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Antinociceptive effects of dichloromethane extract of *Euclea divinorum* Lin.

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Abstract
There are numerous plants whose parts or extracts are used as folklore remedies for disorders such as pain alleviation in Africa. A great majority of these herbal remedies have not been bio- screened for their antinociceptive potential. *Euclea divinorum* is one such plant that falls within this group where extract from the plant parts is traditionally used in treatment of tooth ache, head ache, chest pain, arthritis, cancers and ulcers. Hence this study was motivated by the desire to scientifically evaluate the antinociceptive activity of the dichloromethane extract of the stem and root bark extract in rats. Pain was induced by injecting 5µl of 5% formalin solution in the sub- planta region of the left hind paw. The biting, lifting, licking and flitching of the affected paw was considered as a sign of pain in rats, and spent in that behavior was quantified from 0-5 minutes (acute pain) and 15-30 minutes inflammatory/neurogenic pain.

Keywords: *Euclea, divinorum*, pain, inflammation, anti-nociceptive

Introduction
Euclea divinorum (*Ebenaceae*) is a small tree that grows up to about 6 m in height [1]. It has many folklore uses in Africa such as a remedy for tooth ache, head ache, chest pain, a purgative, constipation, dental hygiene, pneumonia, abscess, anti-helmiths, snake bite [1], wounds, arthritis, cancers, jaundice, ulcers, miscarriage, leprosy, gonorrhea and as a source of fast dyes [2]. Studies on root bark extract raised the frequency of contraction of isolated rabbit uteri strips and augmented oxytocin activity on the same [3] while the acetone root bark extract of related plant *E. undulate* had anti-diabetic activity in rats [4]. The methanol extracts of the root and stem possess antibacterial activity against multi-drug resistant *Streptococcus mutans* [5] while the organic extracts of the root back showed significant antimicrobial effects on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis* and *Lactobacillus acidophilus* [6]. In another study, the plant extract reversed gentamicin-induced nephrotoxicity [7]. The plant contains appreciable amount of alkaloids, however no reported case on their molecular characterization. The phytochemicals isolated from the plant lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl) lup-20(30)-ene [8] and naphthalene derivative, eucleanal (A & B) eucleanal (1 & 2) [9].

Materials and Methods
Plant materials
The fresh stem and root barks of *Euclea divinorum* were collected from Narok County during the day. They were identified, and specimens deposited in the University of Nairobi herbarium. Specimen voucher number 2013/JM02 was obtained. They were then air dried inside a room in the laboratory away from direct sunlight before being ground into a fine powder using a grinding mill.

Organic extraction
One hundred grams of the powder was extracted with dichloromethane (DCM) thrice as follows. It was initially soaked in (DCM), stirred and allowed to stand for two hours, then decanted and the residue re-soaked in DCM and allowed to stand for 24 hours before decanting. The latter procedure was repeated twice in the next 48 hours. The supernatant obtained was filtered using Whatman No.1 paper then concentrated and evaporated to dryness using a rotor evaporator at reduced pressure. The extracts weighed about *E. divinorum* stem and root extract weighed 1.63 g and 1.96 g respectively. The extracts were placed in sealed specimen bottles in the laboratory at 20-25 ºC.

Experimental animals

White Wister rats 160-190 grams in groups of five were placed in cages in the rooms maintained between 20˚C to 25 ˚C were used for the antinociceptive and anti-inflammatory assays. They were fed with standard commercial diet and water *Ad libitum* and a 12 hour day light/dark cycle was maintained throughout the study period and were allowed to acclimatize for seven days before the start of the experiments. All the in *vivo* experiments were carried out in accordance to the guidelines for care and use of laboratory animals [10].

Drugs and chemicals

The following drugs and chemicals were used in the study; normal saline, diclofenac sodium, formalin, morphine, dimethyl sulfoxide (DMSO).

Antinociceptive activity assay

Evaluation of analgesic or antinociceptive effect of the herbs extract was carried out using formalin test. The pull test (a sensory motor test) was used to evaluate the muscle relaxing effect of the herb extracts [11]. Only the animals that showed no sensory motor impairment were used for subsequent experiments.

The formalin test as described in [12, 13] where the sub-plantar region of left hind paw of the rats was injected with 50µl of 5% formalin to induce pain. The animals in groups of five each received intraperitoneal injection of the three doses of extracts, 15 mg/kg diclofenac, 5mg/kg morphine and vehicle (30% DMSO in normal saline). All the treatments were administered 30 minutes prior to formalin injection. They were then placed individually in a transparent plexiglass cage observation chamber. Two mirrors were placed behind and on the side of the cage for ease of visualizing the paws. The lifting, licking, biting, flinching injected paw was considered as indicator of nociception and was recorded for 30 minutes following the formalin injection. The first phase of nociception was measured between 0-5 minutes while late phase took place between 15-30 minutes after formalin injection where the early phase represents neurogenic pain while the late phase is due to inflammatory pain response as well as central sensitization [12, 13, 14].

Qualitative phytochemical analysis

Qualitative screening of the phytochemicals was performed using the procedures used by Rasool *et al.* (2010) [15]. It involved detection of the various secondary metabolites groups present in the plant extract without emphasis on their quantities as follows:

Tannins

Approximately 2 milliliters of 5 percent ferric chloride was added to 2 milliliters of the plant aqueous extracts. The formation of yellow/brown precipitate indicated presence of tannins.

Glycosides

About 1ml glacial acetic acid and 1-2 drops of ferric chloride aqueous was added to about 2 milliliters of alcohol filtrate of the plant extract, followed by 1 ml of concentrated H_2SO_4. Appearance of brown ring at the interface indicated presence of cardiac glycosides**.**

Terpenes

About 5 milliliters of chloroform, 2 milliliters acetic anhydride and concentrated H_2SO_4 was carefully layered onto about 2 milliliters of the aqueous extracts in a test tube. A Reddish brown coloration at the interface was an indication of terpenes.

Flavonoids

Shimoda"s test a few drops of concentrated hydrochloric acid followed by 0.5 g of Zinc turnings were added to 2 milliliters of each of the aqueous extract in a test tube. Then the tube was placed in a boiling water bath for few minutes. Development of magenta, red or pink color indicated presence of flavonoids

Phenolics

1milliliter of 1% $FeCl_3$ solution was added to 2milliliters of the aqueous extract. Presence of blue/green color was an indication of the presence of phenols.

Alkaloids

About 1.5 milliliters of 1% HCl was added to 2 milliliters of methanol extract of the sample and heated. Then six drops of Dragendorff's reagent was added. The appearance of orange precipitate confirmed the presence of alkaloids.

Saponins

$NaHCO_3$ solution was added drop wise to 2 milliliters of the aqueous extract. It was then shaken to facilitate mixing. Presence of saponins was indicated by appearance and persistence of froth.

Results

Table 1: Show the effect the dichloromethane extract *of Euclea divinorum* on formalin induced nociception

Treatment	Early phase	Late phase
50 mg	240±19.2[b]	96±24**[b]
100mg	240±21[b]	72±4.2**[b]
Diclofenac	132±12*[b]	84±19.8**[c]
Vehicle	204±30.6	312±24.6
Formalin	260±12.5	336±40.69
Morphine	42.4±7.49**[b]	23.6±3.08**[b]

**Indicate $p<0.001$ as compared to the vehicle, [b & c] shows $p<0.05$ relative to morphine

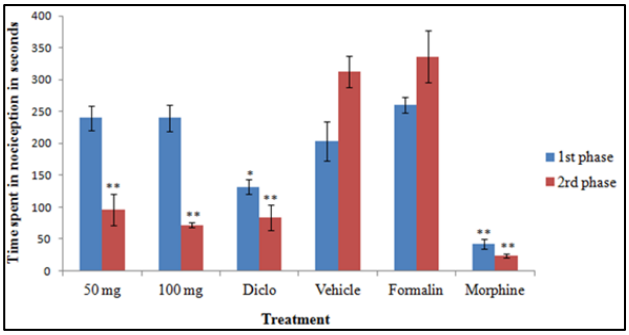

Fig 1: Antinociceptive effect of dichloromethane extract of *E. divinorum* stem *$p<0.05$, **$p<0.001$ relative to vehicle

Table 2: Effect of dichloromethane extract of Euclea divinorum root of formalin induced pain

Treatment	Early phase	Late phase
50 mg	108±12	132±22.45*
100mg	120±18.97	128±14.63*
Diclofenac	132±12*	84±19.8**
Vehicle	204±30.6	312±24.6
Formalin	278±3.74	360±35.21
Morphine	42.4±7.49**[a]	23.6±3.08**[a]

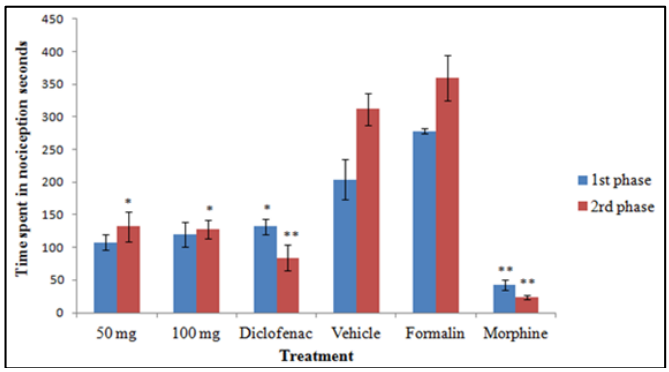

Fig 2: Antinociceptive effect of dichloromethane extract of *Euclea divinorum* root Significant activity relative to vehicle is indicated by **p*<0.05 & ***p*<0.001

Phytochemical	*Euclea divinorum* stem	*Euclea divinorum* root
Alkaloid	++	+
Tannins	+	+
Saponins	+	+
Glycosides	+	+
Terpenes	-	-
Flavonoids	+++	++
Phenolics	++	++

+ Little amount, ++ Moderate amount, +++ Higher amount, - Absent

Discussion

Injection of dilute formalin solution in a paw of the animal produces nociception with two phase that early phase which lasting the first 5 minutes and late phase lasting from 20 to 30 minutes. The two phases have different mechanism where the first phase involves direct action that stimulates of nociceptors [12] and it is mainly mediated via TRPA1 receptors a member of the transient Receptor Potential family of cation channels that is highly expressed by some of C-fiber nociceptors that plays an important role in inflammatory pain. Stimulation of these channels by formalin causes tremendous influx of calcium ions in to the cells expressing this type of ion channel [14].

In the study, the 50 and 100 mg doses of the DCM extract of *E. divinorum* stem had no significant effect on time spent in pain behavior ($p>0.05$) as compared to the vehicle during the first phase of nociception. However they exhibited a highly significant ($p<0.001$) antinociceptive effect in the second phase of nociception (Fig. 1 & Table 1). This activity was not dose dependent but was comparable to diclofenac. Similarly, the 50 and 100 mg doses of root extract had no significant effect in the first phase however it exhibited significant ($p<0.05$) analgesic effect which was not dose dependent in the 2nd phase (Fig. 2). The first phase represents direct action of formalin on nociceptors [12] while the second is mainly due inflammatory activity where inflammatory mediators which also act as mediators of pain such as substance P, prostanoids, 5-hydroxytryptamine and histamine are release [16]. It is also associated with sensitization of nociceptive afferents in the spinal cord [17]. The second phase of formalin induced pain is also associated with inflammatory process. Nevertheless, both IL-1β and TNFα play a key role in inflammatory pain induction in the second phase of formalin test [14]. It is also likely that the extract exerted its analgesic effect via inhibition of inflammatory process or by inhibiting a single or many neural transmitters involved 'gating control of pain' in the dorsal horn of the spinal cord [12, 14].

Qualitative phytochemical analysis showed that the plant material contained relatively high amount of flavonoids (Table 2). Previous studies had led to isolation of several phytochemicals which includes lupeol, lupene, betulin, 7-methyljuglone, isodiospyrin, shinalone, catechin and 3β-(5-hydroxyferuloyl) lup-20(30)-ene [8] Myricitrin [18], Eucleanal A & B [9] glycosides of aromadendrin, quercetin and myricetin [2]. These metabolites include several flavonoids. However studies have shown that the plant possess appreciable amount of alkaloids in both aqueous and methanolic extracts though none has been describe [19, 6]. It is possible that one or several of these metabolites may be responsible for the antinociceptive effects observed in this study. In study, all the doses of the various plant extract showed far less level of pain blocking activity as compared to 5 mg dose of morphine.

Conclusion

The DCM extracts of both stem and root of *Euclea divinorum* possess antinociceptive effects in the second phase which can be attributed to the phytochemicals present. These results support the folklore use of this plants extracts in pain management.

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We declare no conflict of interest.

References

1. Kokwaro JO. Medicinal plants of East Africa. 2rd ed. East African literature bureau. Nairobi, 1993, 222-223.
2. Njuguna PM. *Euclea divinorum* Hiern In: Jansen, P.C.M. & Cardon, D. (Editors). PROTA 3: Dyes and tannins/ Colorants et tanins. [CD-Rom]. PROTA, Wageningen, Netherlands, 2005.
3. Kaingu CK, Oduma JA, Kanui T. Preliminary investigation of contractile activity of Ricinus communis and *Euclea divinorum* extracts on isolated rabbit uterine strips. Journal of Ethno pharmacology. 2012; 142(2):496-502.
4. Deutschländer MS, Lall N, Van de Venter M, Dewanjee S. The hypoglycemic activity of *Euclea undulata* Thunb. var. myrtina (Ebenaceae) root bark evaluated in a streptozotocin–nicotinamide induced type 2 diabetes rat model. South African Journal of evaluating muscle relaxation. Journal of Pharmacological Methods, 2012; 11(2):119-124.
5. Mbanga J, Ncube M, Magumura A. Antimicrobial activity of *Euclea undulata*, *Euclea divinorum* and *Diospyros lycioides* extracts on multi-drug resistant *Streptococcus mutans*. Journal of Medicinal Plants Research. 2013; 7(37):2741-2746.
6. Ngari FW, Gikonyo NK, Wanjau RN, Njagi EM. Safety and antimicrobial properties of *Euclea divinorum* (Hiern), chewing sticks used for management of oral health in Nairobi County, Kenya. Journal of Pharmaceutical and Biomedical Sciences. 2013; 3(3):1-8.
7. Feyissa T, Asres K, Engidawork E. Renoprotective effects of the crude extract and solvent fractions of the leaves of *Euclea divinorum* Hierns against gentamicin-

induced nephrotoxicity in rats. Journal of ethno pharmacology. 2013; 145(3):758-766.
8. Mebe PP, Cordell GA, Pezzuto JM. Pentacyclic triterpenes and naphthoquinones from *Euclea divinorum*. Phyto chemistry. 1998; 47(2):311-313.
9. Ng'ang'a MM, Hussain H, Chhabra S, Langat-Thoruwa C, Al-Harrasi A, *et al*. Eucleanal A and B: Two new naphthalene derivatives from *Euclea divinorum.* Chinese Chemical Letters. 2012; 23(05):576-578.
10. Wolfensohn S, Lloyd M. Small laboratory animals. Handbook of laboratory animal management and Welfare, 2rd ed. Blackwell Science, London, England, 1998, 169-217.
11. Deacon RMJ, Gardner CR. The pull-up test in rats: A simple method for evaluating muscle relaxation. Journal of pharmacological methods. 1984; 11(2):119-124.
12. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain. 1987; 30(1):103-114.
13. Rosland JH, Tjølsen A, Mæhle B, Hole K. The formalin test in mice: effect of formalin concentration. Pain. 1990; 42(2):235-242.
14. McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, Zhao M, *et al*. TRPA1 mediates formalin-induced pain. Proceedings of the National Academy of Sciences. 2007; 104(33):13525-13530.
15. Rasool R, Ganai BA, Akbar S, Kamili AN, Masood A. Phytochemical screening of Prunella vulgaris L.-an important medicinal plant of Kashmir. Pak. J Pharm. Sci. 2010; 23(4):399-402.
16. Damas J, Liegeois JF. The inflammatory reaction induced by formalin in the rat paw. *Naunyn-Schmiedeberg's* Archives of Pharmacology. 1999; 359(3):220-227.
17. Harvey RJ, Depner UB, Wässle H, Ahmadi S, Heindl C. GlyR α3: an essential target for spinal PGE2-mediated inflammatory pain sensitization. Science. 2004; 304(5672):884-887.
18. Hattas D, Hjältén J, Julkunen-Tiitto R, Scogings PF, Rooke T. Differential phenolic profiles in six African savanna woody species in relation to anti-herbivore defense. Phyto chemistry. 2011; 72(14-15):1796-1803.
19. Amusan OO, Sukati NA, Dlamini PS, Sibandze FG. Some Swazi phytomedicines and their constituents. African Journal of Biotechnology. 2007; 6(3):267-272 Botany, 80:9-12.