant were collected, garbled, dried, pulverized, macerated with 70% ethanol for 72 hours, filtered and concentrated. The LD_{50} of the extracts was studied using the method described by Lorke; anti-inflammatory study was carried out using egg albumin-induced oedema and xylene-induced topical oedema models while analgesic study was done using acetic acid-induced writhing, formalin-induced paw licking and hot plate-induced pain models. The result of the study revealed the LD_{50} of 223.60 mg/kg, 273.86 mg/kg and 122.47 mg/kg for the leaf, stem and root extracts, respectively. Also, the leaf, stem and root extracts of *D. arborea* were able to reduce oedema caused by egg albumin and xylene; exhibited high analgesic properties in inhibiting pain induced by formalin, acetic acid and hot plate. These reductions were dose-dependent and statistically significant ($p \leq 0.05$) when compared to distilled water and similar to prototype drugs, acetyl salicylic acid and dexamethasone. The result from this study supports *D. arborea* as anti-inflammatory and analgesic agent in ethnomedicine

Keywords: Comparative, Anti-inflammatory, Analgesic and Effects

INTRODUCTION

D. arborea commonly known as African dragon tree and locally in Nigeria as okono (Ibibio), odo (Igbo), peregun (Yoruba) and akuku (Hausa) is a tree that grows up to 20 meters tall with trunk of 20 to 30 cm in diameter. They are found in semi-arid deserts and distributed throughout Canary Islands, Madeira, Cape Verde Islands, Morocco and tropical Africa (Burkill, 1985; Hodgkiss, 2012). Although pain and inflammation are separate conditions, they are nearly always associated with each other. Pain according to the International Association for the Study of Pain (IASP) is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage'. Inflammation is the tissue's immunologic response to injury, characterized by mobilization of white blood cells and antibodies, swelling and fluid accumulation (Umoh and Nwafor, 2013). The disease of pain and inflammation is a global burden associated with almost all disease conditions thereby affecting the quality of life with attendant societal and economic burdens. Reports from research have also shown that people living with pain are linked with poor health, increased morbidity and mortality (Hochberg, 2008; Torrance *et al*.,2010; McBeth *et al*., 2013). Researches have shown that the leaf of *D. arborea* is antidiabetic, enhances fertility and promotes sexual activity (Watcho *et al*., 2007; Ekere *et al*., 2013; Ogunmodede *et al*., 2015) while the leaf is reported to cause delay in the pro-ejaculatory effect of dopamine and oxytocin in spinal male rats (Watcho *et al*., 2007) . This study was designed to study the involvement of the various parts of *D. arborea* in anti-inflammatory and analgesic processes to validate its ethnomedicinal uses in the management of pain and inflammatory diseases.

MATERIALS AND METHODS

Plant Collection and Identification

The leaf, stem and root of *D. arborea* were collected from the medicinal plants farm of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria and the whole plant specimen was identified by Prof. Mrs Margaret Bassey, a taxonomist in the Department of Botany and Ecological Studies and voucher specimen (UUPHB 30 (i)) was deposited in the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria.

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Maceration of Plant Materials

The leaf, stem and root of *D. arborea* collected were washed, garbled, air dried and pulverized. The pulverized plant materials (0.5 kg each) were macerated with 70% ethanol for 72hours, filtered and concentrated using rotary evaporator to obtain their dry extracts which were preserved in a refrigerator from where they were being used for the studies.

Animal Stock

Adult albino mice weighing 20 – 30 g were obtained from the Animal House of the Department of Pharmacology and Toxicology, University of Uyo, Uyo. They were housed under standard conditions and fed with standard pellets. The animals were starved 24 hr prior to the experiments but given free access to water.

Acute Toxicity Testing

Acute toxicity study was carried out using method similar to the one reported by Lorke (1983). Twenty one albino mice were randomized and divided into groups of three mice per group. Each of the extracts of *D. arborea* was administered in a dose range of 100-500 mg/kg body weight of animals and observed for 24 hours for signs of toxicity.

Anti-inflammatory Study

Egg Albumin-induced Oedema

In this model, albino mice of either sex were randomized and divided into five groups of five animals each. Group one animals were pretreated with distilled water (10 mL/kg), groups 2 to 4 were pretreated with 22.36 mg/kg, 44.72 mg/kg and 67.08 mg/kg of the ethanol leaf extract, respectively, thirty (30) minutes before the induction of oedema with fresh egg albumin while group five animals received the standard drug acetyl salicylic acid (ASA 100 mg/kg). The linear circumference of the injected paws were assessed with venier caliper before and at thirty minutes interval for 5hours following the administration of egg albumin (Okokon *et al*., 2008). This procedure was repeated for ethanol stem extracts (27.39 mg/kg, 54.77 mg/kg and 82.16 mg/kg) and ethanol root extract (12.25 mg/kg, 24.50 mg/kg and 36.75 mg/kg).

Xylene-induced Ear Oedema

Twenty five (25) albino mice were grouped into five groups of five animals per group. They were administered with distilled water (10 mL/kg) for mice in group 1, ethanol leaf extract (22.36 mg/kg, 44.72 mg/kg and 67.08 mg/kg) for animals in groups 2 to 4 and dexamethasone 4 mg/kg for mice in group 5, thirty (30) minutes prior to the topical application of 50 microliter of xylene to the anterior and posterior surfaces of the right ears while the left ears served as control. Fifteen minutes following xylene application, the animals were sacrificed using chloroform anesthesia and both ears removed and weighed. The average weight difference between the two ears were taken as a measure of inflammatory response (Atta and Alkofahi, 1998).

Analgesic Study

Acetic Acid-induced Writhing in Mice

Albino mice of either sex were selected, divided and pretreated as earlier described in the xylene model thirty (30) minutes before the intraperitoneal injection of 2% acetic acid. Analgesic activity was expressed as the reduction of abdominal constrictions between control animals treated with distilled water and mice pretreated with the extracts (Nwafor and Okwuasaba, 2003). This procedure was repeated for ethanol stem extract (27.39 mg/kg, 54.77 mg/kg and 82.16 mg/kg) and ethanol root extract (12.25 mg/kg, 24.50 mg/kg and 36.75 mg/kg).

Formalin-induced Paw Licking in Mice

This method was similar to the one described by Nwafor and Okwuasaba (2003). Albino mice of either sex were randomized and divided into five groups of five animals each and pretreated with the ethanol leaf extract (22.36 mg/kg, 44.72 mg/kg and 67.08 mg/kg), ASA (100 mg/kg) and distilled water (10 mL/kg). Twenty microliters of 2.5% formalin solution (formaldehyde) made up in phosphate buffer was administered subcutaneously under the surface of the right hind paw. The time the animals spent in licking the injected paw was noted and taken as an indicative of pain with the first phase of response at 5 minutes and second phase (15-30 minutes) following formalin injection. This procedure was repeated for ethanol stem extract (27.39 mg/kg, 54.77 mg/kg and 82.16 mg/kg) and ethanol root extract (12.25 mg/kg, 24.50 mg/kg and 36.75 mg/kg).

Hot Plate – induced Pain

The effects of the leaf, stem and root extracts of *D. arborea* on hot plate-induced pain were investigated using adult mice. The animals were grouped and pretreated as earlier mentioned in acetic acid – induced writhing and formalin – induced paw licking models. Hot plate connected to electricity was maintained at a temperature of $45^{o}C \pm 1^{o}C$. The experimental animals were placed into a glass beaker of 50 cm diameter on the heated surface of the hot plate and the time(s) between placement and licking of the paw were recorded (Nwafor and Okwuasaba, 2003).

Statistical Analysis

Data collected were analyzed using one way analysis of variance followed by Primer multiple comparison test and a probability level of $p < 0.05$ was regarded as significant.

Ethical Issues

All animals were handled with humane care in accordance with the international accepted standard guide for the care and use of laboratory animals (1996) and as adopted and promulgated by the National Institute of Health and the related ethics regulation of Faculty of Pharmacy, University of Uyo.

RESULTS

Acute Toxicity

The median lethal doses (LD_{50}) of the leaf, stem and root extracts of *D. arborea* calculated as the geometrical mean of the maximal dose that did not kill any animal and the minimal dose that killed all the animals gave 223.60 mg/kg, 273.86 mg/kg and 122.47 mg/kg for the leaf, stem and root, respectively.

Anti – inflammatory Studies

The result of the anti – inflammatory study of the ethanol extracts of leaf, stem and root of *D. arborea* on egg albumin – induced oedema in mice hind paw as presented in Tables 1, 2 and 3.

Table 1: Effect of ethanol leaf extract of *D. arborea* on egg albumin – induced oedema in mice

	Time Interval (minutes)						
Treatments (mg/kg)	0	30	60	120	180	240	300
Distilled water	2.26±0.01	3.78±0.01	3.79±0.01	3.53±0.02	3.08±0.02	2.95±0.02	2.80±0.02
Extract 22.	2.29±0.01	3.36±0.01*	3.18±0.01*	3.12±0.02*	2.68±0.01*	2.61±0.01*	2.39±0.01*
Extract 45	2.25±0.01	3.42±0.01*	3.14±0.02*	3.09±0.02*	2.77±0.01*	2.65±0.01*	2.50±0.01*
Extract 67	2.25±0.01	3.38±0.01*	3.13±0.01*	3.05±0.01*	2.68±0.01*	2.49±0.01*	2.41±0.01*
ASA 100	2.27±0.01	3.15±0.01*	2.29±0.01*	2.78±0.01*	2.57±0.01*	2.41±0.01*	2.37±0.01*

Values are expressed as Mean ± S E M, significance relative to control *$p < 0.05$, n= 5, ASA = Acetyl Salicylic Acid

Table 2: Effect of the ethanol stem extract of *D. arborea* on egg albumin- induced oedema in mice

	Time Interval (minutes)						
Treatments (mg/kg)	0	30	60	120	180	240	300
Distilledwater	2.26±0.01	3.78±0.01	3.79±0.01	3.53±0.02	3.08±0.02	2.95±0.02	2.80±0.02
Extract 27	2.42±0.01	3.94±0.01	3.65±0.01	3.45±0.02	2.84±0.01	2.75±0.01*	2.51±0.01*
Extract 55	2.47±0.01	3.94±0.01	3.58±0.01*	3.44±0.02*	3.13±0.01	2.78±0.01*	2.56±0.01*
Extract 82	2.38±0.01	3.91±0.01	3.60±0.01*	3.39±0.01*	2.95±0.01	2.80±0.012.54±0.01*	2.54±0.01*
ASA 100	2.27±0.01	3.15±0.01*	2.29±0.01*	2.78±0.01*	2.57±0.01*	2.41±0.01*	2.37±0.01*

Values are expressed as Mean ± S E M, significance relative to control *$p < 0.05$, n= 5, ASA = Acetyl Salicylic Acid

Table 3: Effect of the ethanol root extract of *D. arborea* on egg albumin- induced oedema in mice

	Time Interval (minutes)						
Treatments (mg/kg)	0	30	60	120	180	240	300
Distilled water	2.26±0.01	3.78±0.01	3.79±0.01	3.53±0.02	3.08±0.02	2.95±0.02	2.80±0.02
Extract 12.	2.25±0.01	3.50±0.01*	3.35±0.01*	3.01±0.02*	2.83±0.01*	2.66±0.01*	2.57±0.01*
Extract 25	2.29±0.01	3.73±0.01	3.43±0.02*	3.13±0.02*	2.77±0.01*	2.69±0.01*	2.60±0.01*
Extract 37	2.31±0.01	3.76±0.01	3.51±0.01	3.11±0.01*	2.74±0.01*	2.68±0.01*	2.37±0.01*
ASA 100	2.27±0.01	3.15±0.01*	2.29±0.01*	2.78±0.01*	2.57±0.01*	2.41±0.01*	2.37±0.01*

Values are expressed as Mean ± S E M, significance relative to control *$p < 0.05$, n= 5, ASA = Acetyl Salicylic Acid

The effect of ethanol leaf, stem and root extracts of *D. arborea* on xylene – induced ear oedema on mice ear as shown in Tables 4, 5 and 6 .

Table 4 : Effect of ethanol leaf extract of *D. arborea* on xylene - induced ear oedema in mice

	Treatments (mg/kg)	Weight Difference
Distilled water	10 mL/kg	0.060 ± 0.005
Extract	22	0.030 ± 0.003*
	45	0.030 ± 0.007
	67	0.030 ± 0.003*
Dexamethasone 4 mg/kg		0.017 ± 0.003*

Values are expressed as Mean ± S E M, significance relative to control *$P \leq 0.05$, n = 5

Table 5: Effect of the stem extract of *D. arborea* on xylene - induced ear oedema in mice

Treatments (mg/kg)		Weight Difference
Distilled water	10 mL/kg	0.060 ± 0.005
Extract	27	0.026 ± 0.002*
	55	0.020 ± 0.002*
	82	0.020 ± 0.003*
Dexamethasone	4mg/kg	0.017 ± 0.003*

Values are expressed as Mean ± S E M, significance relative to control *P ≤ 0.05, n = 5

Table 6: Effect of the root extract of *D. arborea* on xylene – induced ear oedema in mice

Treatments (mg/kg)		Weight Difference
Distilled water	10 mL/kg	0.060 ± 0.005
Extract	12	0.030 ± 0.005*
	25	0.037 ± 0.003*
	37	0.023 ± 0.003*
Dexamethasone	4mg/kg	0.017 ± 0.003*

Values are expressed as Mean ± S E M, significance relative to control *p ≤ 0.05, n= 5

Analgesic Study

The effects of the various crude extracts (leaf, stem and root) of *D. arborea* on acetic acid-induced writhing in mice as (Tables 7, 8 and 9) while their effects on formalin-induced paw lickings and thermal-induced pain (Tables 10, 11, 12, 13, 14 and 15) are presented below.

Table 7: Effect of ethanol leaf extract of *D. arborea* on acetic acid- induced writhing in mice

		Time interval (minutes)					
Treatments (mg/kg)		5	10	15	20	25	30
Distilled water		11.00±0.94	18.00±1.89	24.00±1.89	18.00±1.25	11.00±0.94	9.67±1.09
Extract	22	10.00±0.94	11.33±0.54*	20.67±1.66	19.00±1.42	18.00±0.47	10.00±1.70
	45	8.33±0.72*	9.67±1.36*	19.00±1.42	17.00±0.47	15.67±0.54	9.00±1.25
	67	8.33±1.19*	8.00±1.25*	17.00±1.63*	14.00±1.70	14.33±1.23	9.33±1.91
ASA	100	3.00±0.47*	7.00±0.94*	9.00±0.94*	6.00±0.94*	3.33±0.98*	2.00±0.47*

Values are expressed as Mean ± S E M, significance relative to control *P ≤ 0.05, n= 5, ASA = Acetyl salicylic acid

Table 8: Effect of ethanol stem extract of *D. arborea* on acetic acid- induced writhing in mice

	Time Interval (minutes)					
Treatments (mg/kg)	5	10	15	20	25	30
Distilled water	11.00±0.94	18.00±1.8	24.00±1.89	18.00±1.25	11.00±0.94	9.67±1.09
Extract 27	7.00±0.94	10.33±0.72*	18.00±2.16	16.67±1.77	9.67±1.67	10.00±0.94
Extract 55	8.33±0.72	7.00±0.50*	16.00±1.25*	15.67±1.05*	10.00±0.94	9.33±1.19
Extract 82	3.00±0.82*	4.00±0.47*	13.00±1.25*	13.00±1.25*	9.67±1.19*	5.00±0.82*
ASA 100	3.00±0.47*	7.00±0.94*	9.00±0.94*	6.00±0.94*	3.33±0.98*	2.00±0.47*

Values are expressed as Mean ± S E M, significance relative to control *p ≤ 0.05, n= 5, ASA = Acetyl salicylic acid

Table 9: Effect of ethanol root extract of *D. arborea* on acetic acid- induced writhing in mice

	Time Interval (minutes)					
Treatments (mg/kg)	5	10	15	20	25	30
Distilled water	11.00±0.94	18.00±1.80	24.00±1.89	18.00±1.25	11.00±0.94	9.67±1.09
Extract 12	8.00±1.47	10.00±1.16*	20.00±2.06	18.00±0.50	17.67±2.42	7.00±0.82
Extract 25	6.00±1.42*	8.33±1.77*	18.33±1.36*	16.00±1.25	14.00±0.94	8.00±1.42
Extract 37	5.00±0.94*	6.00±0.50*	15.00±1.42*	14.00±0.94*	12.00±2.50	7.00±0.82
ASA 100	3.00±0.47*	7.00±0.94*	9.00±0.94*	6.00±0.94*	3.33±0.98*	2.00±0.47*

Values are expressed as Mean ± S E M, Significance relative to control *p ≤ 0.05, n= 5, ASA = Acetyl salicylic acid

Table 10: Effect of ethanol leaf extract of *D. arborea* on formalin- induced paw licking in mice

	Time Interval (minutes)					
Treatments (mg/kg)	5	10	15	20	25	30
Distilled water	11.00±0.94	18.00±1.80	24.00±1.89	18.00±1.25	11.00±0.94	9.67±1.09
Extract 22	19.00±0.94	23.00±0.47	25.00±1.09	18.00±2.50	18.67±2.68	13.33±0.27
Extract 45	17.00±1.25	18.00±2.05	18.00±1.25	17.00±1.25	13.67±1.42	9.00±0.94
Extract 67	16.00±0.94*	18.00±1.25	17.00±2.06	11.33±0.72*	9.67±0.72*	8.00±0.94*
ASA 100	3.00±0.47*	7.00±0.94*	9.00±0.94*	6.00±0.94*	3.33±0.98*	2.00±0.47*

Values are expressed as Mean ± S E M, significance relative to control *p ≤ 0.05, n= 5.

Table 11: Effect of ethanol stem bark extract of *D. arborea* on formalin- induced paw licking in mice

	Time Interval (minutes)					
Treatments (mg/kg)	5	10	15	20	25	30
Distilled water	11.00±0.94	18.00±1.80	24.00±1.89	18.00±1.25	11.00±0.94	9.67±1.09
Extract 27	15.00±0.94	18.00±1.70	22.00±0.94	16.00±1.89	13.00±0.47	11.33±0.54
Extract 55	13.00±0.94*	12.00±0.94*	16.67±0.98*	12.33±1.52*	8.00±0.82*	9.00±0.94*
Extract 82	10.67±1.91*	8.00±1.70*	11.67±0.54*	9.67±1.66*	5.00±0.94*	7.00±0.50*
ASA 100	3.00±0.47*	7.00±0.94*	9.00±0.94*	6.00±0.94*	3.33±0.98*	2.00±0.47*

Values are expressed as Mean ± S E M, significance relative to control *p ≤ 0.05, n= 5.

Table 12: Effect of ethanol root extract of *D. arborea* on formalin- induced paw licking in mice

	Time interval (minutes)					
Treatments (mg/kg)	5	10	15	20	25	30
Distilled water	19.67±1.79	22.00±2.50	25.67±0.98	19.67±0.98	15.00±0.47	14.33±0.98
Extract 12	16.00±1.19	18.00±1.25	23.00±1.15	17.00±1.25	14.00±1.70	12.00±0.80
25	14.00±2.16*	18.33±1.44	22.00±2.63	13.67±1.25	11.67±1.44	8.00±1.63*
37	13.00±0.94*	12.00±1.63*	13.00±1.25*	11.00±2.05*	6.67±0.27*	7.00±0.50*
ASA 100	3.00±0.47*	7.00±0.94*	9.00±0.94*	6.00±0.94*	3.33±0.98*	2.00±0.47*

Values are expressed as Mean ± S E M, Significance relative to control *p ≤ 0.05, n= 5

Table 13: Effect of ethanol leaf extract of *D. arborea* on hot plate – induced pain in mice

Treatments (mg/kg)		Reaction time (seconds)
Distilled water	10 mL/kg	5.89 ± 0.14
Extract 22		6.59 ± 0.62
45		8.98 ± 0.46
67		12.73 ± 0.33*
ASA 100		29.62 ± 0.62*

ASA = Acetyl salicylic acid, Values are expressed as Mean ± S E M, significance relative to control *p ≤ 0.05, n= 5

Table 14: Effect of stem extract of *D. arborea* on hot plate – induced pain in mice

Treatments (mg/kg)		Reaction time (seconds)
Distilled water	10 mL/kg	5.89 ± 0.14
Extract 27		9.81 ± 0.72*
55		12.18 ± 1.55*
82		13.77 ± 2.70*
ASA 100		29.62 ± 0.62*

ASA = Acetyl salicylic acid, values are expressed as Mean ± S E M, Significance relative to control *p ≤ 0.05, n= 5.

Table 15: Effect of ethanol root extract of *D. arborea* on hot plate – induced pain in mice

Treatments (mg/kg)		Reaction time (seconds)
Distilled water	10 mL/kg	5.89 ± 0.14
Extract 12		9.95 ± 0.72
25		11.39 ± 0.87*
37		15.92 ± 0.95*
ASA 100		29.62 ± 0.62*

ASA = Acetyl salicylic acid, values are expressed as Mean ± S E M, Significance relative to control *p ≤ 0.05, n= 5.

DISCUSSION

The various doses of the extracts used for the study were calculated as 0.1, 0.2 and 0.3 of the lethal doses which represented the low, median and high doses of the extracts. Before the death of the animals, they exhibited decreased locomotion, tremor, uncoordinated body movement and convulsion. Considering that the LD_{50} values of the extracts showed low safety profile, then there was need for precision in the various doses used for the study (Crome, 1993). The result of anti-inflammatory study revealed that the various extracts demonstrated considerable anti – inflammatory effects which were comparable to the standard, acetyl salicylic acid (ASA). The suppression of oedema caused by egg albumin by the extracts was statistically significant ($p < 0.05$) relative to distilled water which served as

negative control and in a dose – dependent manner. The result of this study which indicated the ability of the extracts of *D. arborea* to suppress paw diameter induced by egg albumin, a phlogistic agent in a manner similar to ASA, a cyclo – oxygenase inhibitor suggests that the extracts have systemic potential in the inhibition of oedema which may be associated with the secondary metabolites present in the various parts of *D. arborea* (Chirumbolo, 2010; Dahham *et al*., 2015). In topical inflammatory model, the extracts also significantly ($p \leq 0.05$) inhibited oedema caused by the topical administration of xylene in a dose-related manner, when compared to distilled water in a way similar to a prototype drug, dexamethasone. In systemic anti – inflammatory study, two mechanisms of inhibition of oedema are recognized; the first being the local release or formation of autacoids such as histamine, 5 HT, kinins, prostanoids while the second is the neurogenic stimulation of primary sensory neurons followed by the release of prostaglandins, a mediator of inflammation (Lembeck and Holzer, 1979; AmicoRoxas *et al*., 1984; Ajaghaku *et al*., 2013). Just like ASA, the possible mechanism(s) of the extracts of *D. arborea* in inhibiting oedema induced by egg albumin may in part be due to their ability to block these inflammatory sequences. The induction of oedema by xylene is linked to the release of phospholipase A_2, therefore the ability of the extracts to inhibit oedema caused by xylene may be due to the blocking of the release of phospholipase A_2. In the acetic acid pain model, the extracts also reduced acid-abdominal constrictions and stretchings of mice hind limbs. These reductions were statistically ($p \leq 0.05$) significant relative to distilled water but not as potent as ASA. The result of formalin paw licking also indicated that the extracts were able to reduce the number of paw lickings by the mice in a dose-dependent manner and in a way similar to ASA when compared to distilled water and these reductions were also statistically ($p \leq 0.05$) significant. The various extracts also demonstrated considerable time and dose – dependent analgesia against thermally – induced pains in mice. Acetic acid produced inflammatory pain by capillary permeability; formalin pain types are neurogenic and inflammatory while hot plate pain is indicative of the extracts behaving like narcotics. Thus the ability of the extracts in reducing these pain types may be related to their intrinsic anti – inflammatory, neurogenic and narcotic effects (Nwafor and Okwuasaba, 2003).

CONCLUSION

The result from this study supports the use of the various parts of *D. arborea* in the management of pain and inflammatory diseases in folkloric medicine.

Acknowledgement

The authors are grateful to Mr Nsikan Macauley of the Department of Pharmacology and Toxicology for his technical assistance.

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