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Journal of Ethnopharmacology 75 (2001) 117–124

Journal of ETHNO-PHARMACOLOGY

www.elsevier.com/locate/jethpharm

Analgesic and antipyretic effects of *Dodonaea angustifolia* and *Salvia africana-lutea*

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Received 20 August 1999; received in revised form 30 November 2000; accepted 5 December 2000

Abstract

Water extracts of *Dodonaea angustifolia* L. and *Salvia africana-lutea* L., were investigated for analgesic and antipyretic activities using acetic acid writhing and hot plate tests, and lipopolysaccharide (LP)-induced pyrexia test in mice and rats, respectively. *D. angustifolia* and *S. africana-lutea* significantly inhibited acetic acid-induced writhing and also significantly delayed the time of reaction of mice to thermal stimulation produced by the hot plate. *D. angustifolia* and *S. africana-lutea* significantly reduced fever induced by LP. Paracetamol produced similar effects to *D. angustifolia* and *S. africana-lutea* on the acetic acid-induced writhing but has no effect on hot plate-induced nociception and on pyrexia produced by LP. These data indicate the analgesic and antipyretic potential of *D. angustifolia* and *S. africana-lutea*.

Keywords: Analgesic activity; Antipyretic activity; *Dodonaea angustifolia*; *Salvia africana-lutea*

1. Introduction

The Western Cape Province in South Africa is endowed with numerous plant species which are used for the treatment of various ailments because of their medicinal properties. These include *Dodonaea angustifolia* L. and *Salvia africana-lutea* L. These plants are widely distributed and are found in the wild and nature reserves within the Province. *D. angustifolia* belongs to the family, Compositae, and known locally in Afrikaans as 'ysterhout' and in Venda as 'mutatavhana'. The leaves, bark and roots of the plant are used to treat diarrhoea, croup, fever and painful conditions by traditional medicine practitioners (Mayeng, Oral Communication; van Wyk et al., 1997). *S. africana-lutea* belongs to the family, Labiatae, and known locally in Afrikaans as 'geelblom-salie'. An infusion of the plant had been used by Europeans in the Province for cold and is said to be diaphoretic (Watt and Breyer-Brandwijk, 1962). The Nama use a decoction of the plant for coughs, cold and female ailment (Watt and Breyer-Brandwijk, 1962). Traditional medicine practitioners in the Province use the leaves, in decoction form, to treat headache, fever and digestive disorders (Mayeng, Oral Communication). However, there are no known scientific information or data to validate the claim by the traditional medicine practitioners about the effectiveness of these plants. This study, therefore, intends to investigate the analgesic and antipyretic activities of *D. angustifolia* and *S. africana-lutea* by studying the effects of water extracts of the plants on nociception induced by acetic acid and hot-plate, and on fever induced by lipopolysaccharide (bacterial endotoxin), from *Salmonella typhosa*.

2. Materials and methods

2.1. Plant material

The plants were collected from the Cape Flats Nature Reserve, Bellville, South Africa. The taxonomic identity of the plants was verified by Mr Franz Weitz, a taxonomist, Department of Botany, University of the Western Cape. Voucher specimens of *D. angustifolia*

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0378-8741/01/$ - see front matter
PII: S0378-8741(00)00395-0

(TRAD-1), and *S. africana-lutea* (TRAD-2), were deposited in the University of the Western Cape Herbarium.

2.2. Preparation of plant extracts

Five hundred grams of leaves of *D. angustifolia* or *S. africana-lutea* were washed with 1000 ml of distilled water and rinsed twice with 500 ml of distilled water. They were then dried in a ventilated oven (Memmert oven) at 40°C for 48 h. The dried plant material was milled to a fine powder using the Waring Commercial laboratory blender. To prepare the water extract, 20 g of dried powder of each plant material was refluxed in 500 ml of distilled water for 1 h. The mixture was allowed to cool and filtered. The resultant solution or filtrate was then freeze-dried (LSL Secfroid SR, Model 3021, Switzerland) for 24 h and a yield of about 5.5 g was obtained (27.5%w/w). The dried extracts were freshly dissolved and made up to the appropriate volume with distilled water just before use on each day of the experiment. The extract solution was injected intraperitoneally (i.p.), in a volume of 1 ml/100 g of animal.

Table 1
Effect of water extracts of *D. angustifolia* and *S. africana-lutea* on acetic acid-induced writhing in mice

Treatment groups	Dose (mg/kg)	Writhing (mean ± SEM)
Normal saline	–	35.33 ± 2.29
D. angustifolia	50	22.83 ± 8.37
	100	19.17 ± 5.40[a]
	200	1.67 ± 0.90[c]
S. africana-lutea	50	26.72 ± 1.98
	100	25.21 ± 2.34
	200	13.62 ± 3.78[c]
	400	14.88 ± 4.68[b]
Paracetamol	300	1.17 ± 0.50[c]

[a] $P<0.01$ compared with acetic acid control, Student's t-test ($n=6$).
[b] $P<0.005$ compared with acetic acid control, Student's t-test ($n=6$).
[c] $P<0.001$ compared with acetic acid control, Student's t-test ($n=6$). Writhing is expressed as number per 15 min.

Table 2
Effect of water extracts of *D. angustifolia* and *S. africana-lutea* on hot-plate induced nociception in mice

Treatment groups	Dose	Response time (s)		
	mg/kg	0 min	15 min	30 min
Normal saline	–	5.17 ± 0.15	5.33 ± 0.33	5.17 ± 0.15
D. angustifolia	50	5.83 ± 0.60	5.83 ± 0.44	6.00 ± 0.33
	100	5.17 ± 0.15	9.50 ± 0.46[b]	7.67 ± 0.61[a]
	200	4.83 ± 0.15	5.33 ± 0.38	8.67 ± 0.45[b]
S. africana-lutea	100	6.90 ± 0.08	6.95 ± 0.09	6.94 ± 0.06
	200	5.63 ± 0.12	9.81 ± 0.06[b]	9.91 ± 0.07[b]
	400	6.15 ± 0.01	10.35 ± 0.03[b]	10.34 ± 0.07[b]

[a] $P<0.01$ compared with saline control, Student's t-test.
[b] $P<0.001$ compared with saline control, Student's t-test. The response time in seconds was expressed as mean ± SEM ($n=6$).

2.3. HPLC

The chromatographic system used includes Beckman HPLC system consisting of double pump Programmable Solvent Module model 126, Diode Array detector Module model 168, Samsung computer 386 with management system, Gold (Gold V601) software supplied by Beckman; Column C18 Bondapak 5 μm and dimensions (250 × 4.6 mm^2). The chromatographic conditions are as follows: Mobile phase, solvent A: 1% acetic acid (CH_3COOH); solvent B: methanol (MeOH); Mode: gradient; flow rate 1 ml/min; injection volume: 10 μl; detector: UV at 270 nm; reference standard: Rutin (2.5 g dissolved in 100 ml MeOH). The run time was 30 min.

2.4. Animals

Male albino mice bred in the Animal House, Department of Pharmacology, University of the Western Cape, Bellville, South Africa, weighing 20–30 g and male albino rats bought from the Medical Research Council, Cape Town, South Africa, weighing 135–200 g were used. The animals were kept in groups of six per cage, fasted for 16 h and had free access to water. Each animal was used for one experiment only.

2.5. Drugs and chemicals

Paracetamol (4-Acetamidophenol, Sigma Chemical Co), was dissolved in a minimum amount of propylene glycol (Heynes Mathew), and made up to the appropriate volume with normal saline. LP (bacterial endotoxin), from *Salmonella typhosa* (Sigma Chemical Co), was dissolved in normal saline to an appropriate volume. Paracetamol was injected i.p., and LP injected intramuscularly (i.m.) into the thigh of the rat, all in a volume of 1 ml/100 g of animal.

2.6. Analgesic activity test

2.6.1. Acetic acid writhing in mice

The methods of Koster et al. (1959) and Williamson et al. (1996) were used. Mice were used in groups of six per dose of plant extract or drug. The animals were

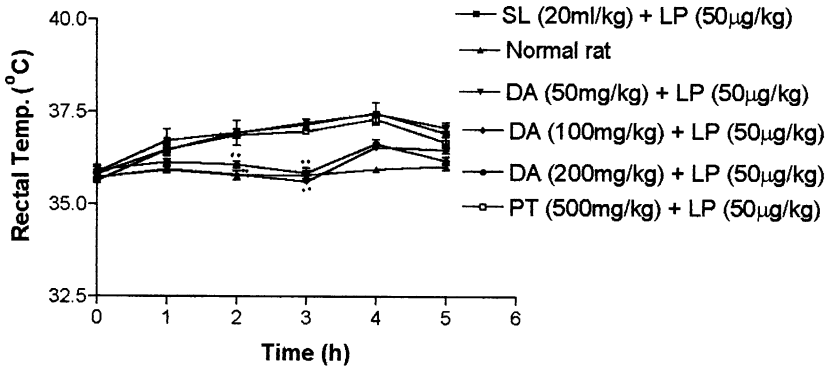

Fig. 1. Effect of *D. angustifolia* (DA) on pyrexia induced by LP, in rats. Pyrexia was induced by i.m. injecton of LP (50 μg/kg), after obtaining the rectal temperature of the rats at 0 h. Different doses of water extract of DA, paracetamol (PT), and saline (SL), were administered i.p. 15 min before LP injection. Rectal temperature of normal rats and those pretreated were measured at different times. Each point and vertical bar represent the mean and SEM ($n = 6$). (b = $P < 0.005$ vs. LP, Student's t-test).

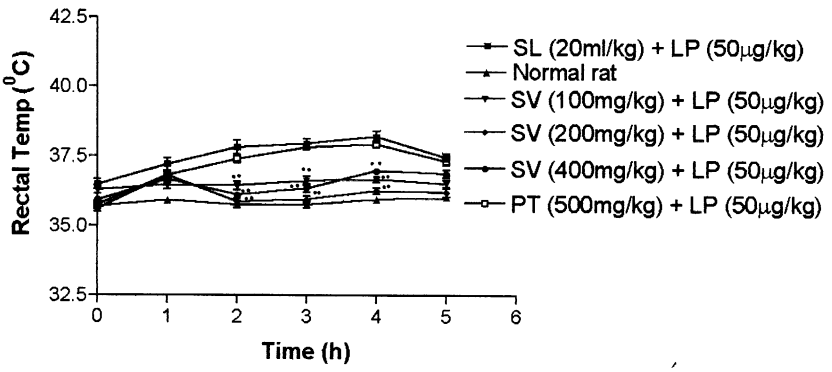

Fig. 2. Effect of *S. africana-lutea* (SV), on pyrexia induced by LP, in rats. Pyrexia was induced by i.m. injecton of LP (50 μg/kg), after obtaining the rectal temperature of the rats at 0 h. Different doses of water extract of SA, paracetamol (PT), and saline (SL), were administered i.p. 15 min before LP injection. Rectal temperature of normal rats and those pretreated were measured at different times. Each point and vertical bar represent the mean and SEM ($n = 6$). (b = $P < 0.001$ vs. LP, Student's t-test).

kept singly in transparent perspex cages ($25 \times 15 \times 15$ cm^3) for 30 min to acclimatize to their new environment before commencement of the experiment. Animals were pretreated with plant extract (i.p.) or paracetamol (i.p.) for 15 min prior to injecting them i.p. with 0.2 ml of 2% acetic acid. Five minutes after the i.p. injection of acetic acid, the number of writhes exhibited by mice was counted for 15 min. Control mice received normal saline in a volume of 1 ml/100 g of animal and the acetic acid experiment was repeated. The doses and pretreatment times were obtained from preliminary studies in our laboratory. All experiments were carried out between 08:00 and 17:00 h in a quiet laboratory with an ambient temperature of 20 ± 1°C.

2.6.2. Hot-plate test in mice

The methods of Eddy and Leimback (1953) and Williamson et al. (1996) were used. Mice were placed individually in a 2 l glass beaker placed on a thermostatically controlled hot plate, HC500 (Bibby Sterilin Ltd, England) maintained at 55°C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The time taken for the mice to react in this fashion was obtained using a stopwatch. The animals were first tested for the paw-lick or jump response and only those that reacted after 4 s were used for the experiment. Mice were tested in groups of six per dose before and 15 and 30 min after i.p. injection of plant extracts or paracetamol. Control animals received equal volume of normal saline and the experiment was repeated. All experiments were carried out between 08:00 h and 17:00 h in a quite laboratory with an ambient temperature of 20 ± 1°C. A cut off time of 60 s was used to avoid harm to the animals.

2.7. Antipyretic activity test

The method of Santos and Rao (1998) was modified and used for the assessment of the antipyretic activity of the plant extracts. Rats were used in groups of six

per dose of plant extract or drug. Animals were kept singly in transparent perspex rat cages for 30 min to acclimatize to their new environment before plant extract or drug treatment. Fever was induced with 50 μg/kg of LP injected i.m. into the thigh of the rats. The animals were pretreated for 15 min with plant extracts, saline or paracetamol, all injected i.p. before the administration of LP. Rectal temperature was measured before the pretreatment of animals and at 1 h intervals for 5 h after the administration of the endotoxin with a digital thermometer (Model BAT-12, Bailey Instruments Inc, USA) inserted 2.3 cm into the rectum of the rats. The rectal temperature of normal rats (normothermic) without any treatment was also measured at 1 h intervals for 5 h. The effects of the different doses of plant extracts used were also studied alone on the rectal temperature of normothermic rats. The control experiment involved animals treated with saline plus LP. All experiments were carried out between 08:00 h and 17:00 h in a quiet laboratory with an ambient temperature of 20 ± 1°C.

2.8. Phytochemical screening

The plant extracts were screened for various constituents using standard screening tests and conventional protocols (Ikhiri et al., 1992).

2.9. Statistical analysis

The data was presented as mean ± standard error of the mean (SEM). It was analysed using paired Student's *t*-test. *P* values of less than 5% ($P < 0.05$), were considered to be significant.

3. Results

3.1. Analgesic activity tests

3.1.1. Acetic acid writhing test

0.2 ml of 2% acetic acid produced a substantial number of writhes in control animals pretreated with

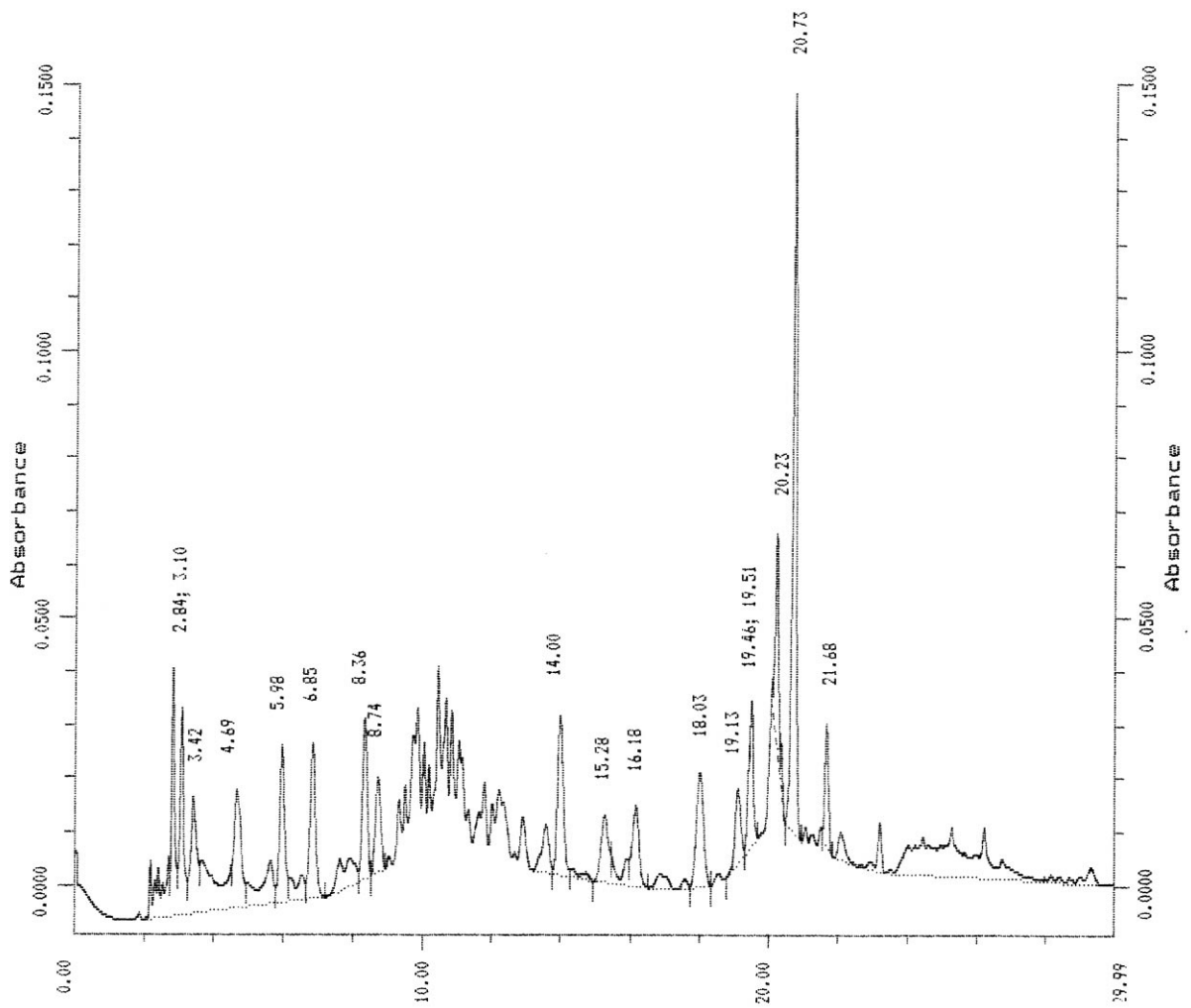

Fig. 3. HPLC fingerprint of water extract of *D. angustifolia*.

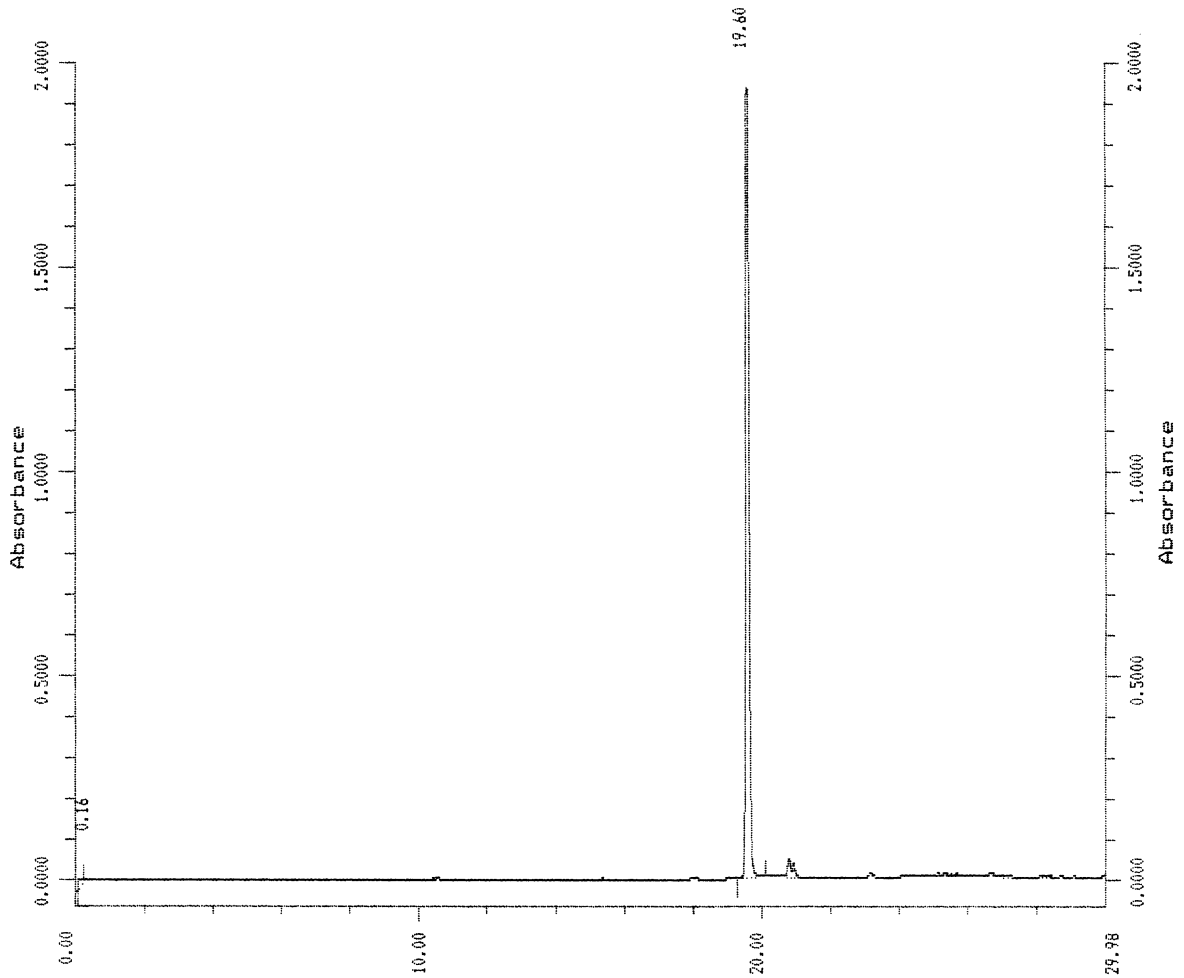

Fig. 4. HPLC fingerprint of Rutin (reference standard for *D. angustifolia*).

normal saline. Water extract of *D. angustifolia* (50–200 mg/kg, i.p.) dose dependently and significantly inhibited the writhes induced by 2% acetic acid. 50 mg/kg (i.p.) of *D. angustifolia* did not affect the acetic acid-induced writhes to any significant extent. Water extract of *S. africana-lutea* (200–400 mg/kg, i.p.) significantly reduced the number of writhes induced by 2% acetic acid whereas 50–100 mg/kg (i.p.) did not alter the number to any significant extent (Table 1). There was no change in the gross behaviour of the animals during the pretreatment period with the plant extracts.

3.1.2. Hot-plate test

The pain threshold was considered to have been reached when the animals lifted and licked their paws or attempted to jump out of the 2 l beaker placed on the hot plate at 55°C. The reaction time of mice to hot-plate thermal stimulation was obtained before (0 min) and 15 and 30 min after pretreatment with normal saline, plant extracts or drug. Water extract of *D. angustifolia* (100 mg/kg, i.p.) significantly delayed the response of animals to hot-plate thermal stimulation 15 and 30 min after treatment. There was no change in reaction time 15 and 30 min after treatment with 50 mg/kg (i.p.) of *D. angustifolia*. 200 mg/kg (i.p.) of *D. angustifolia* significantly delayed the response time to hot-plate thermal stimulation only 30 min after treatment. Water extract of *S. africana-lutea* (200–400 mg/kg, i.p.) significantly delayed the response of mice to hot-plate thermal stimulation. This effect was seen 15 and 30 min after administration. 100 mg/kg (i.p.) of *S. africana-lutea* did not alter the response time to hot-plate thermal stimulation to any significant extent at the different times of measurement (Table 2). Paracetamol (300–500 mg/kg, i.p.), a standard analgesic and antipyretic drug, did not significantly alter the reaction time to hot-plate thermal stimulation 15 and 30 min after treatment (results not shown).

3.2. Antipyretic activity test

LP (50 μg/kg, i.m.) produced a time-dependent increase in rectal temperature of normal saline pretreated rats which reached a maximum at 4 h after which there

was a decrease. The maximum mean rectal temperature was 37.45 ± 1.1°C. The temperature increased by 1.67°C at 4 h. At the same period, the maximum mean rectal temperature of normothermic rats was 35.95 ± 0.04°C, thus, LP significantly increased the rectal temperature ($P < 0.005$). Water extract of *D. angustifolia* (100–200 mg/kg, i.p.) significantly reduced LP induced increase in rectal temperature with a maximum effect at 3 h ($P < 0.005$), after which there was a gradual increase up to 5 h. 100 mg/kg (i.p.) of the extract kept the maximum mean rectal temperature at 35.62 ± 0.05°C at 3 h compared to that of normothermic rats (35.77 ± 0.13°C) and saline plus LP (37.15 ± 0.05°C). The maximum mean rectal temperature with *D. angustifolia* (200 mg/kg, i.p.) at 3 h was 35.83 ± 0.13°C. 50 mg/kg (i.p.) of the extract and paracetamol (500 mg/kg, i.p.) did not significantly alter the fever induced by LP over 5 h (Fig. 1). In the second study, LP (50 g/kg, i.m.) significantly increased the rectal temperature of saline pretreated animals with a maximum mean rectal temperature of 38.18 ± 0.18°C at 4 h after which there was a decrease up to 5 h. The mean rectal temperature increased by 1.65°C at 4 h. Water extract of *S. africana-lutea* (100–400 mg/kg, i.p.) significantly reduced the increased rectal temperature produced by LP ($P < 0.001$), over a period of 5 h with the maximum reduction at 2 h. The maximum mean rectal temperatures produced by LP in the presence of 100 mg/kg, 200 mg/kg and 400 mg/kg of *S. africana-lutea* were 36.43 ± 0.13°C, 35.87 ± 0.1°C and 36.1 ± 0.07°C, respectively. Paracetamol (500 mg/kg, i.p.) did not significantly affect the fever induced by LP (Fig. 2). The different doses of *D. angustifolia* and *S. africana-lutea*, when used alone, did not alter the rectal temperature of normothermic rats (results not shown).

4. Discussion and conclusion

The results of the phytochemical studies carried out in our laboratory using the method of Ikhiri et al. (1992), show that the leaves of *D. angustifolia* contain

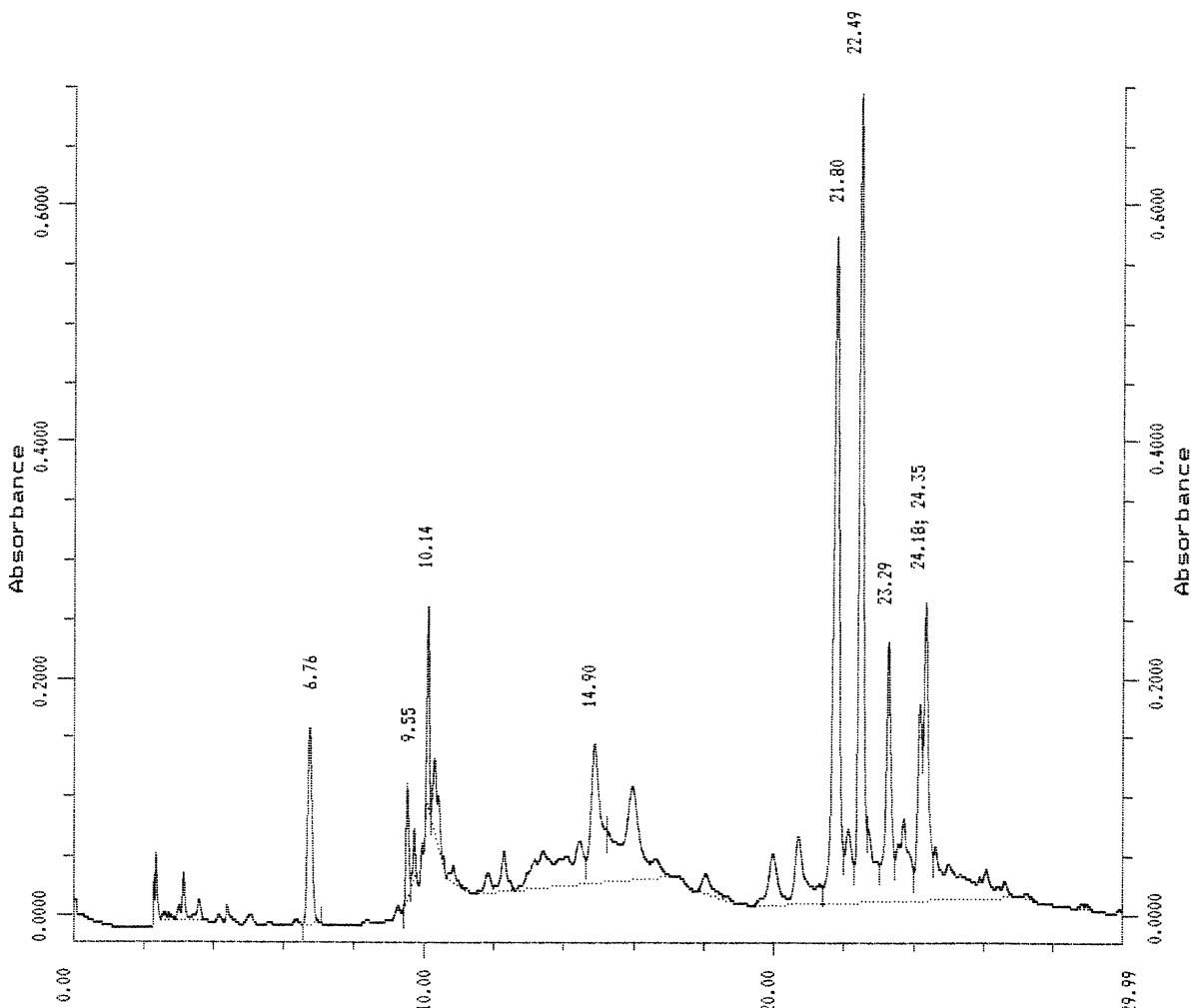

Fig. 5. HPLC fingerprint of water extract of *S. africana-lutea*.

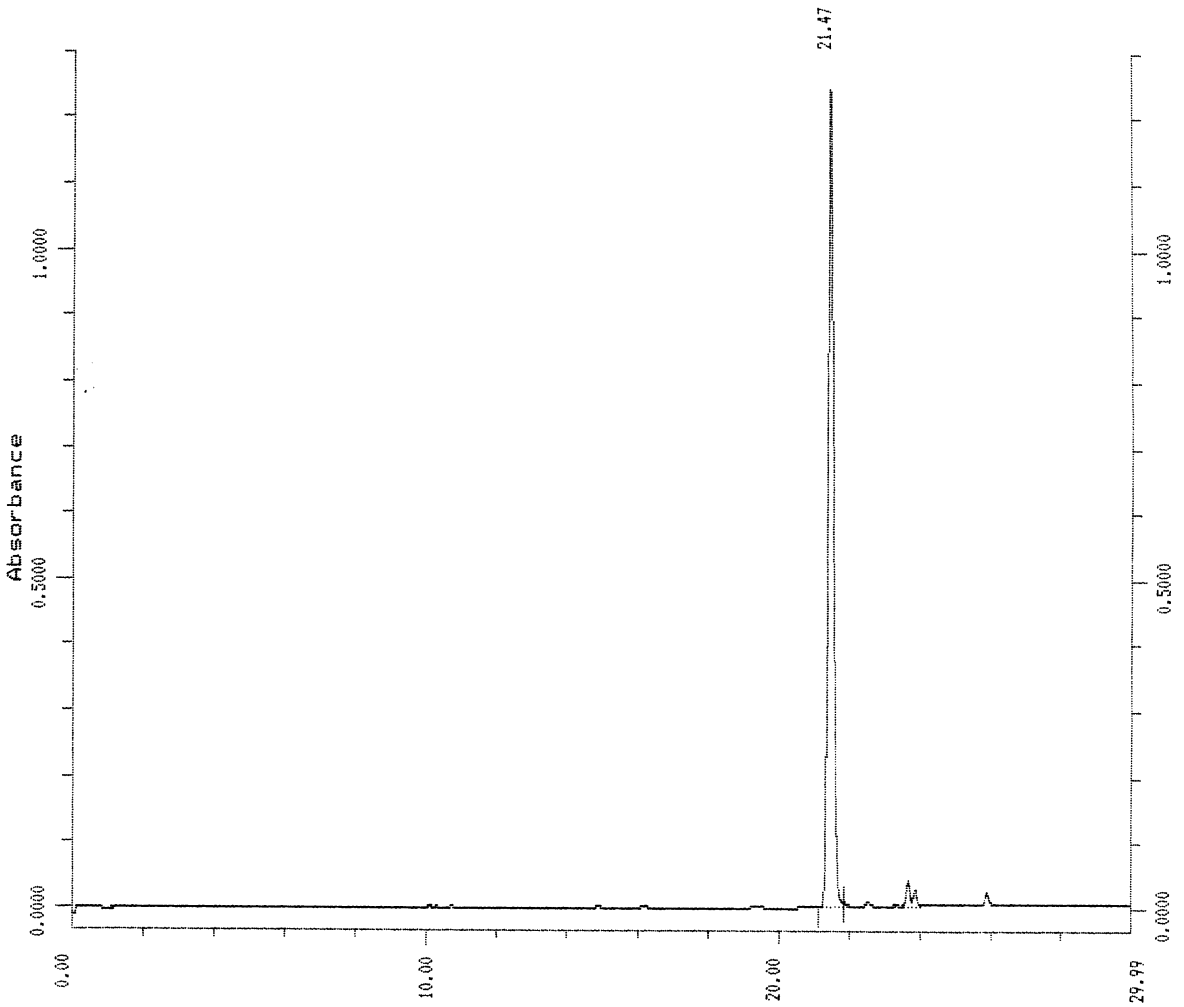

Fig. 6. HPLC fingerprint of Rutin (reference standard for *S. africana-lutea*).

flavonoids, reducing sugars, alkaloids, saponins and tannins while *S. africana-lutea* contain reducing sugars, quinones, saponins and tannins. The HPLC fingerprints of the water extract of *D. angustifolia* show major peaks at the retention times (min) of 2.84, 5.98, 6.85, 8.36, 14.00, 20.23 and 20.73 with the major peak for the reference standard, Rutin appearing at the retention time (min) of 19.60. The major peaks from the water extract of *S. africana-lutea* appeared at the retention times (min) of 6.76, 10.14, 21.80, 22.49, 23.29 and 24.35 with the major peak for the reference standard, Rutin appearing at the retention time (min) of 21.47 (Figs. 3–6).

The present study shows that water extracts of both *D. angustifolia* and *S. africana-lutea* significantly antagonised acetic acid-induced writhing and significantly attenuated the nociception produced by hot-plate thermal stimulation as well as reduced the pyrexia induced by LP from *Salmonella typhosa*. Paracetamol, a standard analgesic and antipyretic drug (Rang et al., 1995), also significantly antagonised the acetic acid-induced writhing but did not significantly affect the nociception produced by hot-plate thermal stimulation or the pyrexia induced by LP.

Acetic acid writhing and hot-plate tests are normally used to study the peripheral and central analgesic effects of drugs, respectively (Eddy and Leimback, 1953; Koster et al., 1959). In the present study, both *D. angustifolia* and *S. africana-lutea* attenuated the acetic acid writhing and hot-plate thermal stimulation. It is probable, therefore, that these plants could be producing their effects both peripherally and centrally.

Fever is thought to be produced by certain endogenous substances which include tumour necrosis factor-α (TNF-α) and prostaglandins (Kluger 1991). According to Kluger (1991) and Roth and Zeisberger (1995), LP produced fever in guinea-pigs and rabbits by stimulating the production of endogenous TNF-α. Paracetamol has been shown to suppress fever by inhibiting prostaglandin synthetase resulting in the blockade of synthesis of prostaglandin in the brain (Flower and

Vane, 1972). It is not surprising, therefore, that paracetamol (500 mg/kg, i.p.) did not alter LP-induced fever to any significant extent. The present study shows that water extracts of both *D. angustifolia* (100–200 mg/kg, i.p.) and *S. africana-lutea* (100–400 mg/kg, i.p.) attenuated pyrexia or increased rectal temperature caused by LP. It is tempting, therefore, to suggest that *D. angustifolia* and *S. africana-lutea* might partly be affecting TNF-α production to attenuate LP-induced pyrexia. It is significant to note that all the doses of *D. angustifolia* and *S. africana-lutea*, when used alone, did not alter the rectal temperature of normothermic rats.

In conclusion, the data obtained in this study indicate that *D. angustifolia* and *S. africana- lutea* may have both analgesic and antipyretic activities and affirm the claim by traditional medicine practitioners of their use in headache or some painful conditions and fever. However, further studies are necessary to fully elucidate the mechanism of action of the plants.

Acknowledgements

The study was financially supported by the Medical Research Council, Cape Town, South Africa. We wish to thank Ms C. Farmer, Senior Laboratory technician, and our project students for their technical assistance.

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