Phytopharmacological Assessment from Two Medicinal Plants Used for Analgesic and Anti-inflammatory Purposes in Burkina Faso

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Abstract: The purpose of this investigation was to elucidate the anti-nociceptive and anti-inflammatory properties of aqueous acetone extracts from Cienfuegosia digitata Cav. and Sida Alba L. in Swiss mice, with an aim to provide a scientific basis for the traditional use of these plants in the treatment of inflammation disorders. In anti-inflammatory activity, the carrageenan-induced paw edema and oil croton-induced ear edema in Swiss mice. As for analgesic effects, acetic acid writhing and formalin test methods were used in mice. About anti-inflammatory potential, the extracts at doses of 100; 200 and 400 mg/kg body weight produced significant comparatively to the control groups (p<0.05; p<0.01 and p<0.001) and we noticed a dose-dependent anti-inflammatory activity. The dose-dependent inhibition of edema was observed at 1; 2 and 3 h. However, extracts showed dose-dependent inhibition of croton oil induced ear oedema, at doses of 200; 300 and 500 µg/ear. As for analgesic activity, extracts produced significant analgesic effects in acetic acid writhing and formalin test method (p<0.05; p<0.01 and p<0.001) compared to the control groups and a dose-dependent inhibition was observed. The present study concludes that Cienfuegosia digitata Cav. and Sida Alba L. have anti-inflammatory and analgesic properties.

Keywords: Analgesic, Cienfuegosia digitata cav., inflammation, mice, Sida Alba L

INTRODUCTION

World over, at least 35.000 plant species are used for medicinal purposes (Kong et al., 2003). The most important industrial medicines nowadays are based on about 90 species of herbs and in developing countries (Ikpme et al., 2007). Focus on plant research has increased worldwide in recent time and a large body of evidence has been collected to show the immense capacities of medicinal plants used in various traditional systems. Many data on the phytopharmacology have showed medicinal plants capacities in certain area of pharmacology (Osadalor et al., 2011) and researchers are also beginning to appreciate the role of medicinal plants in health care delivery (Kolwole et al., 2011). Medicinal plants and herbs have been used for many centuries for the treatment or prevention of diseases and for the promotion of good health. Certainly, herbal medicine as old as the human species itself and before the availability of synthetic drugs, man was completely dependent on natural medicinal plants for curing diseases as inflammatory diseases (Soods et al., 2009).

In African traditional medicine, ethno medicines prepared from plants materials are used to treat a wide range of disease conditions including pain and inflammation. These ethno medicines are relied on by local West African dwellers for their primary health care since the plant materials used in their preparation are cheap and readily available (Jodi et al., 2008). Ethnobotanical investigations in the central region of Burkina Faso have shown that some herbaceous such as Cienfuegosia digitata Cav. and Sida alba L. (Malvaceae) are frequently used in traditional medicine to treat various kinds of diseases such as malaria, fever, pain, variola, as well as having antibacterial, anti-inflammatory, anti-viral activities and hepatoprotective properties (Nacoulma, 1996). Phytochemical analysis of these Malvaceae species under study has mainly demonstrated the presence of saponosides, coumarins, polyphenols compounds, terpenoid/steroid and alkaloids compounds (Nacoulma, 1996).

In the previous study, the extracts and fractions from Cienfuegosia digitata Cav. and Sida alba L. were evaluated for their in vitro antioxidant and anti-inflammatory activities (Konaté et al., 2010a, b). Most
of the synthetic drugs used at present for analgesic and anti-inflammatory effects that cause many side and toxic effects. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs. Medicinal plants are in use since a long time for analgesic and anti-inflammatory activities because of the reason that they are devoid of side effects (Ahmad et al., 1992). According to WHO there is about 82% population that depends upon herbal drugs and these are gaining popularity because of less side effects and low-priced (Kumara, 2001).

There is yet no scientific report validating the ethnomedicinal uses of those Malvaceae in the treatment of inflammation pain. This led us to investigate the anti-inflammatory and analgesic potential of Cienfuegosia digitata cav. and Sida Alba L. in experimental animal models.

**MATERIALS AND METHODS**

**Plants material:** Cienfuegosia digitata Cav. and Sida Alba L. were collected in August 2008 in Gampela, 25 Km east of Ouagadougou, capital of Burkina Faso. The plants were identified in the Laboratory of plants Biology and Ecology, University of Ouagadougou, where a voucher specimen was deposited.

**Preparation of extracts:** Fifty grams (50 g) of powdered plant material was extracted with 80% aqueous acetone (500 mL) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R-200, Switzerland) at approximately 40°C and freeze-dried by a being Telstar Crysodos 50 freeze-dryer. The extract residues were weighed before packed in waterproof plastic flasks and stored at 4°C until use.

**Animals handling:** Swiss NMRI mice (25-30 g) of both sexes were used for this study. All animals were housed in cages under controlled conditions of 12-h light and 12 h without light and 25°C. They received pellets of food enriched with 20% protein and water ad libitum. They were deprived of food for 15 h (but with access to drinking water) and weighed before the experiments. Experiments on the animals were performed according to the protocols already approved by the Institute of Health Sciences Research/University of Ouagadougou (Burkina Faso) and met the international standards for animal study.

**Anti-inflammatory property:**

Carrageen-induced paw edema test: The anti-inflammatory activity was evaluated according to Ninghan et al. (1994). The acute inflammation was induced by injection of 50 µL of 1% w/v carrageenan in normal saline into the subplantar region of right hind paw Swiss mice were divided into five groups, each containing six mice. Extracts (100; 200 and 400 mg/kg body weight), phenylbutazon and distilled water were orally administered 1 h prior to injection of carrageenan injection using plethysmometer (Ugo Basile, No 7141, Italy). The average volumes of the right hind paw of each mouse was calculated from three readings. The inhibitory activity was calculated according to following formula:

\[
\text{% Inhibition} = \frac{(A - B) \text{control} - (A - B) \text{treated}}{(A - B) \text{control}} \times 100
\]

A is the paw circumference at time t, B is the paw circumference before carrageen injection, A - B is edema, (A - B) control is edema or paw size after carrageenan injection to control mice at time.

Ear edema induces by croton oil: Topical inflammation was carried out according to Miller and Tainter (1994). Swiss mice were divided into four groups, each containing six mice. Animals were anaesthetized with 100 mg/kg ketamine hydrochloride; inflammation was induced in the morning between 10.00 a.m. and 12.00 noons to avoid inflammatory response variation due to circadian fluctuation of endogenous corticosteroids.

Cutaneous inflammation was induced by applying 5 µL of solution of croton oil dissolved in 42% alkaloid extracts and the samples (hydrocortisone) on the inner surface of the right ear. Control mice received only the irritant solution. Six hours later, the mice were sacrificed and the plug (diameter = 7 mm) was removed from both the treated (right) and the untreated (left) ears. Edematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as percentage reduction of edema in treated mice compared with the control mice.

**Analgesic capacity:**

Acetic acid-induced writhing test: The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice model (Winter et al., 1962). Nociception was induced by an intraperitoneal injection of 0.6% acetic acid solution in a value of 10 ml/kg body weight. The animals were divided into five groups with six mice in each group. Group I, animals
Table 1: Effect of oral administration of Aqueous Acetone Extract (AAE) from *Cienfuegosia digitata* Cav; on carrageenin-induced hind paw edema

| Samples         | Doses (mg/kg bw) | Increase in paw volume (ΔV mL) | Edema inhibition (%) |
|-----------------|------------------|--------------------------------|-----------------------|
|                 |                  | 1 h    | 3 h    | 5 h    | 1 h    | 3 h    | 5 h    |
| Control         | ---              | 0.22±0.02 | 0.37±0.01 | 0.40±0.02 | --- | --- | --- |
| AAE_Cien        | 100              | 0.13±0.00* | 0.21±0.02* | 0.17±0.01*** | 40.90 | 43.24 | 57.5 |
| AAE_Cien        | 200              | 0.10±0.01** | 0.17±0.01* | 0.14±0.007*** | 54.54 | 54.05 | 65.00 |
| AAE_Cien        | 400              | 0.07±0.01*** | 0.14±0.01* | 0.12±0.01*** | 68.18 | 62.16 | 67.00 |
| Phenyl butazone | 10               | 0.12±0.01* | 0.21±0.01* | 0.18±0.01*** | 45.45 | 43.24 | 55.00 |

Values are Mean±SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test: compare all vs, control group (reference drug): p>0.05; *p<0.05; **p<0.01; ***p<0.001 compared with control

Table 2: Effect of topical administration of Aqueous Acetone Extract (AAE) from *Cienfuegosia digitata* Cav; on croton-induced ear edema

| Samples          | Doses (µg/mL) | Volume of edema (mg) | Inhibition (%) |
|------------------|---------------|----------------------|---------------|
| Control          | ---           | 6.66±0.13            | ---           |
| AAE_Cien         | 100           | 5.75±0.10*           | 13.66         |
| AAE_Cien         | 200           | 4.21±0.10**          | 36.78         |
| AAE_Cien         | 400           | 2.47±0.10***         | 62.91         |
| Hydrocortisone   | 100           | 3.13±0.10***         | 53.00         |

Values are Mean±SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test: compare all vs, control group (reference drug): p>0.05; *p<0.05; **p<0.01; ***p<0.001 compared with control

RESULTS

**Anti-inflammatory property:**

**Carrageenan induced paw edema test:** Carrageenan-induced paw edema was markedly inhibited by intraperitoneal treatment with the extracts or phenyl butazone (10 mg/kg bw). The extracts at doses of 100; 200 and 400 mg/kg body weight produced significant compared to the control groups (p<0.05; p<0.01 and p<0.001) and a dose-dependent anti-inflammatory activity. The dose-dependent inhibition of edema was observed at 1; 2 and 3 h. The results were shown in the Table 1 and 3.

**Ear edema induced by oil croton:** Results obtained from extracts and hydrocortisone significantly reduced the ear edema comparatively to the control groups (p<0.05; p<0.01 and p<0.001). In addition, extracts showed dose-dependent inhibition of croton oil induced ear edema, at doses of 100; 200 and 400 mg/kg bw. Results are summary in the Table 2 and 4.

**Antinociceptive capacity**

**Acetic acid-Induce writhing test:** As for acetic acid-induced writhing test, the extracts effectively reduced the number of abdominal muscle contractions induced by 0.6% acetic acid solution. The extracts have a dose...

Statistical analysis: The data were expressed as Mean±Standard Deviation (SD) of six determinations (n = 6). Results were analyzed by one-way ANOVA followed by Dunnett’s t-test using Prism 4 software. The level of significance was accepted at p≤0.05.
Table 3: Effect of oral administration of Aqueous Acetone Extract (AAE) from Sida Alba L. on carrageenin-induced hind paws edema

| Samples       | Doses (mg/kg) | Volume of edema (µg/ear) | Inhibition (%) |
|---------------|---------------|--------------------------|----------------|
| Control       | ---           | 5.92±0.12                 | ---            |
| AAE Cien      | 100           | 3.3±0.48                 | 43.47          |
| AAE Cien      | 200           | 1.9±0.29                 | 48.97          |
| AAE Cien      | 400           | 0.9±0.19                 | 58.97          |
| Butazon       | 10            | 0.4±0.12                 | 96.47          |
| Paracetamol   | 100           | 2.2±0.14                 | 44.47          |
| Paracetamol   | 200           | 1.1±0.09                 | 51.47          |
| Paracetamol   | 400           | 0.5±0.09                 | 58.47          |
| Sida Alba     | 10            | 0.2±0.08                 | 98.47          |
| Sida Alba     | 20            | 0.1±0.08                 | 99.47          |
| Sida Alba     | 40            | 0.1±0.08                 | 99.47          |
| AAE Cienfuegosia digitata Cav. | 100 | 1.2±0.09 | 51.47 |
| AAE Cienfuegosia digitata Cav. | 200 | 0.7±0.09 | 61.47 |
| AAE Cienfuegosia digitata Cav. | 400 | 0.3±0.09 | 71.47 |
| AAE Cienfuegosia digitata Cav. | 1000 | 0.1±0.08 | 99.47 |

Values are Mean ± SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test; compare all vs; control group (reference drug): p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001 compared with control

Table 4: Effect of topical administration of Aqueous Acetone Extract (AAE) from Sida Alba L. on croton-induced ear edema

| Samples       | Doses (µg/ear) | Inhibition (%) |
|---------------|---------------|----------------|
| Control       | ---           | ---            |
| AAE Cien      | 100           | 55.12          |
| AAE Cien      | 200           | 65.12          |
| AAE Cien      | 400           | 75.12          |
| Hydrocortisone | 1000     | 85.12          |
| Paracetamol   | 100           | 50.12          |
| Paracetamol   | 200           | 60.12          |
| Paracetamol   | 400           | 70.12          |
| Sida Alba     | 10            | 90.12          |
| Sida Alba     | 20            | 99.12          |
| Sida Alba     | 40            | 99.12          |

Values are Mean ± SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test; compare all vs; control group (reference drug): p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001 compared with control

Table 5: Effect of Aqueous Acetone Extract (AAE) from Cienfuegosia digitata Cav. on writhing-induced by acetic acid

| Compounds     | Doses (µg/kg b.w.) | Number of writhing | Inhibition (%) |
|---------------|--------------------|--------------------|----------------|
| Control       | ---                | 20.0±1.00          | ---            |
| AAE Cien      | 100                | 10.0±1.0           | 50.00          |
| AAE Cien      | 200                | 5.0±1.0            | 70.00          |
| AAE Cien      | 400                | 1.0±1.0            | 90.00          |
| AAE Cienfuegosia digitata Cav. | 100 | 5.0±1.0 | 80.00 |
| AAE Cienfuegosia digitata Cav. | 200 | 2.5±1.0 | 95.00 |
| AAE Cienfuegosia digitata Cav. | 400 | 1.0±1.0 | 99.00 |
| AAE Cienfuegosia digitata Cav. | 1000 | 0.5±1.0 | 99.50 |

Values are Mean ± SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test; compare all vs; control group (reference drug): p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001 compared with control

Table 6: Effect of Aqueous Acetone Extract (AAE) from Sida Alba L. on writhing-induced by acetic acid

| Compounds     | Doses (µg/kg b.w.) | Number of writhing | Inhibition (%) |
|---------------|--------------------|--------------------|----------------|
| Control       | ---                | 20.0±1.00          | ---            |
| Paracetamol   | 100                | 10.0±1.0           | 50.00          |
| Paracetamol   | 200                | 5.0±1.0            | 70.00          |
| Paracetamol   | 400                | 1.0±1.0            | 90.00          |
| Sida Alba     | 10                 | 1.0±1.0            | 99.00          |
| Sida Alba     | 20                 | 5.0±1.0            | 95.00          |
| Sida Alba     | 40                 | 1.0±1.0            | 99.00          |
| AAE Cienfuegosia digitata Cav. | 100 | 1.0±1.0 | 99.00 |
| AAE Cienfuegosia digitata Cav. | 200 | 0.5±1.0 | 99.50 |
| AAE Cienfuegosia digitata Cav. | 400 | 0.1±1.0 | 99.90 |

Values are Mean ± SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test; compare all vs; control group (reference drug): p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001 compared with control

DISCUSSION

In Burkina Faso system of medicine, certain herbs are claimed to provide relief of pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. In the present study one such drug extracts from Cienfuegosia digitata and Sida Alba were taken for the study to assess for its in vivo anti-inflammatory and analgesic activities in mice.

Preliminary phytochemical screening revealed the presence of polyphenols compounds, saponosides, coumarins, terpenoid and steroid compounds, and alkaloids in the extracts (Nacoulma, 1996; Konaté et al., 2010b). These constituents may be responsible for the anti-inflammatory and analgesic activities (Hanasaki et al., 1994). Flavonoids have been reported to possess potent inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid (Oweyele et al., 2005; Saleem et al., 2011). Many anti-inflammatory plants and agents modify inflammatory responses by accelerating the destruction or antagonizing the action of the mediators of inflammatory reaction. Foods and fruits rich in flavonoids and other phenolic compounds have been associated with decreased risk of developing inflammatory and other related diseases (Sies et al., 2005; Shrivastava and Patel, 2007), thus suggesting that the flavonoids in garden egg might be part of the active anti-inflammatory constituents in the plant. Flavonoids isolated from medicinal plants possess anti-inflammatory capacity (Musa et al., 2007). Certain hypothesis strongly supported that flavonoids and saponins are well known for their ability to inhibit pain perception as well anti-inflammatory properties (Hossain et al., 2011). According to Annegowda et al. (2010), bioactive compounds derived from plants have been utilised since from the earlier time for the various purposes including
The treatment of pain. Terpenoid and steroid compounds are widely distributed in the plant and exhibit distinctive pharmacological properties. Naturally occurring terpenoids were known to possess anti-inflammatory and analgesic properties (Asmawi et al., 2011).

The antinociceptive effect was assessed by two different models: the formalin test and acetic acid-induced writhing test in mice, whereas the anti-inflammatory effects were examined with ear edema model. The formalin test is a valid and reliable model of nociception and is sensitive for various classes of analgesic drugs. Formalin is known to produce biphasic pain behaviours (Abbadie et al., 1997). The first transient phase is ascribed to the direct effect of formalin on sensory C fibers and the second prolonged phase is associated to the development of an inflammatory response and the release of analgesic mediators (Buritova et al., 2005). It was reported that substance P and bradykinin participate in the manifestation of the first phase responses and histamine, serotonin, prostaglandin and bradykinin are involved in the second phase response (Otuki et al., 2001; Choi et al., 2003).

In the present study, acetic acid injection was demonstrated to induce a characteristic writhing response in the mice. Acetic acid-induced writhing is a highly sensitive and useful test for analgesic drug development especially peripherally acting analgesics. Acetic acid induces pain by liberating endogenous substances (bradykinin, serotonin, histamine, substance P) (Lu et al., 2007). Antinociceptive activity of these Malvaceae was tested by acetic acid-induced writhing model causing pain sensation by triggering localized inflammatory response and formalin induced nociception. Acetic acid, which is used to induce writhing, causes analgesia by liberation of endogenous substances, which in turn excite the pain nerve endings (Taestikul et al., 2003). Increased levels of PGE2 and PGE2α in the peritoneal fluid have been reported to be responsible for pain production caused by intraperitoneal administration of acetic acid (Derardt et al., 1980). The result of the test showed that the extracts of these Malvaceae at dose 500 mg/kg bw exhibit significant writhing inhibition (p<0.001) as compared with the control. The compounds present in the plant extracts may be responsible for the obtained antinociceptive activity. According to the basis of these results it can be concluded that the extracts have an possesse antinociceptive activity.

The most widely used primary test for the screening of new anti-inflammatory agents is the carragenin-induced mice paw edema model (Sawadogo et al., 2006). The edema formation is a biphasic event. The initial phase, observed during the first hour, is attributed to the release of histamine and serotonin (Vinegar et al., 1969) and the delayed edema is due to the release of bradykinin and prostaglandins. It has been reported that the second phase of edema is sensitive to steroidal and non-steroidal anti-inflammatory agents (Di Rosa et al., 1971). The extracts reduced the paw volume significantly from 1 to 5h in which the highest effects were found at the third hour. These results tend to suggest the probable anti-inflammatory activity of the extracts.

The ear edema model permits the evaluation of anti-inflammatory steroids and is less sensitive to non-steroidal anti-inflammatory agents. In croton oil induced ear edema test, mediators of anti-inflammation are released following stimulation. The extracts have significant anti-inflammatory effects in this test, thus it may have a membrane stabilizing effect that reduces capillary permeability and/or has inhibitory effects on mediators. Intraperitoneal administration of the

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Table 7: Effect of Aqueous Acetone Extract (AAE) from *Cienfuegosia digitata* Cav. on licking the hind paw-induced by formalin injection

| Compounds      | Doses (mg/kg b.w.) | First phase (0 to 5 min) | Inhibitions (%) | Second phase (15 to 30 min) | Inhibitions (%) |
|----------------|--------------------|--------------------------|----------------|-----------------------------|----------------|
| Control        | ---                | 86.60±1.34               | ---            | 127.00±2.24                | ---            |
| AAE *C. digitata* Cav | 100               | 69.20±5.17**             | 20.00          | 92.20±1.92***              | 24.70          |
| AAE *C. digitata* Cav | 200               | 72.00±1.58*              | 16.86          | 74.8±2.05***               | 41.10          |
| AAE *C. digitata* Cav | 400               | 63.00±1.41***            | 27.25          | 68.00±1.58***              | 46.46          |
| Paracetamol    | 100                | 46.60±1.14***            | 46.19          | 55.60±1.82***              | 56.22          |

Values are Mean ± SEM (n = 6) one-way ANOVA followed by Dunnett’s t-test: compare all vs; control group (reference drug): p<0.05; *p<0.05; **p<0.01; ***p<0.001 compared with control.

Table 8: Effect of Aqueous Acetone Extract (AAE) from *Sida Alba* L. on licking the hind paw-induced by formalin injection

| Compounds      | Doses (mg/kg b.w.) | First phase (0 to 5 min) | Inhibitions (%) | Second phase (15 to 30 min) | Inhibitions (%) |
|----------------|--------------------|--------------------------|----------------|-----------------------------|----------------|
| Control        | ---                | 86.8±2.39                | ---            | 121.2±1.79                 | ---            |
| AAE *S. alba*   | 100                | 71.4±1.67*               | 17.74          | 95.4±2.70***               | 21.28          |
| AAE *S. alba*   | 200                | 73.00±2.28*              | 15.89          | 75.00±1.58***              | 38.11          |
| AAE *S. alba*   | 400                | 63.40±1.52***            | 26.95          | 66.40±2.07***              | 45.21          |
| Paracetamol    | 100                | 47.40±1.14***            | 45.39          | 54.00±1.58***              | 55.45          |

Values are Mean±SEM (n = 6) one-way ANOVA Followed by Dunnett’s t-test: Compare all vs; control group (reference drug): p<0.05; *: p<0.05; **p<0.01; ***p<0.001 compared with control.
extracts, 30 min before topical application of croton oil, dose dependently inhibited the development of ear edema (Vogel and Vogel, 1997).

CONCLUSION

This study on these Malvaceae confirms that Cienfuegosia digitata Cav. and Sida Alba L. are good candidates for anti-inflammatory and analgesic uses. Thus, which many explain the traditional basis of using these plants in the treatment of various ailments like fever, inflammatory and analgesic disorders in Burkina Faso? Further pharmacological investigations are required to identify the active constituents of the plant extracts responsible for the antimicrobial and anti-inflammatory and effects.

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