Full Length Research Paper

Studies on the use of *Zizyphus spina-christi* against pain in rats and mice

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*Zizyphus spina-christi* (Rhamnaceae) grows wild in tropical Africa and Asia and can be domesticated. It has folkloric usage in pain related ailments throughout these regions. In view of the claimed therapeutic potentials, investigation of the plant’s root bark was initiated in our laboratory. The plant material was first sequentially extracted with hexane, chloroform, ethylacetate and methanol, and in this report, a fraction (numbered) ZS-4D from the methanol extract eluted with 70:30% (chloroform : methanol) using flash column chromatography was apparently traced to be responsible for its main analgesic, and in addition, anti-inflammatory activities. The fraction (25, 50 and 100 mg/kg, i.p.) was tested on chemical (acetic acid-induced writhing, formalin), mechanical (analgesy-meter) and thermal (tail-flick) analgesic tests with the aim of elucidating both central and peripherally mediated action in rats and mice. Its anti-inflammatory action against egg albumin-induced hind paw oedema was also tested in rats. Results show that the fraction has some levels of dose related effect on all the models except the tail-flick test in which the activity was not statistically significant.

Key words: *Zizyphus spina-christi*, Fraction ZS-4D, pain, inflammation.

INTRODUCTION

Traditional herbal remedies have a long and popular usage in treatment of pain related ailments (Almeida et al., 2001). Interestingly, the World Health Organization have recommended the integration of traditional medicines proved to be useful into national health care programmes (WHO, 1976), set up guidelines for its study (WHO, 1991; 2001) and defined its role under what it termed ‘Traditional Medicine/Complementary and Alternative Medicine (TM/CAM)’ by developing a strategy to address issues of policy, safety, efficacy, quality, access and rational use (WHO Traditional Medicine Strategy 2002-2005). Traditional medicine practice is most popular in developing countries (Elisabet sky, 1991) and unique in its own way because it takes a holistic view of the patient’s situation (Jager, 2005). Several bioactive agents in modern pharmacopoeia were derived from products initially used in traditional medicine (Farnsworth, 1985; Astin, 1998; De Smet, 1998; De Silva, 2005). In view of the popular and widespread use of herbal products used in such practice, important technical aspects such as standardization and quality control need development (Fansworth, 1980; Bonati, 1991; De Smet, 1991; Elisabetesky and Shanley, 1994; Patwardhan, 2005), since some are suspected to be interacting with synthetic drugs (Ernst, 2000).

*Zizyphus spina-christi* Willd (Rhamnaceae) is a plant that grows wild in Asia and tropical Africa. The plant is originally of the Middle East south of the Euphrates and spread to Saharan Oases across Africa into the Sahel (Burkill, 1997). It has several physiological and morphological characteristics (Keay et al., 1964) that assures its ability to adapt to arid environment. It derived its name from kinnara, a sweet edible fruit produced from Kris’s thorn tree, one of the prickly/thorny shrubs found in Palestine believed to have been used for Christ’s crown of thorns (Arndt, 2000). The plant has versed medicinal and nutritional values (Dalzell, 1937; Hutchens, 1973; Duke, 1985; Al-Yahya, 1986; Eden Foundation, 1992;
Burkill, 1997; Al-Khalifa and Al-Arify, 1999). It was reported to exhibit antibacterial, anticancer, anti-diabetic, anti-nociceptive, antihypertensive, antiarrhoheal and CNS effect from previous studies (Fleurentin and Pelt, 1982; Friedman et al., 1986; Tanira et al., 1988; Shah et al., 1989; Hussain and Deeni, 1991; Glombitza., 1994; Ali-Shtayeh et al., 1998; Ali et al., 2001; Shahat et al., 2001; Adzu et al., 2001, 2002a, 2003; Lev and Amar, 2002; Nazif, 2002; Abdel-Zaher et al., 2005; Adamu et al., 2005; Abdel-Wahhab et al., 2007). Some useful phytochemicals that include flavonoid, tannins, lipids, terpenes, alkaloids, steroids and carbohydrates have been isolated from the plant (Grindley, 1948; Aynehchi and Mahoodian, 1973; Tschesche et al., 1974; Ikram and Tomlinson, 1976; Tawfik et al., 1978; Rizk, 1982; Ali et al., 1985; Al-Yahya, 1986; Shah et al., 1986; Abdel Galil and El-Jissry, 1991; Mahran et al., 1994; Weinges and Schick, 1995; Shahat et al., 2001).

Among the popular use of *Zizyphus spina-christi* in traditional medicine is in the treatment of pain and inflammatory related ailments across regions in which the plant grows. This includes Saudi Arabia (Shah et al., 1989; Al-Said, 1993), Oman (Ghazanfar and Al-Sabahi, 1993), Israel (Friedman et al., 1986), northern Nigeria (Adzu et al., 2001) and a related specie (*Z. jujuba*) used in Japan (Watanabe et al., 1973; Shibata and Fukushima, 1975; Morishita et al., 1987). Of recent, we initiated fraction-alization and pharmacological evaluation of useful components of the plant's root bark in line with the 31st WHO Assembly involving inventory, evaluation and standard-ization of traditional medicines (Farnsworth, 1980). A previous study (Adzu et al., 2001), which suggested potent anti-nociceptive activity prompted this work. In this report, we presented the potentials of a fraction (num-bered ZS-4D) against pain and inflammatory models in rats and mice. The root bark was first sequentially ex-tracted with four solvents along increasing polarity to give hexane (ZS-1), chloroform (ZS-2), ethylacetate (ZS-3) and methanol (ZS-4) extracts. The combined extract of each solvent was concentrated and evaporated to dryness. It gave yields of 4.623, 27.834, 4.443 and 245 g for hexane, chloroform, ethylacetate and methanol extracts respectively. The total yield of the extracts was 21.86% w/w of crude starting material.

**MATERIALS AND METHODS**

**Plant material**

*Z. spina-christi* materials were collected locally at Midiu, Adamawa State, Nigeria between April and May, 2004. It was authenticated at Taxonomy Unit, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen (NIPRD #4108) was deposited at the herbarium of the Institute. The root bark of the plant was carefully removed, cleaned and dried under shade. The dried root was grounded into powder and 1.29 kg of the powdered material sequentially extracted to obtain hexane (ZS-1), chloroform (ZS-2), ethylacetate (ZS-3) and methanol (ZS-4) extracts. The combined extract of each solvent was concentrated and evaporated to dryness. Some useful phytochemicals that include flavonoid, tannins, lipids, terpenes, alkaloids, steroids and carbohydrates have been isolated from the plant (Grindley, 1948; Aynehchi and Mahoodian, 1973; Tschesche et al., 1974; Ikram and Tomlinson, 1976; Tawfik et al., 1978; Rizk, 1982; Ali et al., 1985; Al-Yahya, 1986; Shah et al., 1986; Abdel Galil and El-Jissry, 1991; Mahran et al., 1994; Weinges and Schick, 1995; Shahat et al., 2001).

**Phytochemical test**

Phytochemical constituents present were screened using standard procedures (Harborne, 1998; Evans, 2002). TLC was carried out to determine spot zones which were viewed under UV light (254/365 nm Eagle Scientific Limited, UK).

**Flash column chromatography**

Twenty grams of the methanol extract (ZS-4) was mixed with 20 g of silica gel (Aldrich Chemicals; 230-400 Mesh) and loaded on top of a flash column (Still et al., 1978) containing another 150 g of silica gel. It was flush with hexane, and eluted in increasing polarity with hexane, combinations of chloroform and methanol, and metha-nol in multiples of 100 ml using an air pump (ABM, Germany). Each of the fractions consisting of 100 ml was individually collected. A total of 73 fractions were collected. Fractions detected to contain the same product using TLC were combined together, concentrated over water bath, and allowed to evaporate at room temperature. This results in 6 fractions (ZS-4A to ZS-4F). Fraction ZS-4D eluted with 70:30 (chloroform: methanol) which presented both analgesic and anti-inflammatory activity according to analgesic activity monitoring was thus fully investigated.

**Vacuum column chromatography**

The chromatography (O’Donnell et al., 2006) was carried out using Sinta funnel (Quickfit, England) attached to an air pump (ABM, Germany) to get enough of the fraction (ZS-4D) for pharmacological studies. Eighty grams of the methanol extract (ZS-4) was mix with 60 g of silica gel and flush with mixture of 75:25 (chloroform: methanol) followed by elution of 21 of the fraction of interest that is, 70:30 (chloroform:methanol). It was concentrated over water bath and allowed to dry at room temperature. This gave 13.01 g.

**Animals**

Swiss albino mice and Wistar rats of both sexes obtained from Animal Facility Centre, NIPRD, Abuja, were used for the study. They were housed in plastic cages with saw dust as beddings and given food and water *ad libitum*. The mice were used in accordance with NIH Guide for the Care and Use of Laboratory Animals; published by the US National Institute of Health-NIH Publication (No 83-23) revised (1985), ethical guidelines for investigation of experimental pain in conscious animal (Zimmermann, 1983) and NIPRD’s in-house Standard Operation Procedures (NIPRD-SOPs) on laboratory animal usage.
Chemicals, drugs and test agents

All the chemicals used for the extraction, fractionalisation and chromatography (hexane, chloroform, ethylacetate and methanol) were of analytical grade and were purchased from Sigma-Aldrich sales representative in Nigeria (Zayo International Limited, Jos, Nigeria). Other chemicals used are formaldehyde, Triton-X and acetic acid (BDH, England). Three dose levels of the fractions (25, 50 and 100 mg/kg, i.p.) were used throughout the study. The negative control received normal saline (10 ml/kg, i.p.) while acetylsalicylic acid (ASA) (100 mg/kg, i.p.) (Osadepe and Okoye, 2003) served as the positive control. ASA was chosen for the fact that it has anti-nociceptive action independent of their anti-inflammatory effect which does not have any delay of onset of action (Capetola et al., 2003). The safety of the methanol extract (ZS-4) was tested using Lorke's method (1983). Briefly, the extract was administered in geometric doses of 10, 100, 1000 and 2000 mg/kg, i.p. to four groups of mice. Another mouse was given normal saline to serve as the control and all the mice were kept under same conditions and observed for toxic signs, and mortality within 24 h was noted as index of acute toxicity. LD₅₀ was estimated from the square root of the lowest lethal dose and the highest non-lethal dose (Osadepe and Okoye, 2003; Vongtau et al., 2004).

Acute toxicity test

The safety of the methanol extract (ZS-4) was tested using Lorke's method (1983). Briefly, the extract was administered in geometric doses of 10, 100, 1000 and 2000 mg/kg, i.p. to four groups of mice. Another mouse was given normal saline to serve as the control and all the mice were kept under same conditions and observed for toxic signs, and mortality within 24 h was noted as index of acute toxicity. LD₅₀ was estimated from the square root of the lowest lethal dose and the highest non-lethal dose (Osadepe and Okoye, 2003; Vongtau et al., 2004).

Acetic acid-induced writhing

The test was carried out using the methods described by Koster et al. (1959) and Collier et al. (1968). Two sets (1 and 2) of five groups of mice (n = 5) were pretreated with ZS-4D, saline and ASA. Each mouse was injected with 0.7% of an aqueous solution of acetic acid (10 ml/kg, i.p.) after the predetermined time (30 and 60 min for sets 1 and 2 respectively) and placed in transparent observational cage. The number of writhes (abdominal constriction) exhibited by each mouse was cumulatively counted for 15 min after 5 min (lag time) of the aqueous acid injection (Loro et al., 1999) and the mean count of each group taken.

Formalin test

Dubuisson and Dennis (1977) method modified by Tijssen et al. (1992) was adopted. Five groups of rats (n = 5) were pretreated with either the fraction, saline or ASA. They were injected s.c. with 50 μl of 2.5% solution formalin into the sub-plantar surface of rat left hind paw 30 min after the treatment. Severity of pain was rated in two distinct phases for 1 h: the first phase (0 – 10 min) taking every two min and late phase (15 – 60 min) every 5 min using 3 prominent pain-induced behaviour in the following scoring manner: (0) normal weight baring on the injected paw; (1) light resting on the paw on the floor; (2) elevation of the injected paw and (3) for licking, biting or grooming of the injected paw. The mean (±SEM) of the readings were recorded as the pain score.

Anaesthesy-meter

The experiment was carried out using Ugo Basile Anaesthesy-Meter (No. 7200) on normal rats as described by Vongtau et al. (2004). Twenty five rats were grouped into five (n = 5) and treated with saline, ZS-4D or ASA. The rat paw was placed on a plinth under a cone-shaped pusher of the instrument and increased pressure app- lied to the middle dorsum of the rat’s left hind paw (Chipkin et al., 1983; Kitchen, 1984; Nguelfack et al., 2004). Stimulus was terminated and force threshold readings in grams (Anseloni et al., 2003) taken as soon as nociceptive response were elicited by the rats (Iwalewa et al., 2003; Srinivasan et al., 2003). Readings were taken at 0, 15, 30 and 60 min after treatment.

Tail-flick test

The study (D‘Amour and Smith, 1941), as subsequently modified for rats using hot water bath (Janssen et al., 1963; Asongalem et al., 2004a, 2004b; Rabanal et al., 2005; Sanchez-Mateo et al., 2006) was adopted with minor modification for our local laboratory settings. The test was carried out by immersing the terminal 2 cm of the rat tail in hot water contained in a 500 ml beaker maintained at 55 ± 1°C using a hot plate (Ugo Basile, Socrel DS-37). A thermometer was placed inside the water to monitor the temperature reading. Twenty five rats that shows response (withdrawing the tail) within 3s were selected and grouped into five (n = 5). The rats were then treated with either ZS-4D, saline or ASA and their responses taken at 30 and 60 min after treatment.

Anti-inflammatory activity

In this procedure, fresh undiluted egg albumin was used as in vivo model of acute inflammation in rats (Akah et al., 1993). The rats were divided into five groups (n = 5) and treated with saline, ZS-4D or ASA. Oedema was then induced 30 min later in the rats by sub-plantar injection of 0.1 ml of raw egg-albumin to the left hind paw and oedema volume measured with a LETICA (Spain) digital plethsmometer (LE 7500) calibrated with 0.1% Triton X-100. Readings were taken 5 min later in an interval of 20 min for a total of 120 min after treatment (Adzu et al., 2002b).

Data analysis

Results were expressed as mean ± standard error of mean (SEM). Student t-test was used to analyze level of statistical significance between groups and Analysis of Variance (ANOVA) among groups. All level of significance were set at p < 0.05; F [(4, 24) = 2.78].

RESULTS

The methanol extract of the extract (ZS-4) gave positive test for balsams, carbohydrates, saponins and tannins. The extract produced 100% lethality in doses higher than 793.74 mg/kg, i.p. Tremor, then sedation was observed to the middle dorsum of the rat’s left hind paw (Chipkin et al., 1983; Kitchen, 1984; Nguelfack et al., 2004). Stimulus was terminated and force threshold readings in grams (Anseloni et al., 2003) taken as soon as nociceptive response were elicited by the rats (Iwalewa et al., 2003; Srinivasan et al., 2003). Readings were taken at 0, 15, 30 and 60 min after treatment.
Table 1. Effect of *Zizyphus spina-christi* ZS-4D fraction on acetic acid-induced writhing (mean ± SEM/15 min) in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>39.0 ± 3.4</td>
<td>36.2 ± 2.9</td>
</tr>
<tr>
<td>ZS-4D</td>
<td>25</td>
<td>31.4 ± 2.2</td>
<td>32.8 ± 2.7</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>21.0 ± 1.9</td>
<td>19.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>15.0 ± 2.1</td>
<td>13.0 ± 1.2</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>4.6 ± 0.8</td>
<td>2.8 ± 0.8</td>
</tr>
</tbody>
</table>

Table 2. Effect of *Zizyphus spina-christi* ZS-4D fraction on formalin induced noxious stimulus score

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>First phase (0-10 min)</th>
<th>Second phase (15-60 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>ZS-4D</td>
<td>25</td>
<td>2.2 ± 0.2</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2.0 ± 0.3</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>1.4 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>2.0 ± 0.3</td>
<td>0.9 ± 0.1</td>
</tr>
</tbody>
</table>

Maximum score = 3.

DISCUSSION

We have shown in previous study that the crude aqueous extract of *Z. spina-christi* exhibited anti-nociceptive potency with both central and peripheral effect (Adzu et al., 2001). The plant has been implicated in the folkloric treatment of inflammatory and pain related ailments (Friedman et al., 1986; Shah et al., 1989; Al-Said, 1993; Ghazanfar and Al-Sabahi, 1993). In this study, we fractionated the extracts of the plant material and trace the main analgesic and in addition, an anti-inflammatory activity to either fraction (numbered) ZS-4D eluted with 70:30 (chloroform : methanol) along or in combination with components of methanol extract of the root bark. Although there are some advantages to the medical use of extracts as oppose to isolated entities (Phillipson, 2001), there is the need to get to the closest of which component is responsible for the observed effect (Farnsworth, 1980). Such search for analgesic agents have been a priority of some pharmaceutical industries for some time now (Mattison et al., 1988), and plant sources are one of the logical and productive strategies, especially for the fact that important analgesic prototypes like salicylic acid and morphine were originally derived from plant kingdom (Elisabetsky et al., 1995). The experimental models were chosen in such a way that the existence of both central and peripherally mediated pain relief could be measured.

The results show that the fraction exhibited potent analgesic activity against aqueous acetic acid-induced writhing. The acid produces abdominal writhing response in animals through chemosensitive nociceptors by increasing fluids of PGE$_2$ and PGF$_2$ (Daraedt et al., 1980) and sympathetic nervous system mediators (Duarte et al., 1988; Barsato et al., 2000) leading to production of algogenic substances at the peritoneal receptors (Bentley et al., 1983). The test is a sensitive procedure in detecting analgesic effects of medicinal substances and can detect antinociceptive effect that may appear inactive in other methods (Collier et al., 1968; Bently et al., 1981). However, the test cannot indicate the exact mechanism of analgesic effect of test substances (Stai et al., 1995) with false positive occurring with agents like sedatives (Elisabetsky et al., 1995). The fraction gave a similar effect on the formalin test inhibiting both the first and the second phase. Formalin test is biphasic, and measures pain of both neurogenic (first phase) and of inflammatory origin (second phase). The first phase (0 – 10 min) being a result of direct stimulation of nociceptors measures centrally mediated effects and is insensitive to anti-inflammatory agents while the second phase (15 – 60 min) which is qualitatively different from
Table 3. Effect of *Zizyphus spina-christi* ZS-4D fraction on analgesy-meter in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>Threshold (mean ± SEM X 20 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>4.0 ± 0.5</td>
</tr>
<tr>
<td>ZS-4D</td>
<td>25</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>3.0 ± 0.8</td>
</tr>
</tbody>
</table>

Table 4. Effect of *Zizyphus spina-christi* ZS-4D fraction on thermal stimulus stimulus-induced tail-flick test (withdrawal time (s)) in rats (mean ± SEM).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>30 min</th>
<th>60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>3.8 ± 0.7</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>ZS-4D</td>
<td>25</td>
<td>5.0 ± 0.7</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>6.2 ± 0.6</td>
<td>6.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6.4 ± 0.7</td>
<td>5.6 ± 0.9</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>5.8 ± 1.4</td>
<td>5.3 ± 0.8</td>
</tr>
</tbody>
</table>

Table 5. Effect of *Zizyphus spina-christi* ZS-4D fraction on egg albumin-induced paw oedema in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg, i.p.)</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
<th>100</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZS-4D</td>
<td>25</td>
<td>0.13±0.04</td>
<td>0.158±0.04</td>
<td>0.188±0.03</td>
<td>0.208±0.03</td>
<td>0.222±0.03</td>
<td>0.244±0.03</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.160±0.05</td>
<td>0.154±0.06</td>
<td>0.135±0.05</td>
<td>0.134±0.05</td>
<td>0.130±0.05</td>
<td>0.114±0.05</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.136±0.04</td>
<td>0.130±0.04</td>
<td>0.100±0.04</td>
<td>0.116±0.03</td>
<td>0.124±0.03</td>
<td>0.108±0.04</td>
</tr>
<tr>
<td>ASA</td>
<td>100</td>
<td>0.140±0.04</td>
<td>0.120±0.04</td>
<td>0.118±0.04</td>
<td>0.106±0.04</td>
<td>0.114±0.04</td>
<td>0.102±0.03</td>
</tr>
</tbody>
</table>

Values in parenthesis are paw oedema volume (cm³).
models is devoid of specific morphine-like activity. This central action is presently not completely understood, but a preliminary work earlier done on the crude aqueous extract, shows that it has CNS activity that is sedative in nature (Adzu et al., 2002a). This might have been responsible for the central effects seen in the formalin and analgesy-meter tests.

The fraction exhibited anti-inflammatory effect by inhibiting oedema induced by egg albumin in rat paw. The test is used as an in vivo model of inflammation to screen agents for anti-inflammatory effect (Akah et al., 1993; Okoli et al., 2007) especially if inflammation wasn't intended to be sustained for long (Williamson et al., 1996; Osadebe and Okoye et al., 2003). This may also be an immense advantage of the fraction since inflammatory diseases are currently treated with steroidal and NSAIDs, often associated with adverse effects (Fung and Kirschenbaum, 1999) hence the need for safe compounds. Inflammation, a common occurrence in infective cases, the fraction is worth probing for development into phytochemical component is responsible for the central effects seen in the formalin and analgesy-meter tests.

Inflammation, a common occurrence in infective and trauma conditions is a complex array of responses of tissue to injury (Vane and Bolting, 1995) which is usually associated with pain as a secondary process, resulting from the release of algesic mediators (Hunskaar and Hole, 1987) with therapy often directed at the inflammation process. The anti-inflammatory effect of the fraction therefore was perhaps elicited through prostaglandins synthesis, the most frequently encountered mechanism of action amongst anti-inflammatory drugs (Vane, 1971) that exert their effect through spectrum of different mode of actions (Samuelson et al., 1978). Conclusively, these observations provide evidence for analgesic and anti-inflammatory activity of the fraction (ZS-4D) as the primary component of the plant material that exhibit pain relief action. It is difficult (at this stage) to pin down which of the phytochemical component is responsible for the activity of the fraction because of the little correlation between anti-inflammatory activity and the chemical nature of agents (Sertie et al., 1990). On the mechanism, the fraction exhibited activity similar to NSAIDs in a complementarity manner, most likely through an interwined action of blocking the stimulus propagation in the pain nervous fibers or decrease in the production of prostaglandins responsible for pain mediation (Matheus et al., 2005). This shows, with some certainty, that the anti-inflammatory component may in addition involve the observed peripheral analgesic activity (Suessoto et al., 2003) and vice versa. Whereas all these explains the basis of the use of plant in the treatment of pain related cases, the fraction is worth probing for development into phytodrug with the hope that it will be in agreement with the folkloric experience of its crude usage.

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