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# Analgesic and anti-inflammatory activities of herranone, spinasterol and crude extract of *Pachystela msolo* in rat and mice

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**ABSTRACT**

The present study aims to isolate bioactive principles from the methylene chloride-methanol extract of *Pachystela msolo* and examine the compounds and the extract for their analgesic and anti-inflammatory activities. 1D NMR, 2D NMR and mass spectra data were used to characterize the isolates from the extract. Spinasterol and herranone along with the extract have been evaluated for their analgesic and anti-inflammatory activities using models of acetic acid induced writhing response, formalin test, carrageenan and dextran-induced paw oedema in mice and rats respectively. The extract was administered orally at 50, 100 and 200mg/kg while herranone and spinasterol were administered at 1 and 2mg/kg each through intraperitoneal route. The results demonstrated that the extract, herranone and spinasterol produced significant ($P<0.01$, $P<0.001$) analgesic activity in acetic acid-induced writhing response in mice. *In vivo* anti-inflammatory activities of the compounds, the co-administered product and the extract showed significant ($p<0.01$) reduction of formalin-induced paw oedema in mice. Statistically significant ($p<0.01$) results were found in *in vivo* anti-inflammatory activities test for the co-administered product in the dextran-induced paw oedema in rats. The in vivo anti- inflammatory activities of the extract also showed significant ($p<0.01$, $p<0.05$) reduction of carrageenan-induced paw oedema in rats. Taken together, the identified constituents and the extract exert significant analgesic and anti-inflammatory activities which may be due to inhibition of serotonin and histamine. The investigation validates the folkloric usage of *Pachystela msolo* as phytomedicine in the treatment of pain and the identified compounds could serve as useful tool for standardization.

**Keywords:** *Pachystela msolo*, Herranone, Spinasterol, Analgesic, Anti-inflammatory.

## 1. INTRODUCTION

*Pachystela msolo* (Engler) also known as *Synsepalum msolo* (Engler) T.D. Penn (Sapotaceae) is a medium or tall evergreen tree with many branches which grows up to 20-50m high. In the flora of Cameroon, it is found widely distributed in the Bali Ngeumba forest in the North-west region and in the North-east region in Bertoua and Nanga-Eboko. It is also found in East and Tropical regions of Africa in Tanzania, Uganda, Kenya, Gabon, D.R. Congo, Ivory Coast and Ghana [1]. *P. msolo is* known as "Msamvia'' in Swahili language in Tanzania. The fruits of the plant are eaten raw as a snack, especially by children and herdsmen. The ripe fruits can be soaked in water, squeezed then filtered; sugar is added to the juice and served as beverage [2]. The decoction of the dried stem bark of *P. msolo* alone or in combination with sugarcane is taken orally as a galactogogue in Tanzania [3-4]. In Bali community, the plant is known as "Bangbali" and the stem bark is currently claimed to have folkloric usage in the treatment of pain related ailments such as fever, headache and stomach ache. However, no report has been documented on the pain relief properties

of the plant. In addition, no scientific work has been carried out to ascertain the properties related to pains. Therefore, investigation of such properties is necessary by the use of experimental methods to identify the potentially active principles. Hence, the aims of this study were to isolate bioactive principles from the stem bark of *P. msolo* and evaluate the effect of the compounds and the extract for their analgesic and anti-inflammatory activities using animal models.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals and reagents

All biomarkers were purchased from Pharma factory, India. The chemicals and solvents were of analytical grade and were procured from Merck (Darmstadt, Germany).

### 2.2. Identification of plant

*Pachystela msolo* Engler stem bark was collected from the Bali Nguemba forest, North West Region of Cameroon, in March 2012. The plant was identified by Dr Barthélémy Tchiengué, botanist at the National Herbarium, Cameroon and a voucher specimen was deposited under the number (3849 / SRFK) at the National Herbarium, Yaounde, Cameroon.

### 2.3. Phytochemical screening

The plant extract was screened for the presence of various secondary metabolites like alkaloids, anthraquinone glycosides, fixed oils, flavonoids, phenols, saponins, steroids, sterols, tannins and triterpenoids using standard methods [5].

### 2.4. Extraction and isolation

The air-dried stem bark of *P. msolo* (3300 g) was blended to a fine powder and exhaustively extracted with $CH_2Cl_2$-MeOH (1:1) for 48 h at room temperature. After filtration, the filtrate was concentrated in a vacuum with rotary evaporator at 40 $^0$C to afford 209 g of a red viscous residue (yield 6.33%). This residue (86g) was subsequently chromatographed on silica gel [60(0.063-0.04mm)] using hexane and ethyl acetate gradient solvent systems. The fractions were collected progressively in a 75 mL flask at a flow rate of 8.33 mL/min. TLC permitted the combination of fractions into group of fractions. Fractions 38-42 coded A, and 46-50 coded B eluted from hexane/ethyl acetate (80:20 V/V) and (75:25 V/V) gradient system, afforded compound **1** (12 mg) and compound **2** (10mg) respectively.

### 2.5. Animals

Analgesic test were carried out on male and female swiss mice (20-30g). For the anti-inflammatory test male and female wistar rats (90-180g) were used. Animals were bred in the animal house of the Faculty of Science, University of Yaounde I, Cameroon, under standard condition: (12/12h, light/dark cycle, at 22$^o$C ± 2$^o$C). They were fed with standard commercial diet and water *ad libitum*. The animals were fasted (with free access to water) overnight before the experiments. All experiments were performed according to guidelines for the care of laboratory animals from the Cameroon National Ethical Committee (Ref. no Fw-IRB00001954).

#### 2.5.1. Pharmacological tests

##### 2.5.1.1. Analgesic activity

*Acetic acid induced writhing in mice*

The test was carried out using the method described by Koster et al., 1959 [6]. The writhing response was recorded over a period of 30 minutes, immediately after intraperitoneal administration of acetic acid solution (1%, 10mL/kg) to mice (5 per group). Three doses of crude extract (50, 100 or 200mg/kg, *p.o.*), aspirin (100mg/kg, *p.o.*), distilled water (10mL/kg, *p.o.*), herranone or spinasterol (1 or 2mg/kg, *i.p.*) were administered to mice 30 minutes before the injection of acetic acid. The number of writhing responses was recorded and percentages of protection were expressed using the following ratio: (control mean-treated mean) x 100/control mean [7].

*Formalin test*

The method used in this work was similar to that previously described by Okpo et al., 2001 [8]. The formalin solution (1%, 20µL) was injected subcutaneously into the right hind paw of 50 mice divided into 10 groups of 5 animals each. Animals were treated orally with the plant extract (50, 100 or 200mg/kg), indomethacin (10mg/kg) or distilled water. Herranone, spinasterol (1 or 2mg/kg) or the mixture of these compounds (1 mg/kg each) were administered to mice through intraperitoneal route. The tested products were administered to animals 30 minutes before injection of formalin. The time of paw licking, as an indicator of pain response was recorded the first 5 minutes and between 15-30 minutes after formalin injection Percentages of protection was expressed using the following ratio: (Tc-Tt) x 100 / Tc, where Tc = mean time of licking paw by control mice and Tt = mean time of licking paw by treated mice [7].

##### 2.5.1.2. Anti-inflammatory activity

*Carrageenan-induced rat paw oedema*

The method used was described by Winter et al., 1962 [9]. The crude $CH_2Cl_2$-MeOH

(1:1) extract of *P. msolo* (50, 100 or 200mg/kg), indomethacin 10mg/kg or distilled water were orally administered to rats 30 minutes before the injection of 0.1 mL of carrageenan (1 % in 0.9 % NaCl), into the sub-plantar aponeuvrosis of the right hind limb of each rat. Measurement of paw size was done using a digital caliper 150 mm (6") before carrageenan injection, and 0.5, 1, 2, 3, 4, 5 and 6 h after carrageenan injection. Percentages of inhibition were obtained for each group using the following ratio:$[(D_t - D_0)_{control} - (D_t - D_0)_{treated}] \times 100/(D_t - D_0)_{control}$, where $D_t$ is the average diameter for each group at a time "t" and $D_0$ is the average diameter for each group before any treatment [10].

*Dextran- induced rat paw oedema*

This test was carried out according to the method described by Gupta et al., 2005 [11]. Group of 5 rats were orally administered different doses (50,100 and 200mg/kg) of the crude extract of *P. msolo*, cyproheptadin (10mg/kg) or distilled water. Tested products were given to rats 30 min before the injection of dextran (0.1 mL, in 0.9 % NaCl). Measurement of paw diameter was done using a digital caliper 150 mm (6″) at 0, 30 min, 1 h and 2 h after injection of dextran.

### 2.5.2. Statistical analysis

Data was expressed as Mean ± SEM (Standard error of Mean). One way analysis of variance (ANOVA) followed by Dunnets test were used for statistical evaluation of the results. Results below $p<0.05$ and $p<0.01$ are considered statistically significant.

## 3. RESULTS AND DISCUSSION

The chemical structures of the compounds were determined by comprehensive analysis of their 1D NMR, 2D NMR, and Mass spectra data in comparison with literature set equivalence.

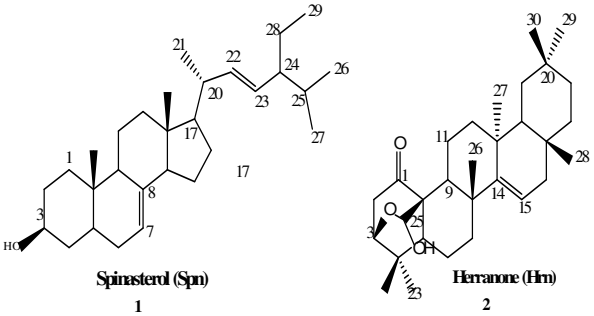


**Figure - 1: Chemical structures of isolated compounds from the extract.**

Compound **1** designated as (3**β**, 5**α**, 22E)-stigmasta-7, 22-dien-3ol is commonly known as spinasterol [12]. Spinasterol precipitated as white crystals and gave a blue to green coloration in the Lieberman Burchard test indicating the presence of sterol. Compound **2** designated as 3**β**, 25-epoxy-25-hydroxy-14-taraxerene-1-one is commonly known as herranone [13]. Herranone was obtained as white non-crystalline needles and gave a red/violet coloration in the Liebermann Burchard test indicating the presence of triterpenoid. Purity of compounds was determined using Nuclear Magnetic Resonance spectra data and Sharpness of melting points.

### 3.1. $^1$H NMR and $^{13}$C NMR data of spinasterol and herranone

#### 3.1.1. β-spinasterol (1)

White crystals (12mg), m.p. 280.5$^o$C, $^1$H NMR ($^1$H: 300 MHz, $CDCl_3$) δ: 1.13 (1H, m, H-1), 1.04 (1H, m, H-1), 1.65 (1H, m, H-2), 1.37 (1H, m, H-2), 3.60 (1H, m, H-3), 1.42 (1H, m, H-4), 1.38 (1H, m, H-5), 1.75 (2H, m, H-6), 5.15 (1H, m, H-7), 1.70 (1H, s, H-9), 1.57 (1H, m, H-11), 1.44 (1H, m, H-11), 1.99 (1H, m, H-12), 1.25 (1H, m, H-12), 1.81 (2H, m, H-14), 1.33 (2H, m, H-15), 1.29 (1H, m, H-16), 1.27 (1H, m, H-16), 0.55 (3H, s, H-18), 0.81 (3H, s, H-19), 2.02 (1H, m, H-20), 1.08 (3H, d, *J* = 6 Hz, H-21), 5.15 (1H, dd, *J* = 8.7, 6.8 Hz, H-22) 5.01 (1H, dd, *J* = 15.3, 8.7 Hz, H-23), 1.50 (1H, m, H-24), 3.01(1H, m, H-18),1.64 (1H, m, H-19), 1.74 (1H, m, H-21), 1.28 (1H, m, H-21), 1.56 (1H, m, H-22), 0.80 (3H, d, *J* = 1.8 Hz, H-26), 0.86 (3H, d, *J* = 4.5 Hz, H-27), 0.81 (3H, d, *J* = 3.6, 1.8 Hz H-29). $^{13}$C NMR (75 MHz, $CDCl_3$) δ: 37.2 (C-1), 31.5 (C-2), 71.1 (C-3), 38.2 (C-4), 40.3 (C-5), 29.7 (C-6), 117.5 (C-7), 139.6 (C-8), 39.5 (C-9), 34.3 (C-10), 21.6 (C-11), 39.5 (C-12), 43.3 (C-13), 55.2 (C-14), 23.1 (C-15), 28.6 (C-16), 55.9 (C-17), 12.1 (C-18), 13.1 (C-19), 40.1 (C-20), 29.2 (C-21), 138.2 (C-22), 129.5 (C-23), 51.2 (C-24), 29.5 (C-25), 19.0 (C-26), 21.6 (C-27), 23.0 (C-28), 12.3 (C-29). ESI-MS spectrum (positive mode), m/z = 413.53 $[M+H]^+$, corresponding to the molecular formula [$C_{29}$ $H_{48}$ O]. The physical and spectra data showed complete resemblance with literature values [12].

#### 3.1.2. Herranone (2)

White non-crystalline needles (10mg), m.p. 280-281.5$^o$C, $^1$H NMR ($^1$H: 600 MHz, $CDCl_3$) δ: 2.90 (1H, *J* = 18.6 Hz, H-2), 2.53 (1H, dd, *J* = 18.6 Hz, H-2), 3.75 (1H, m, H-3), 2.63 (1H, t, *J* = 6 Hz, H-5), 2.90 (1H, d, *J* = 6 Hz, H-6), 1.60 (1H, m, H-7), 1.25 (1H, m, H-7), 2.35 (1H, d, *J* = 6 Hz, H-9), 2.90 (1H, dd, *J* = 18.6Hz, H-11), 2.53 (1H, dd, *J* = 18.6Hz, H-11), 1.60 (1H, m, H-12), 1.25 (1H, m, H-12), 0.81(1H, m, H-13), 1.21(1H, m, H-13), 5.57(1H, m, H-15), 0.90 (1H, m, H-19), 1.25(1H, m, H-19), 1.60 (1H, m, H-21), 1.25 (1H, m, H-21), 1.25 (1H, m, H-22), 1.60 (1H, m, H-22), 1.16 (3H, s, H-23), 1.11 (3H, s, H-24), 5.81 (1H, s, H-25), 0.88 (3H, s, H-26), 1.01 (3H, s, H-27), 0.82 (3H, s, H-28), 0.95 (3H, s, H-29), 0.82 (3H, s, H-30). $^{13}$C NMR (150 MHz,

$CDCl_3$) δ: 210.1 (C-1), 42.0 (C-2), 78.5 (C-3), 35.2 (C-4), 50.0 (C-5), 22.0 (C-6), 29.5 (C-7), 38.0 (C-8), 37.0 (C-9), 57.0 (C-10), 18.0 (C-11), 35.0 (C-12), 35.4 (C-13), 157.4 (C-14), 117.7 (C-15), 38.0 (C-16), 36.5 (C-17), 49.0 (C-18), 34.6 (C-19), 29.2 (C-20), 37.0 (C-21), 35.4 (C-22), 30.5 (C-23), 24.5 (C-24), 93.7 (C-25), 25.6 (C-26), 21.0 (C-27), 29.5 (C-28), 33.0 (C-29), 29.5 (C-30). Molecular weight [454 g/mol] corresponding to the molecular formula [$C_{30}H_{46}O_3$]. The physical and spectra data showed complete resemblance with literature values [13].

### 3.2. Effects of *P. msolo* extract, Herranone and Spinasterol on acetic acid-induced abdominal constrictions

Administration of $CH_2Cl_2$-MeOH (1:1) extract of *P. msolo* inhibited acetic acid-induced writhing response in mice, with a maximal inhibition of 53.55% at 100mg/kg (Figure 2). Herranone and spinasterol (2mg/kg) exhibited significant ($p< 0.01$) anti-nociceptive activity with 49.32%, and 48.11% inhibition respectively. The effect of *P. msolo* (100 and 200mg/kg) extract was similar to that of aspirin, which produced 51.73 %, inhibition of acetic acid-induced pain in mice.

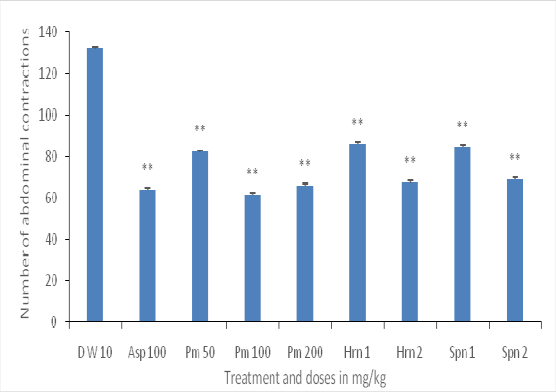


**Figure - 2: The analgesic activity of herranone, spinasterol, and *P. msolo* extract on acetic acid-induced writhing (**Each column represents the mean abdominal constrictions ± SEM (n = 5), (**p < 0.01) significantly different when compared with the corresponding value of standard group. Asp = Aspirin, Pm = *Pachystela msolo*, Hrn = herranone, Spn = Spinasterol, DW = Distilled Water).

### 3.3. Effects of Herranone, Spinasterol and $CH_2Cl_2$-MeOH (1:1) extract of *P. msolo* on formalin-induced pain

The values presented in table 1, showed that the crude extract of *P. msolo* (50, 100 and 200 mg/kg) produced a significant and dose dependent protection against formalin-induced neurogenic pain, with 27.86%, 29.69% and 44.49% inhibition respectively. During the second phase of formalin-induced pain, the plant extract induced significant inhibition with 71.53% activity at 50 mg/kg. The effect of the plant extract was similar to that of indomethacin, responsible of 77.97% inhibition of inflammatory pain. Herranone (1 mg/kg) induced an anti-nociceptive effect of 46.10% and 53.76 % during the first and the second phase of formalin-induced pain respectively. Spinasterol showed a significant and dose dependent inhibition of formalin-induced pain, exhibiting 49.48% and 58.23% inhibition of neurogenic and inflammatory pain respectively at 2 mg/kg. The mixture of the compounds (herranone and spinasterol) reduced the time of paw-licking by 32.15% and 43.54 % during the first and the second phase of formalin-induced pain respectively.

### 3.4. Effects of *P. msolo* extract, Herranone and Spinasterol on dextran-induced paw oedema

The results presented in table 2, showed that *P. msolo* extract (100mg/kg) produced a significant ($p<0.01$) effect on dextran-induced paw oedema, with 56.19%, and 64.15% of inhibition 1 and 2 h after induction of inflammation respectively. Herranone and spinasterol did not exhibit any significant effect on dextran-induced inflammation. However, co-administration of the mixture of compounds significantly ($p<0.01$) reduced inflammation throughout the experimentation, with 72% 71.42% and 71.69% inhibition 30min, 1 and 2h respectively after dextran injection. Cyproheptadin (10 mg/kg) showed 78 % and 81.13% ($p<0.01$) of inhibition respectively one and two hours after induction of inflammation.

### 3.5. Effect of *P. msolo* extract on Carrageenan-induced paw oedema

The effect of the crude extract of *P. msolo* on carrageenan-induced inflammation is presented in table 3. The plant extract reduced the paw oedema all over the experimentation, with a maximal anti inflammatory effect of 56.41% 30 min after carrageenan injection at 50 mg/kg. Indomethacin used as reference drug showed a significant inhibition during the 6 hours of experimentation.

Two different analgesic testing methods were employed with the objective of identifying possible peripheral and central effects of the test substances. Using both acetic acid-induced writhing response and formalin test, it was observed that the extract, spinasterol and herranone possessed analgesic effects against both models. It is known that acetic acid-induced abdominal constriction is very sensitive and able to detect analgesic effects of extracts or compounds that may appear inactive in other methods like the tail flick test [14-15]. In acetic acid-

induced writhing response and formalin test, it was observed that the extract, spinasterol and herranone possessed analgesic effects against both models. It is known that acetic acid-induced abdominal constriction is very sensitive and able to detect analgesic effects of extracts or compounds that may appear inactive in other methods like the tail flick test [14-15]. In acetic acid-induced abdominal writhing, a visceral pain model, the processor which releases arachidonic acid via cyclo-oxygenase and prostaglandin biosynthesis plays a role in the nociceptive mechanism [16]. Results of the present studies showed that the crude extract, herranone and spinasterol possessed significant analgesic effect which may be due to inhibition of cyclo-oxygenase and/or lipoxygenase activity. The results of this test alone do not allow us to conclude whether origin of the analgesic activity lays on the central or on the peripheral action.

**Table – 1: Effects of Herranone, Spinasterol and $CH_2Cl_2$-MeOH extract of *P. msolo* on formalin-induced paw oedema**

| | | Licking Time (S) | | | | | |
|---|---|---|---|---|---|---|---|
| **Group** | **Doses (mg/kg)** | **Neurogenic Phase (0-5 minutes)** | **Percent Inhibition** | **Inflammatory Phase (15-30minutes)** | **Percent Inhibition** | | |
| Dist water | 10 | 85.78 ± 1.18 | - | 141.78 ± 1.88 | - | | |
| Indomethacin | 10 | 89.72 ± 2.33 | -4.59 | 31.22 ± 2.22** | 77.97 | | |
| *P. msolo* | 50 | 61.88 ± 2.16** | 27.86 | 40.36 ± 1.37** | 71.35 | | |
| *P. msolo* | 100 | 60.31 ± 1.42** | 29.69 | 56.02 ± 2.51** | 60.48 | | |
| *P. msolo* | 200 | 47.61 ± 2.08** | 44.49 | 61.88 ± 2.87** | 56.35 | | |
| Herranone | 1 | 46.23 ± 1.49** | 46.10 | 65.55 ± 1.99** | 53.76 | | |
| Herranone | 2 | 63.83 ± 1.67* | 25.59 | 117.31 ± 1.58* | 17.25 | | |
| Spinasterol | 1 | 47.42 ± 2.13** | 44.71 | 70.61 ± 1.30** | 50.29 | | |
| Spinasterol | 2 | 43.33 ± 2.10** | 49.48 | 59.21 ± 1.62** | 58.23 | | |
| (Hrn + Spn) | 1+1 | 58.20 ± 1.11** | 32.15 | 80.08 ± 2.48** | 43.52 | | |

The values represent the licking time ± SEM (n = 5), (*p <0.05), (**p <0.01) significantly different when compared with corresponding value of standard group. Herranone (Hrn); Spinasterol (Spn); *P. msolo* = *Pachystela msolo;* Dist water = Distilled water.

**Table – 2: Effects of Herranone, Spinasterol and $CH_2Cl_2$-MeOH extract of *P. msolo* on dextran-induced paw oedema**

| **Group** | **Doses (mg/kg)** | **Change in Paw diameter Δd ± SEM (mm)** | | |
|---|---|---|---|---|
| | | **0.5(h)** | **1(h)** | **2(h)** |
| Dist water | 10 | 0.20 ± 0.03 | 0.21 ± 0.04 | 0.21 ± 0.03 |
| Cyproheptadin | 10 | 0.07 ± 0.01** | 0.05 ± 0.01** | 0.04 ± 0.01** |
| *P. msolo* | 50 | 0.11 ± 0.03 | 0.14 ± 0.03 | 0.08 ± 0.03* |
| *P. msolo* | 100 | 0.11 ± 0.02 | 0.09 ± 0.02* | 0.08 ± 0.02* |
| *P. msolo* | 200 | 0.07 ± 0.02** | 0.08 ± 0.01** | 0.09 ± 0.02** |
| Herranone | 1 | 0.20 ± 0.03 | 0.14 ± 0.02 | 0.14 ± 0.03 |
| Herranone | 2 | 0.17 ± 0.03 | 0.14 ± 0.02 | 0.12 ± 0.03 |
| Spinasterol | 1 | 0.18 ± 0.03 | 0.16 ± 0.02 | 0.16 ± 0.02 |
| Spinasterol | 2 | 0.18 ± 0.03 | 0.17 ± 0.02 | 0.16 ± 0.02 |
| (Hrn+Spn) | 1+1 | 0.05 ± 0.01** | 0.06 ± 0.02** | 0.06 ± 0.01** |

The values represent the variation of paw diameter ± SEM, (n = 5), (*p < 0.05), (**p < 0.01) significantly different when compared with corresponding value of standard group. *P. msolo* = *Pachystela msolo*, Hrn = Herranone, Spn = Spinasterol, Dist water = Distilled water.

**Table -3: Effects of $CH_2Cl_2$-MeOH (1:1) extract of *P. msolo* on Carrageenan-induced paw oedema in rats**

| Treatments | Doses (mg/kg) | Change in Paw diameter Δd ± SEM (mm) | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.5(h) | | | 1(h) | | | 2(h) | | | 3(h) | | | 4(h) | | | 5(h) | | | 6(h) | | |
| Dist water | 10 | 0.15 | ± | 0.01 | 0.17 | ± | 0.02 | 0.33 | ± | 0.02 | 0.44 | ± | 0.02 | 0.41 | ± | 0.03 | 0.44 | ± | 0.03 | 0.38 | ± | 0.02 |
| Indomethacin | 10 | 0.05 | ± | 0.01** | 0.04 | ± | 0.01** | 0.16 | ± | 0.01** | 0.18 | ± | 0.01* | 0.18 | ± | 0.03* | 0.21 | ± | 0.01* | 0.17 | ± | 0.02** |
| *P.* msolo | 50 | 0.06 | ± | 0.01** | 0.12 | ± | 0.02* | 0.32 | ± | 0.03 | 0.37 | ± | 0.02 | 0.41 | ± | 0.03 | 0.38 | ± | 0.03 | 0.38 | ± | 0.01 |
| *P. msolo* | 100 | 0.06 | ± | 0.02** | 0.10 | ± | 0.02* | 0.23 | ± | 0.01* | 0.32 | ± | 0.03* | 0.39 | ± | 0.04 | 0.37 | ± | 0.06 | 0.25 | ± | 0.03** |
| *P. msolo* | 200 | 0.08 | ± | 0.01** | 0.09 | ± | 0.01* | 0.22 | ± | 0.02* | 0.27 | ± | 0.01* | 0.26 | ± | 0.01** | 0.29 | ± | 0.02** | 0.35 | ± | 0.03 |

The values represent the variation of paw diameter ± SEM (n=5), (*p< 0.05), (**p< 0.01) significantly different when compared with corresponding value of standard group. *P. msolo = Pachystela msolo;* Dist water = Distilled water

The formalin test may be more useful as model of chemical pain in which the first phase is due to direct chemical stimulation of nociceptors, whereas the second phase is dependent of peripheral inflammation and changes in central processing [17]. Substance P, bradykinin, nitric oxide and prostaglandins are involved in the inflammatory phase [18]. The extract of *P. msolo,* spinasterol and the mixture of heranone and spinasterol exhibited a more potent analgesic effect during the inflammatory phase compared to the neurogenic phase of formalin-induced pain. This result suggests that the activities of the plant extract and compounds (herranone and spinasterol) could be due to peripheral action. The mixture of the compounds (herranone and spinasterol) resulted in a decrease in activity in both phases of the formalin-induced pain. However, more investigations will be carried out on the proper mechanism of action to determine whether this low activity could be due to the interaction with the binding receptors or competition between herranone and spinasterol.

The carrageenan-induced paw oedema is a routine and valid animal model for assessing the anti-oedematous effect of natural products. It is known to be biphasic; the first phase (1h) involves the release of serotonin and histamine and second phase (over 2h) is mediated by prostaglandins, cyclo-oxygenase products and the continuity between the two phases is provided by kinins [19]. The plant by impairing the inflammation during the first phase of inflammation may have anti-serotonin and anti-histamine properties. In addition, the extract of *P. msolo* has slightly inhibited the release of bradykinin and prostaglandins during the 4th and 5th hour of inflammation induced by carrageenan.

Dextran is known to induce serotonin and histamines liberation from mast cells [20]. The plant extract (200 mg/kg) significantly reduced the release of serotonin and histamine. In addition, the result on the dextran test within the first and second phases proved that the mixture of herranone and spinasterol are able to act synergistically. Both histamine and serotonin are characterized by the increase in vascular permeability. The dextran-mediated inflammation (oedema) was reduced probably as a result of inhibition of histamine and serotonin liberation and or activity by these compounds.

## 4. CONCLUSIONS

The present study on *P. msolo* stem bark extract and tested compounds has demonstrated a significant analgesic and anti-inflammatory effects which may be through the inhibition of histamine and serotonin production. In addition, the isolated constituents indicate the potentially active ingredients that may play a significant role in the plant. Finally, the experimental model

applied validates the claim of the usage of *P. msolo* as phytomedicine in painful disorders.

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