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Short Communication

Phytochemical and analgesic activity of root crude extracts of D*icoma niccolifera* wild (Asteraceae)

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ABSTRACT

The study aimed at evaluating the phytochemical profile and anti-nociception activity of root crude extracts of *D. niccolifera*, a plant commonly used to alleviate painful conditions by local communities. Standard phytochemical screening tests revealed presence of tannins, alkaloids, flavonoids, terpenoids, reducing sugars, cardiac glycosides and anthraquinones. Anti-nociception was assessed using the hot plate model on Swiss Albino mice. Mice intraperitoneally injected with root crude extract showed that the *D. niccolifera* root extract had analgesic activity by taking longer to react to the thermal stimulus than the control group. The extract higher doses of 500 and 1000mg/kg showed peak mean latency times of 2.39 and 2.12 seconds respectively. These latency times were found to be significantly different ($p<0.05$) from the control. The anti-nociception activity may be attributed to the phenolic compounds in the extract. The study validates the use of *D. niccolifera* in managing painful conditions.

INTRODUCTION

The genus *Dicoma* Cass. (Asteraceae) comprises approximately 35 species, 16 of which are confined to southern Africa (Ortiz, 2001). Seven species are recorded for Zimbabwe (Wild, 1972). Pharmacological studies on *Dicoma* have focused on the widely distributed and highly polymorphic *Dicoma anomala* which is known to have several ethno-medicinal uses. The tubers of *Dicoma* species are commonly used to treat the following painful conditions: backache, toothache, sores and wounds (Watt and Breyer-Brandwijk, 1962), abdominal pains, general body pains, sore throat (Gelfand *et al.* 1985; Drummond *et al*., 1975) and stomach complaints (Kokwaro, 1976). Previous phytochemical studies on *D. anomala* and *D. tomentosa* have revealed the presence of several bioactive compounds including sesquiterpene lactones known to exhibit anti-tumor, cytotoxic, anti-microbial (Rodriguez *et al*., 1976), antiplasmodial (Becker *et al.,* 2011), anti-inflammatory, anticancer and antibacterial activities (Steenkamp *et al*., 2004; Khalid *et al.*, 1995).

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Dicoma niccolifera is a near endemic mostly confined to serpentine soils of the Great Dyke Mountains of Zimbabwe, but also known to occur on non serpentine areas from one locality in Mutare in the east of the country and another site near Lusaka in neighbouring Zambia.

The plant is reported to be a heavy metal hyperaccumulator (Wild, 1974) and has been studied for its potential in phytoremediation (Brooks and Yang, 1984). *D. niccolifera* is a straggling prostrate bushy perennial herb bearing a woody taproot. The stems scramble across the ground, forming small mats of narrow, grey-green leaves covered with dense white hairs.

Flower heads are variable in number, terminal on branches or short shoots and are subtended by showy spiky involucral bracts. Outer florets are sterile and lack corollas while the inner are fertile and bear pale purplish corollas. *D. niccolifera* is known to be used in pain alleviation (Mavi, 1996), but its phytochemistry and pharmacological activities have never been investigated.

The present study evaluates the phytochemical composition of the species and at the same time tests its anti-nociception activities.

MATERIAL AND METHODS

Plant material

The woody taproots of *D. niccolifera* were dug out from several plants growing at the Mutorashanga Pass, Great Dyke, in Zimbabwe on the 19th December 2011.

The plant was identified by taxonomists in the Department of Biological Sciences and a voucher specimen was prepared and deposited in the University of Zimbabwe teaching herbarium. The cleaned root material was chopped into smaller pieces and sun-dried for two weeks.

Crude extraction

The dried root samples were ground into a fine powder using a hand held blender. Some 10 g of ground material were placed in a thimble and extracted with 200 ml analytical grade methanol in a Soxhlet apparatus for 16 h in a water bath at 80^0 C. The crude extract was then concentrated using a rotary evaporator at 40^0C under reduced pressure.

Phytochemical screening

Standard qualitative methods (Sofowora,1983) were adopted for phytochemical screening. The crude extract was tested for phytochemical constituents using the following tests and reagents: reducing sugars with Fehlings test, anthraquinones with Borntragor's test, terpenoids with Salkowski test, flavonoids with ammonia and suphuric acid, saponins with foam test, tannins with Ferric Chloride test, alkaloids with Mayer's and Draggendorff's tests and cardiac glycosides with Keller- Killian's test. The phytochemicals were identified by characteristic colour changes.

Anti-nociception activity

Animals

Adult Swiss albino male mice (18-24 g) were used for this experiment. The mice were obtained from the Animal House at Central Veterinary Laboratories in Harare, Zimbabwe. The animals were housed in standard cages, and were allowed free access to standard pellets and water. All experiments were carried out with strict adherence to ethical guidelines (Zimmerman, 1983).

Hot Plate test

The anti-nociception activity was evaluated using the Hot Plate test (Wilson *et al*., 2003). The experimental mice were fasted for 18 h and the hot plate latency of each determined by placing each mouse in a beaker on a hot plate maintained at a temperature between 52^0C and 55^0C. The time between placement of the mouse on the hot-plate and the occurrence of either a hind paw lick or a jump off the surface was recorded as the hot-plate latency. All mice with baseline latencies greater than 15 seconds were excluded from the experiment following recommendations by (Nkomo *et al.,* 2010). Asprin (200mg/kg), distilled water (10ml/kg) and root extract concentrations of 1000, 500, 250 and 125mg/kg were then administered intraperitoneally using a syringe. For each extract concentration and control, 4 mice were used as replicates in a group. Hot plate latencies were determined at 30 minute intervals for 2½ h.

Data analysis

For each group, the average mice reaction times were determined, and the percentage increase in pain threshold P calculated using the following formula: $\mathbf{P} = \{(Pt - Po)/Po\} \times 100$, where **Pt** = pain threshold at time, **t**, and **Po** = pain threshold at time zero (**0**). A graph of the percentage increase in pain threshold was plotted against time. The results are presented as mean ± SEM. Data were statistically analyzed by analysis of variance (ANOVA) and Student t-test using Excel 2007. Levels of significance were set at $p < 0.05$.

RESULTS

Phytochemical screening

The result of the phytochemical screening revealed the presence of tannins, alkaloids, terpenoids, reducing Sugars, cardiac glycosides, anthraquinones and flavonoids (Table 1). Tests for saponins yielded negative results.

Table. 1: Results of phytochemical screening tests on methanol extract of *D. niccolifera* root extract.

Test	Observation
Reducing sugars	+
Terpenoids	++
Anthraquinones	++
Flavonoids	++
Saponins	_
Cardiac glycosides	++
Alkaloids	++
Tannins	++

Key - = negative, + = slightly present, ++ = present

Hot plate test

The effect of various *D. niccolifera* extracts on hot plate latencies in mice is shown in Table 2. Mice treated with the root extract and standard drug (Aspirin) showed longer latency times than the negative control group, with the higher doses of 500 and 1000mg/kg showing high mean latency times of 2.39 and 2.12 seconds respectively and the standard 4.05 seconds.

The percentage increase in mice reaction times over time for the different experimental groups is shown in Figure 1. The mice groups reached their peak reaction times at different times with the standard and highest extract dose of 1000mg/kg peaking within 30 minutes, 500 mg/kg after 1½ hours, 250 mg/kg after 2½ hours and the 125 mg/kg dose after 2 hours. Generally the reaction times were much lower than the standard drug and appeared to be dose dependent.

The ANOVA results showed that there were significant differences ($p<0.05$) between the negative control and the treatments. T-tests showed that there were significant differences in hot latencies between the control and the highest doses of 500 mg/kg ($p=0.044$, $p<0.05$) and 1000 mg/kg ($p=0.023$, $p <0.05$) and that there were no significant differences with the lower doses of 250mg/kg ($p=0.132$, $P>0.05$) and 125 mg/kg ($p=0.176$, $p>0.05$).

Table. 2: Mean reaction times of mice injected with different concentrations of *D. niccolifera* root extracts (n=4, ±SEM).

Time	Reaction times(s)/*Increase in pain threshold (%)					
	Distilled water	Aspirin	Extract concentrations (mg/kg)			
			1000	500	250	125
0	0.43±0.12	1.26±1.75	0.73±0.47	0.85±0.55	0.49±0.14	0.58±0.25
30	0.63±0.41	7.23±10	2.12±1.82	1.60±0.73	0.71±0.14	0.75±0.158
	46.5*	473.8*	190.4*	88*	44.9*	29.3*
60	0.60±0.21	4.05±4.6	1.16±0.74	0.73±0.13	1.07±0.76	0.68±0.11
	39.5*	221.4*	58.9*	-14.1*	118.4*	17.2*
90	0.62±0.21	1.99±1.78	1.67±1.21	2.39±1.97	0.88±0.396	0.81±0.064
	44.2*	57.9*	128.8*	181.2*	79.6*	39.7*
120	0.66±0.04	1.57±0.49	1.57±0.83	0.71±0.02	0.74±0.12	1.13±0.60
	53.5*	24.6*	115.1*	-16.5*	51.0*	94.8*
150	0.73±0.29	0.94±0.37	0.78±0.08	1.80±1.12	1.29±0.34	0.75±0.08
	67.7*	25.4*	6.9*	111.8*	163.3*	29.3*

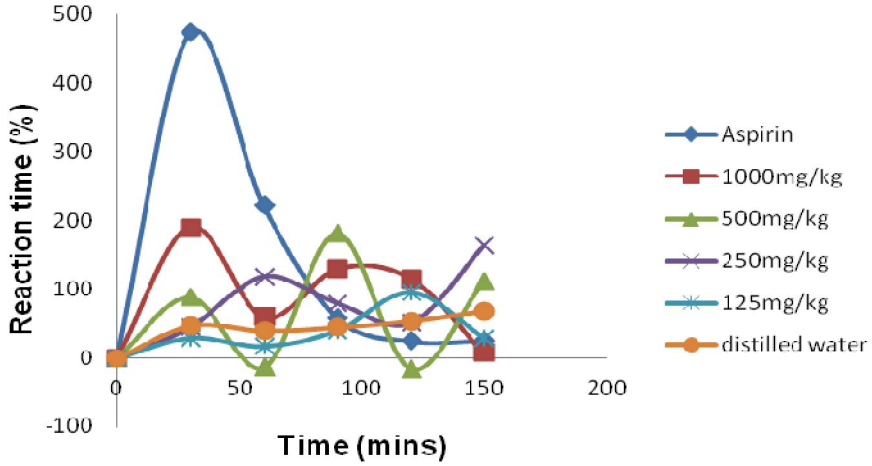

Fig. 1: Percentage increase in reaction time in mice treated with different dose levels of *D. niccolifera* root extract.

DISCUSSION

The hot plate test is one of the models used in elucidating centrally mediated antinociceptive responses (Sabina *et al.*, 2009) and any agent that causes a prolongation of the hot plate latency is considered to be acting centrally (Ibronke and Ajiboye, 2007). The results presented in this study show that intraperitoneal administration of *D. niccolifera* extract increased pain threshold in mice in a similar way to Aspirin, a standard Nonsteroidal anti-inflammatory drug (NSAID), which indicates that the activity may be through a centrally mediated analgesic mechanism. NSAIDs produce analgesia through the inhibition of the synthesis and release of prostaglandin thereby reducing the sensitivity of neurons to pain stimuli (Prempeh and Mensa-Attipoe, 2008). However, prostaglandin could not be implicated in the mediation of pain in this study since responses of mice to the thermal stimulus occurred within 30 minutes, a time too short to permit the release of prostaglandins, which are normally released 2 h after the induction of inflammation. This suggests the involvement of other chemical mediators in the alleviation of pain.

From the activity time profiles of the extracts (Figure 1) it appears that higher doses of extract are required to achieve reaction times comparable to those of the standard drug. The active principle responsible for analgesia probably occurs in minute quantities in the crude extracts and doses higher than the 1000 mg/kg are needed for the extracts to be effective.

The observation that the reaction times of mice injected with the various extracts peak at different times may be a result of different rates of absorption and build-up of the extracts in the plasma. Similar observations were made by (Prempeh and Mensa-Attipoe, 2008). Screening for phytochemicals in plants is important as a first step in elucidating the pharmacological properties of a plant species.

The phytochemicals identified in *D. niccolifera* belong to large diverse groups with varied pharmacological activities. Analgesic effects have already been established in flavonoids, tannins and alkaloids (Musa *et al*., 2008; Zulfiker *et al*., 2010) therefore it is possible that the anti-nociceptive effects observed in the extract may be attributed to its phytochemical constituents. Studies on other *Dicoma* species like *D. anomala (*Becker *et al., 2011), D. tomentosa (*Khalid *et al., 1995), D. capensis*, *D. zeyheri(*Van der Merwe, *2008)* yielded similar phytochemical profiles.

CONCLUSIONS

Methanolic root extract of *D. niccolifera* exhibits analgesic activities due to the presence of phenolic compounds. However, further studies are required to elucidate the precise mechanism of analgesia and to isolate the active principle. This study has validated the traditional use of *D. niccolifera* root extract in pain alleviation.

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