ORIGINAL ARTICLE

In vivo anti-inflammatory and antinociceptive activities of the aerial part extract of *Dicliptera laxata*

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Introduction: *Dicliptera laxata* (*Acanthaceae*) is a perennial herb with stems creeping and rooting and its aerial parts are used for the treatment of headache, by using the nasal route of administration. A blood red decoction of the aerial part of *D. laxata* is taken for orofacial inflammation. **Aims:** In the present study, the *in vivo* anti-inflammatory and antinociceptive effects of the aqueous aerial part extract of *D. laxata* were investigated. **Materials and Methods:** The anti-inflammatory effect was evaluated using the carrageenan-induced mouse pedal (paw) edema model, while the formalin test in mice was employed to study the antinociceptive activity. **Results:** Administration of 400 mg/kg p.o. of the aqueous extract of the aerial parts of *D. laxata* produced significant ($P < 0.05 - 0.01$) anti-inflammatory effects against carrageenan-induced acute inflammation and formalin-induced nociceptive pain stimulus in mice. Bioassay-guided fractionation of the total extract indicated that the water fraction was by far the most potent in both models. **Conclusions:** The present findings indicate that *D. laxata* possesses genuine anti-inflammatory and antinociceptive properties, lending pharmacological support to the folkloric or anecdotal use of the plant in the treatment and/or management of painful inflammatory conditions. To the best of our knowledge, this is the first report on the anti-inflammatory and antinociceptive activities of the extracts and isolated compound (DL-1) of *D. laxata*.

Key words: Anti-inflammatory, antinociceptive, *Dicliptera laxata*

INTRODUCTION

Many people in Africa (approximately 75% of the population) still rely on traditional healing practices and medicinal plants for their daily healthcare needs, despite the immense technological advancement in modern medicine.[1]

Dicliptera laxata (*Acanthaceae*) is a perennial herb with stems creeping and rooting at the nodes, ascending above, or erect. It grows up to 1 m in height. The leaves are blade-like and narrowly ovate to elliptic. They are broadest below the middle and glabrous or nearly so.[2]

A report also described the ethnobotanical use of *D. laxata* by the Meinit (Me'en) people in the Bench Maji Zone, southwest of Ethiopia.[3] The parts of the herb above the ground are used for headache, using the nasal route of administration. In a study on medicinal plant diversity and the local uses in southern Uganda, the infusion made from the leaves of *D. laxata* has been reported to find application as a poison antidote.[4]

D. laxata is known by its vernacular names '*Omoro*' in Hadiyegna and Kembatigna or '*Togo*' in Kefigna (Jimma area). A blood red decoction of the aerial part of *D. laxata* is consumed for orofacial inflammation by the local people. To the best of our knowledge, there is a paucity of such an ethnopharmacological report in the literature.

Due to its usage as a folk medicine, many phytochemical studies have been carried out on other *Dicliptera* species. For example, compounds like C_{15-31} fatty acids, flavonoids, carotenoids, α-amino acids, betulin, daucosterol and long-chain aliphatic hydrocarbons, have been isolated from *D. roxburghiana* and *D. chinensis*.[5]

The aim of the present study was to investigate the anti-inflammatory and antinociceptive activity of the aerial part extract of *D. laxata* in an animal experimental model, with a view to providing a pharmacological justification (or otherwise) for the claimed folkloric use of this plant in the treatment of orofacial inflammation by the local communities around the Hadiya zone, in the southern part of Ethiopia.

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MATERIALS AND METHODS

Plant Materials

Fresh aerial parts of *D. laxata* were collected from Hossaena, Hadiya zone, southern Ethiopia, 230 km from Addis Ababa, in November 2008. No specific permission was required for collecting this plant from this location, as it was a widely growing weed and this study did not involve any endangered or protected species. The authenticity of the plant material was confirmed by Mr. Melaku Wondafrash, Plant Taxonomist, National Herbarium, Addis Ababa University and a voucher specimen of *D. laxata* (MW02) was deposited for future reference [Figure 1].

Chemicals and Solvents

The following chemicals and solvents were purchased and used as received: Carrageenan and indomethacin (Sigma Chemcials Co., USA), silica gel G F_{254} (BDH Chemicals Ltd., England), formalin (BDH Chemicals Ltd., England), *n*-butanol (BDH Chemicals Ltd., England), chloroform (E. Merck, Stockholm), methanol (Loba Chemie Pvt. Ltd., India) and acetic acid (BDH Chemicals Ltd., England). The other reagents were of analytical grade and obtained from different commercial sources.

Animals

Swiss Albino mice of either sex with a body weight of 20-35 g were used for the experiments and housed under standard laboratory conditions and fed commercial mice feed and tap water *ad libitum*. All animal experiments were conducted in accordance with the internationally accepted laboratory animal use, care and guidelines (ILAR, 2011).[6]

Method of Extraction

Preparation of aqueous extracts of D. laxata

Fresh aerial parts of *D. laxata* were washed with distilled water and then chopped into pieces. The plant material (1 kg) was then extracted with 2 L of boiling distilled water (H_2O) for 30 minutes. After filtration (Whatmann No 1), the extract was concentrated to dryness in a ventilated oven at a temperature not exceeding 40°C. The dried extract was kept in a refrigerator before use. At the time of use the extract was reconstituted in distilled water at the required concentrations.

Preparation of solvent fractions

The crude aqueous extract (20 g) of *D. laxata* was suspended in 100 mL of distilled water, which was fractionated successively by partitioning with organic solvents to obtain the following fractions: Chloroform (3 × 100 mL), chloroform: Methanol (3:1, 3 × 100 mL) and the remaining water fractions. After being dried under reduced pressure at 40°C, each fraction was used for bioassay and analysed by thin layer chromatography (TLC).

Figure 1: Aerial parts of *Dicliptera laxata*

Preparation of test samples for the bioassay

The crude extract, fractions and isolated compound were administered *per os* to the test animals in 400, 200 and 100 mg/kg doses, respectively, after suspending in distilled H_2O or 1% carboxymethyl cellulose (CMC) as vehicles. The control group animals were treated in a similar manner as those in the test groups, except that the drug treatment was replaced with appropriate volumes of the vehicle (10 mL/kg). Indomethacin (10 mg/kg in distilled H_2O) for carrageenan-induced hind paw edema and morphine HCl (5 mg/kg in distilled H_2O) for formalin-induced nociception were used as reference drugs.

Acute toxicity

Animals employed in the carrageenan-induced paw edema and formalin-induced nociception experiments were observed for seven days and mortality was recorded, if any, for each group, at the end of the observation period.

Chromatographic Techniques

Thin layer chromatography

Analytical and preparative TLC procedures utilised adsorption chromatography. Normal phase analytical TLC was performed using silica gel 60 F_{254} pre-coated plates (0.20 mm) (E. Merck, Darmstadt). For preparative TLC, a glass measuring 20 × 20 cm was prepared using the slurry of silica gel G suspended in distilled water (1:2 w/v), which was spread to a thickness of 0.50 mm. The plates were activated for one hour at 110°C and allowed to cool to room temperature and humidity before use.

Solvent system used in thin layer chromatography

The solvent system used for both analytical and preparative TLC contained butanol:acetic acid:water (4:1:1).

Visualisation

For analytical TLC, the developed chromatograms were air-dried and then examined in daylight and under $UV_{254\ nm}$ (short wavelength) and $UV_{366\ nm}$ (long wavelength) prior to spraying with the detecting reagents. In the case of preparative TLC, the chromatograms were air-dried

after development and bands corresponding to the major compounds were scraped off on the basis of the colours observed in the daylight and/or under UV light fluorescence. Separate chromatograms were also sprayed with various spraying reagents (Dragendorff's reagent, vanillin-sulphuric acid, antimony (III) chloride, 25% aqueous lead acetate and ferric chloride-potassium ferriccyanide), for phytochemical screening.[7,8]

Preparation of isolated compound(s) from active fractions

Isolation was carried out by making bands on the preparative TLC and scraping off the silica gel corresponding to a major band and eluting with methanol. The eluates were evaporated to dryness under reduced pressure and repeated preparative plates were made in an effort to get a single pure compound(s) for further bioactivity testing.

In vivo *anti-inflammatory activity testing*

According to a previously described report the *in vivo* anti-inflammatory activity was evaluated on the basis of the inhibition of carrageenan-induced mouse hind paw edema, with some modifications.[9] The mice were fasted for 12 hours with free access to water until the experiment started. The extract, fractions and isolated compound, at doses of 400, 200 and 100 mg/kg, respectively, were administered orally to the test groups, using an oral gavage. Animals in the reference group received indomethacin (10 mg/kg p.o.), while control animals received distilled water (10 ml/kg p.o.). One hour later, edema was induced by injecting 0.1 mL of 1% carrageenan solution in normal saline into the right hind paw of each mouse. The volume of paw edema was measured before and one, two, three, four and five hours after induction of inflammation, using the Plethysmometer (UgoBasile 7140, Italy).

Antinociceptive activity testing

The antinociceptive activity of the extract was determined using the formalin test.[10] The animals were categorised into control-treated (vehicle — distilled water) (10 mL/kg), reference-treated indomethacin (10 mg/kg p.o.) or morphine (5 mg/kg, s.c.) and test groups (extract-, fraction-, or isolated compound-treated). Each group consisted of six mice (three males and three females) each weighing 20-35 g.

Formalin test

The formalin test was carried out according to the modifications of the test for mice and had two phases.[10]

A. Early phase

The experiment was carried out on mice that had been individually exposed to the observation chambers for an adaptation period of two hours; the animals were deprived of food and water once they were in the observation chambers. The extract, fractions and isolated compound at 400, 200 and 100 mg/kg doses, respectively, indomethacin (10 mg/kg, p.o.) or morphine (5 mg/kg, s.c.) and the vehicle were given orally (10 mL/kg) to the test, reference and control groups, respectively. One hour after administration of the extract, indomethacin, or vehicle, or 30 minutes after the morphine injection, each mouse was taken out of the cage, with minimum restraint, and 20 µL of 2.5% formalin was injected just under the skin of the dorsal surface of the right hind paw using a micro-syringe. Next, the mouse was put back into the chamber and the time that the animal spent licking the injected paw or leg, in seconds, was recorded, for 0-5 minutes after the injection of formalin.

B. Late phase

Exposure of the animals to the observation chambers and administration of the extract, fractions, or isolated compound, indomethacin or morphine and vehicle, for the test, reference and control groups, respectively, was done in the same manner as the early phase test. When testing in the late phase, recording of the licking time was done 20-30 minutes after the formalin injection.

Data Analysis

Anti-inflammatory activity

For the anti-inflammatory test, the increase in paw volume, that is, inflammation (%*I*) was calculated according to the equation:[11]

$$\%I = \left[\frac{V_f - V_i}{V_i}\right] \times 100$$

Where V_f and V_i were the final and initial paw volumes of each animal, respectively. The mean percent inflammation (%*I*) was then calculated and a curve of mean %*I* versus time was plotted. In addition, the anti-inflammatory effect (%*A*) was calculated according to the formula given below[11] and data were presented as mean ± SEM (standard error of the mean).

$$\%A = \frac{[\%I_c - \%I_e]}{\%I_c} \times 100$$

where $\%I_c$ and $\%I_e$ were the mean inflammation values reached in the control and experimental groups, respectively. The significance of drug-induced changes was estimated using one-way analysis of variance (ANOVA) followed by the Tukey's test, to analyse the data and $P < 0.05$ was taken as statistically significant[12] by using the software package INSTAT.

Antinociceptive activity

Formalin test

The mean number of seconds the animals spent licking the injected paw and the SEM in each treatment group were calculated for the formalin test of both phases. Data were presented using a bar graph and plotted as treatment

group versus the mean number of seconds spent in paw licking.

RESULTS AND DISCUSSION

Extraction

The percentage yields of the aqueous extract from the aerial part of *D. laxata* and solvent–solvent fractions from the extract are shown in Table 1.

Preliminary Phytochemical Screening

As indicated in Table 2, the preliminary phytochemical screening of the aqueous extract of *D. laxata* (aerial parts) strongly indicated the possible presence of polyphenolics and saponins, with the absence of tannins and anthraquinones. However, a weak positive result was obtained for carotenoids in *D. laxata.*

Analytical Thin Layer Chromatography

Analytical TLC performed on the extract of the aqueous aerial part of *D. laxata* revealed at least three major separated spots (R_f = 0.86, 0.73 and 0.68) with very minor ones at the bottom and all appearing as dark spots when detected under UV light of long wavelength (366 nm) [Figure 2]. Only one of these spots (R_f = 0.86) appeared intensely pink when viewed under UV light of short wavelength (254 nm).

The analytical TLC profile of the aqueous extract of the plant is summarised in Table 3. According to the preliminary chemical and analytical TLC tests the major compound in the extract of *D. laxata* appears to be a terpenoid. Moreover, unsaturated compounds were also detected in the plant.

Isolation of a Compound from Active Fraction of *D. Laxata*

Preparative TLC of the aqueous fraction of *D. laxata* (AFD) revealed a pink band (R_f = 0.87) when viewed under UV light of short wavelength (254 nm). The analytical TLC of the compound isolated from *D. laxata* initially appeared pink [Figure 3] and then gradually changed to a violet colour when the chromatogram was sprayed with antimony (III) chloride (Carr–Price reagent) followed by heating, suggesting the possible presence of a steroidal nucleus in the compound. However, the analytical chromatogram of this

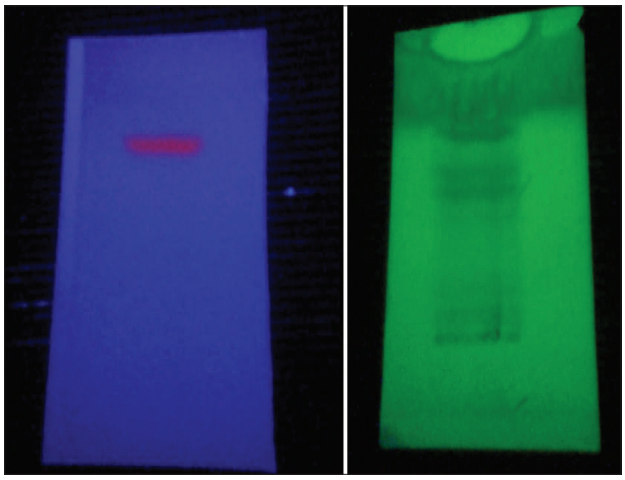

Figure 2: Analytical TLC of the aqueous aerial part extract of *Dicliptera laxata*; solvent system: butanol:acetic acid:water (4:1:1); detected at 254 nm (Right) and 366 nm (Left) of UV light

Table 1: Percentage yields of extract and fractions obtained from the extract of the aerial part of *Dicliptera laxata*

Plant material	Extraction or fractionation solvent	Method of extraction or fractionation	Percentage yield (w/w, %)
D. laxata (Aerial part)	Distilled water	Decoction	4.26[a]
	Chloroform	Solvent-solvent fractionation	4.15[b]
	Methanol chloroform (1:3)	Solvent-solvent fractionation	18.05[b]
	Distilled water	Solvent-solvent fractionation	77.80[b]

[a]Extract yields in grams of dry extracts per 100 g of fresh plant material, [b]Fraction yields in grams of dry fractions per 100 g of aqueous extract

Table 2: Preliminary phytochemical screening of the aqueous extract of the aerial part of *Dicliptera laxata*

Chemical compounds	Tests/reagents	*D. laxata*
Alkaloids	Dragendorff's reagent/Mayer's reagent	–
Polyphenols	1% Ferric chloride and 1% potassium ferrocyanide	+ +
Saponins	Honeycomb froth; 10% sodium nitrate and conc. sulphuric acid	+
Tannins	1% Gelatin	–
Anthraquinones	Borntrager's test	–
Cardiac glycosides	Liebermann-Burchard's reagent	–
Carotenoids	Antimony trichloride in chloroform	+

Key: – – negative; + – weakly positive; + + – moderately positive; + + + – strongly positive

Table 3: TLC profile of the aqueous extract of the aerial part of *Dicliptera laxata*

Chemical compounds	Spray reagents	*D. laxata*
Alkaloids	Dragendorff's reagent	-
Phenolics	1% Aluminium chloride in ethanol	-
Terpenoids	Vanillin in ethanolic sulphuric acid (3%) with heating at 115°C for two minutes	Pink spot changed to light green, but faded away upon heating
Anthraquinone	10% ammonium hydroxide vapor	Pink spot changed to violet with strong fluorescence at 366 nm and a slightly blue coloured spot just above the pink spot in daylight
Unsaturated compounds	Iodine crystal	Pink spot and two yellow spots beneath

TLC – Thin layer chromatography

compound gave a faint blue coloured spot when exposed to 10% NH_4OH vapor, which made it difficult to pinpoint the class of compound it belonged to. Furthermore, when left in air for some time, the freshly prepared chromatogram of this pink coloured isolate gradually changed its colour to purple, suggesting the instability of the compound.

Anti-inflammatory Activity

As shown in Table 4 and Figure 4, the aqueous extract of the aerial part of *D. laxata* showed a significant ($P < 0.05$) effect on the percent reduction of carrageenan-induced inflammation in mice, with a percent inhibition of 68.43 and 69.18, respectively, in the second hour. The activities were also highly significant ($P < 0.001$) on the delayed stages of the edematogenic response (third and fourth hours) and continued to the fifth hour, 59.67-52.39% when compared to the vehicle. Indomethacin, the standard drug used in the study also exhibited a very significant ($P < 0.01$) activity against carrageenan-induced edema at the third and fourth hours, with a percent reduction of 58.76 and 48.24, respectively. Indomethacin is a known cyclo-oxygenase inhibitor which is experimentally shown to attenuate a carrageenan response in the second phase of inflammation, mainly mediated by prostaglandins.[13-15]

The carrageenan-induced hind paw edema model is a widely used screening protocol to test the anti-inflammatory activity of natural products.[16] It is believed to be bi-phasic. The first phase, within two hours, involves the release of serotonin and histamine, and the second phase, after two hours, is mediated by prostaglandins and cyclo-oxygenase products.

Thus, as shown in Table 5, the mean percent anti-inflammatory effect of the aqueous extract of *D. laxata* (20.26 and 20.99) displayed pronounced activity in the delayed stages (three and four hours, respectively) of the edematogenic response induced by carrageenan, when compared to the vehicle. Indomethacin, the positive control used in this study, exhibited a better anti-inflammatory activity (25.63 and 32.25%) at three and four hours, respectively.

As the anti-inflammatory effect of the aqueous extract of *D. laxata*, like indomethacin, occurs mainly in the second phase of inflammation, it suggests that the anti-inflammatory activity might be due to the inhibition of prostaglandin release. However, the early anti-inflammatory effect of the aqueous extract of *D. laxata* (10.36 and 9.37%) in the second hour, against the inflammation induced by carrageenan due to histamine, serotonin and a kinin-like substance release should also not be ruled out.[17]

Thus, based on the results obtained at this stage, it is difficult to explain the exact mechanism of action of the

Figure 3: Analytical TLC of the major compound isolated from the aqueous fraction of *Dicliptera laxata*; solvent system: butanol:acetic acid: Water (4:1:1); detected as a pink band in daylight

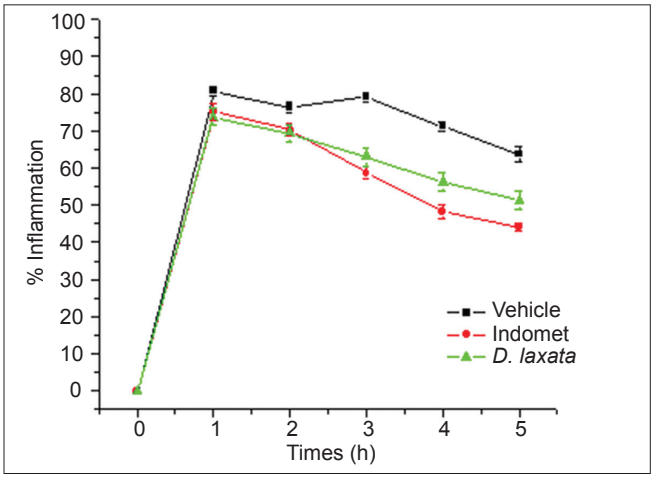

Figure 4: Reduction in percent inflammation (%*I*) by the aqueous extract of *Dicliptera laxata* (400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.) compared to the vehicle on carrageenan-induced mouse paw edema, Indomet = indomethacin

Table 4: Effect of the aqueous extract of *Dicliptera laxata* (400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.) on % inflammation over a period of five hours after carrageenan mouse paw injection

Treatment	% Inflammation				
	1 h	2 h	3 h	4 h	5 h
Vehicle	80.67±1.31	76.33±1.40	79.01±1.23	71.20±1.33	63.74±1.94
Indomet	75.05±2.39	70.28±1.56	58.76±1.74***	48.24±1.99***	44.21±1.15**
D. laxata	73.75±2.26	69.18±2.27*	63.00±2.44***	56.25±2.34***	51.31±2.37*

Indomet – Indomethacin; Values are mean±SEM, n=6, *P<0.05, **P<0.01, ***P<0.001 compared to vehicle

aqueous extract of *D. laxata* against the carrageenan-induced inflammation in mice.

The active aqueous extract of *D. laxata* was fractionated through solvent–solvent extractions with increasing polarity and three fractions were obtained; chloroform ($CHCl_3$), methanol–chloroform (MeOH–$CHCl_3$,1:3) and the remaining water fraction. Each fraction was administered to the mice in doses of 200 mg/kg orally in the same way as the total aqueous extracts.

As shown in Table 6, the AFD, unlike the chloroform fraction of *D. laxata* (CFD) and the methanol–chloroform fraction of *D. laxata* (1:3) (MCFD), showed a significant ($P < 0.05$) anti-inflammatory activity (9.85%) against carrageenan-induced edema in mice starting from the second hour. Such an effect also became very significant ($P < 0.01$) in the subsequent hours (three to five hours), with the activity increasing from 23.34 to 30.08%.

Despite being inactive in the early stage, the MCFD showed a significant ($P < 0.05$) anti-inflammatory effect in the late stages of inflammation. The CFD, however, did not show any significant effect. As the aqueous extract and particularly the AFD were found to be most active, further bioassay-guided anti-inflammatory activity was carried out on these fractions in an attempt to isolate the compound(s) responsible for such a bioactivity.

Thus, as shown in Table 7, the band scraped off from the AFD designated as DL-1 was found to be more active against carrageenan-induced paw edema in mice.

DL-1, at a dose of 100 mg/kg given orally, very significantly inhibited ($P < 0.01$) carrageenan-induced edema formation by 23.36%, three hour after administration of carrageenan. The anti-inflammatory activity of DL-1 became more potent (25.14 and 26.58%) during the fourth and fifth hour respectively. Similarly, the group treated with indomethacin showed good activity, decreasing edema formation by 25.79 and 28.17% in the fourth and fifth hour respectively. The results were statistically significant compared to the percent inflammation of vehicle-treated group (71.20 and 63.74% during the fourth and fifth hour).

Table 5: Anti-inflammatory effect of the aqueous extract of *Dicliptera laxata* (400 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.) on carrageenan-induced mouse paw edema

Test substance	Percent of anti-inflammatory activity (%*A*)				
	1 hour	2 hours	3 hours	4 hours	5 hours
D. laxata	8.58	9.37	20.26	20.99	19.56
Indomethacin	6.97	7.93	25.63	32.25	30.69

Table 6: Anti-inflammatory effect of the fractions of *Dicliptera laxata* (200 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.) on carrageenan-induced mouse paw edema

Test substance	Percent anti-inflammatory activity (%*A*)				
	1 hour	2 hours	3 hours	4 hours	5 hours
CFD	4.36	4.97	10.12	8.02	7.87
MCFD	6.13	8.04	16.83*	14.88*	17.41*
AFD	5.67	9.85*	23.34**	26.21**	30.08**
Indomethacin	5.89	8.05	25.29***	30.03***	34.61**

AFD – aqueous fraction of *D. laxata*; CFD – chloroform fraction of *D. laxata*; and MCFD – methanol-chloroform fraction (1:3) of *D. laxata*; Values are mean %*A* calculated from %*I*; n=6; *P<0.05; **P<0.01; ***P<0.001 compared to vehicle

Antinociceptive Activity

As shown in Figure 5, the aqueous extract of *D. laxata* (400 mg/kg) produced inhibition on the formalin-induced pain responses in mice. The extract significantly ($P < 0.05$) reduced the licking time (82.60 and 91.83 seconds, respectively) at the late phase of formalin-induced pain. The positive control drug, morphine (5 mg/kg), very significantly ($P < 0.001$) attenuated the pain responses of the early and late phases (30.17 and 21.67 seconds, respectively), whereas, indomethacin (10 mg/kg) was very efficient in the late phase (83.00 seconds licking time) in comparison to the vehicle, with a licking time of 127.33 and 133.33 seconds in the early and late phases, respectively.

As reported by Umukoro and Ashorobi (2007), formalin-induced paw licking is often used to distinguish between central and peripheral analgesic actions.[18]

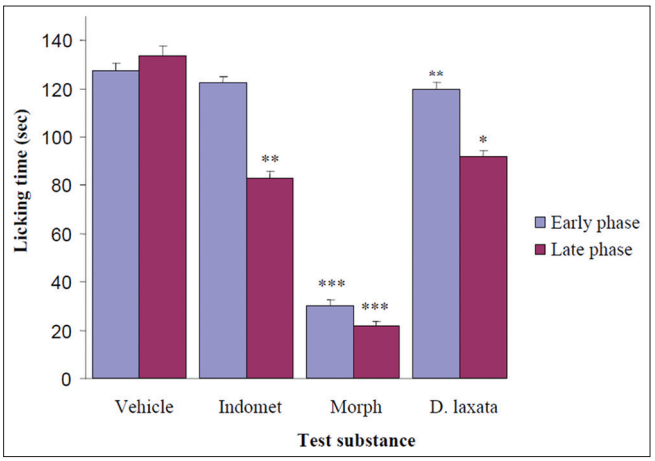

Figure 5: Antinociceptive activities of the aqueous extract of *Dicliptera laxata* (400 mg/kg, p.o.), morphine (morph, 5 mg/kg, s.c.) and indomethacin (indomet, 10 mg/kg, p.o.) compared with the vehicle on the early and late phases of formalin-induced nociception in mice, the values are means ± SEM, n = 6, *P < 0.05, **P < 0.01, ***P < 0.001 compared to the vehicle

Table 7:Anti-inflammatory effect of the isolated compound of *Dicliptera laxata* (100 mg/kg, p.o.) and indomethacin (10 mg/kg, p.o.) on carrageenan-induced mouse paw edema

Test substance	Percent anti-inflammatory activity (%A)				
	1 hour	2 hours	3 hours	4 hours	5 hours
DL-1	7.48	6.85	23.36***	25.14***	26.58**
Indomethacin	5.74	6.41	21.27***	25.79***	28.17**

DL-1 – Compound isolated from *D. laxata;* Values are mean %*A* calculated from %*I*; n=6; *P<0.05; **P<0.01; ***P<0.001 compared to vehicle

In the present study, the aqueous extract of *D. laxata* was found to be highly potent against the nociceptive response in the late phase of the formalin test. Thus, these results tended to suggest that the antinociception effect might be due to their peripheral anti-inflammatory effect. Moreover, the results suggested the presence of phytochemically active constituents in the aqueous extract of the plant, with prostaglandin synthesis inhibitory activity. Similarly, indomethacin showed significant inhibition of pain induced by formalin in the second phase.

As shown in Figure 6, at a dose of 200 mg/kg, p.o. the AFD produced a significant ($P < 0.05$) antinociceptive effect (113.67 seconds) in the early phase. It also showed a very significant ($P < 0.01$) inhibitory effect (84 seconds) in the late phase compared to the vehicle (127 and 135.8 seconds in the early and late phases, respectively).

However, at a dose of 200 mg/kg p.o., the methanol–chloroform and chloroform fractions of *D. laxata* did not significantly alter the nociceptive responses associated with the first phase (neurogenic pain) and the second phase (inflammatory pain) of the formalin test. Even as morphine very significantly ($P < 0.001$) inhibited the first and late phases (33 and 26.33 seconds, respectively) of formalin-induced paw licking, indomethacin was only effective (78.17 seconds) against the second phase.

As depicted in Figure 7, at an oral dose of 100 mg/kg, the isolated compound of *D. laxata* (DL-1) displayed good antinociceptive activity, very significantly decreasing ($P < 0.001$) the licking and biting time by 65.5 and 60.33 seconds, respectively, in the second phase of formalin-induced pain. In the control group, the licking times induced by 2.5% formalin were 129.17 and 136.83 seconds during the first and second phases, respectively. Although not pronounced as in the late phase, DL-1 also showed significant ($P < 0.05$) antinociception (116 seconds) in the first phase when compared with the vehicle.

The mechanism of antinociception by DL-1 is also correlated with that of AFD, in that, DL-1 is also very active in the second phase of formalin-induced pain. Thus, the result of the present study suggests that the peripheral antinociceptive effects of DL-1 against the nocifensive responses of the mice induced by formalin may be mediated via inhibition of cyclo-oxygenases and/or lipoxygenases (and other inflammatory mediators), which corroborate the anti-inflammatory activity of DL-1. However, the additional activity of DL-1 in the early phase of formalin suggests that its action may also be mediated through inhibition of the central pain receptors.

CONCLUSION

It can be concluded that the extract obtained from *D. laxata* possesses significant anti-inflammatory and antinociceptive activities. It can also be proposed that the antinociceptive activities of this plant are mainly due to its anti-inflammatory effect. The pink compound isolated from the aerial part of *D. laxata* shows both anti-inflammatory and antinociceptive activities; however, the structural elucidation of the isolated compound needs further study. The results of this study support the traditional use of this medicinal plant for the treatment of orofacial inflammation. To the best of our knowledge, this is the first report on the anti-inflammatory and antinociceptive activities of the extract and the isolated compound (DL-1) of *D. laxata*.

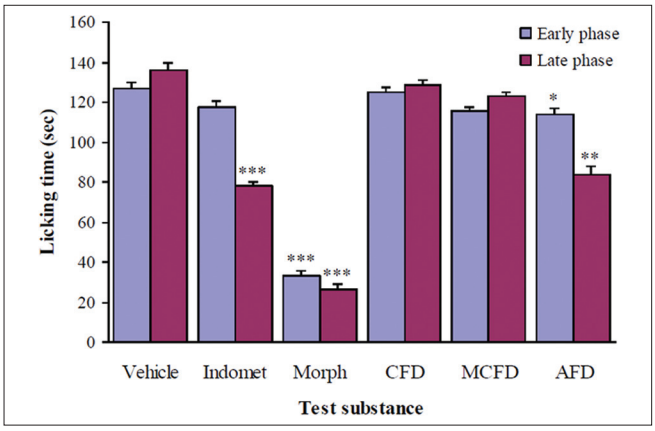

Figure 6: Antinociceptive activities of fractions of *Dicliptera laxata* (200 mg/kg, p.o.), morphine (morph, 5 mg/kg, s.c.) and indomethacin (indomet, 10 mg/kg, p.o.) compared with the vehicle on the early and late phases of formalin-induced nociception in mice; the values represent means ± SEM, $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle; AFD = aqueous; MCFD = methanol–chloroform (1:3) and CFD = chloroform fractions of *D. laxata*

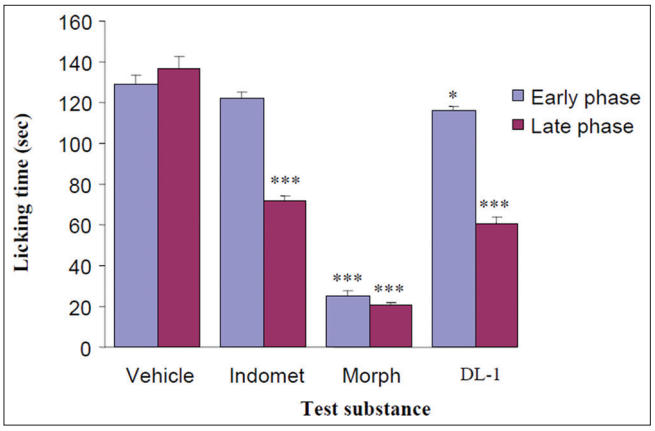

Figure 7: Antinociceptive activities of the isolated compound of *Dicliptera laxata* (100 mg/kg, p.o.), morphine (morph, 5 mg/kg, s.c.) and indomethacin (indomet, 10 mg/kg, p.o.) compared with the vehicle in the early and late phases of formalin-induced nociception in mice; the values represent means ± SEM, $n = 6$, $^{*}P < 0.05$, $^{***}P < 0.001$, compared to vehicle; DL-1 = compound isolated from *D. laxata*

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