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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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A B S T R A C T

Pachystalamsolo Engler is a plant used in traditional medicines to treat various devices. The present study aims to evaluate herranone, spinasterol and the CH_2Cl_2-MeOH extract of *Pachystela msolo* for their analgesic and anti-inflammatory activities. Spectroscopic data were used to characterize the isolates from the extract. The analgesic activity was assayed using acetic-acid induced writhing response and formalin test in mice. Anti-inflammatory activities were accessed using model of dextran and carrageenan induced paw oedema in rats. In the study of the analgesic effect, the extract (50, 100, or 200 mg/kg; p.o), herranone and spinasterol (1 or 2 mg/kg; i.p) significantly ($p < 0.001$, $p < 0.01$) reduced acetic acid-induced writhing response in mice. *In vivo* anti-inflammatory activities of the extract, herranone, spinasterol, and the co-administered product (1 mg/kg each; i.p) showed significant ($p < 0.01$) reduction of formalin-induced paw oedema in mice. Statistically the extract (200 mg/kg) and the co-administered product significantly ($p < 0.01$) decreased dextran-induced paw oedema in rats. The extract (200 mg/kg) also showed significant ($p <0.01$) reduction of carrageen an-induced paw oedema in rats for 6 hours. To conclude, the tested products and the extract exerted analgesic and anti-inflammatory activities, which may be due to inhibition of cyclooxygenase, serotonin, histamine, and prostaglandins. The investigation validates the folkloric usage of *Pachystela msolo* as phytomedicine in the treatment of pain and the identified constituents could serve as useful tool for standardization.

Keywords: *Pachystela msolo*, herranone, spinasterol, analgesic, anti-inflammatory.

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Contents

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 20-50 m 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 (**Aubréville, 1964**). *P. msolo is* known as "Msamvia'' in Swahili language in Tanzania. The ripe fruits can be soaked in water, squeezed then filtered; sugar is added to the juice and served as beverage (**Ruffo et *al*, 2002**). The decoction of the dried stem bark of *P. Msolo* alone or in combination with sugarcane is taken orally as a galactogogue in Tanzania. In Taiwan the dried root decoction is drunk as a remedy for diabetes mellitus (**Ruffo et *al*, 2002; Newmark, 2002; Ivan, 2005**). In Bali community, the plant is known as "Bangbali" and the stem bark and root is currently claimed to have folkloric usage in the treatment of pain related ailments such as fever, headache, stomach ache and malaria. In our previous work some chemical constituents that include pachysteloside A, taraxeryl acetate, β-taraxerol, spinasterol, betulinic acid, and spinasterol-3-O-β-D-glucopyranoside isolated from the roots of the plant were cited to present varying pharmacological properties with respect to report from literature sources (**Turibio et *al*, 2015**). However, no scientific work has been carried out to ascertain the *in vivo* analgesic and anti-inflammatory properties of the plant extract or some of its constituents. 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

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).

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.

Phytochemical Screening

The plant extract was screened for the presence of various secondary metabolites like alkaloids, anthra quinone glycosides, fixed oils, flavonoids, phenols, saponins, steroids, sterols, tannins, sugar, and triterpenoids using standard methods (**Kokate, 1994**).

Extraction and Isolation

The air-dried stem bark of *P. msolo* (3300 g) was blended to a fine powder and extracted with CH_2Cl_2-MeOH (1:1) for 48 h at room temperature. After filtration, the filtrate was concentrated in a vacuum at reduced pressure to afford 86 g of the extract. The extract (80 g) was subsequently chromatographed on silica gel [60(0.063-0.04 mm)] using n-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 sub fractions into group of fractions. Fractions 38-42 coded A, and 46-50 coded B eluted from n-hexane/ethyl acetate (80:20 V/V) and (75:25 V/V) gradient system, afforded compound **1** (12 mg) and compound **2** (10 mg) respectively.

Animals

Analgesic test were carried out on male and female swiss mice (20-30 g). For the anti-inflammatory test male and female wistar rats (90-180 g) 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).

Pharmacological tests

Analgesic activity

Acetic acid induced writhing in mice

The test was carried out using the method described by **Koster et *al*, (1959**). The writhing response was recorded over a period of 30 minutes, immediately after intraperitoneal administration of acetic acid solution (1%, 10 mL/kg) to mice (5 per group).Three doses of crude extract (50, 100 or 200 mg/kg, *p.o.*), aspirin (100 mg/kg, *p.o.*), distilled water (10 mL/kg, *p.o.*), herranone or spinasterol (1 or 2 mg/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 (**Dongmo et *al*, 2005**).

Formalin test

The method used in this work was similar to that previously described by **Okpo et *al*, (2001**).The formalin solution (1%, 20 µL) 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 200 mg/kg), indomethacin (10 mg/kg) or distilled water. Herranone, spinasterol (1 or 2 mg/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 (**Dongmo et *al*, 2005**).

Anti-inflammatory activity

Carrageenan-induced rat paw oedema

The method used was described by **Winter et *al*, (1962**).The crude CH_2Cl_2-MeOH (1:1) extract of *P. msolo* (50, 100 or 200 mg/kg), indomethacin 10 mg/kg or distilled water were orally administered to rats 30 minutes before the injection of 0.1 mL of carrageen an (1 % in 0.9 % NaCl), into the sub-plantar aponeurosis of the right hind limb of each rat. Measurement of paw size was done using a digital calliper 150 mm (6") before carrageenan injection, and 0.5, 1, 2, 3, 4, 5 and 6 h after carrageen an 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 (**Lanhers et *al*, 1991**).

Dextran- induced rat paw oedema

This test was carried out according to the method described by **Gupta et *al*, (2005**). Group of 5 rats were orally administered different doses (50,100 and 200 mg/kg) of the crude extract of *P. msolo*, cyproheptadin (10 mg/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.

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. Result and Discussion

The structures of the isolates were determined by comprehensive analysis of their 1D NMR, 2D NMR, and Mass spectra data in comparison with literature set equivalence. Compound 1 designated as (3β, 5α, 22E)-stigmasta-7, 22-dien-3ol is commonly known as spinasterol (**Wandji et al, 2002)**. 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 3β,25-epoxy-25-hydroxy-14-taraxerene-1-one is commonly known as herranone (**Wiedemman et al, 1999**). 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.

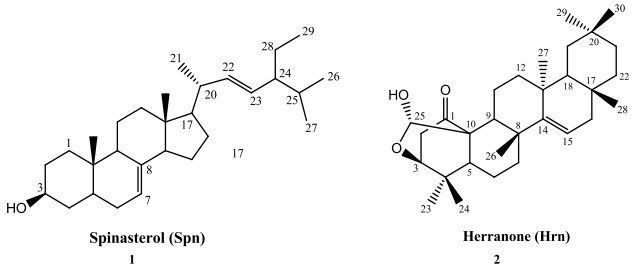

Figure 1: Chemical structures of isolated compounds from the extract.

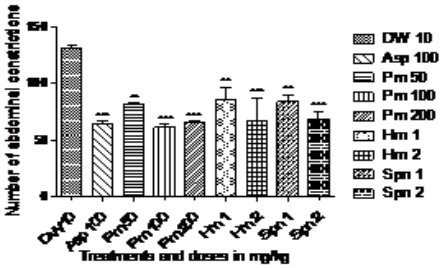

Figure 2: The analgesic activity of herranone, spinasterol, and *P. msolo* extract on acetic acid-induced writhing

1H NMR and 13C NMR data of spinasterol and herranone

β-spinasterol (1)

White crystals (12 mg), m.p. 280.5 oC, 1H NMR (1H: 300 MHz, CDCl3) 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). 13C NMR (75 MHz, CDCl3) 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 (**Wandji et al, 2002**).

Herranone (2)

White non-crystalline needles (10 mg), m.p. 280-281.5oC, 1H NMR (1H: 600 MHz, CDCl3) 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). 13C NMR (150 MHz, CDCl3) 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 (**Wiedemman et al, 1999**).

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 100 mg/kg (Figure 2). Herranone and spinasterol (2 mg/kg) exhibited significant ($p < 0.001$) anti-nociceptive activity with 49.32 %, and 48.11 % inhibition respectively. The effect of *P. msolo* (100 and 200 mg/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), (***$p < 0.001$), (**$p < 0.01$) significantly different when compared with the corresponding value of standard group. Asp = Aspirin, Pm= *Pachystela msolo*, Hrn = herranone, Spn= Spinasterol, DW = Distilled Water.

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.

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 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. 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.

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

The results presented on table 2, showed that *P. msolo* extract (100 mg/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 72.00 %, 71.42 %, and 71.69 % inhibition 30 min, 1 and 2 hours respectively after dextran injection. Cyproheptadin (10 mg/kg) showed 78.00 % and 81.13 % ($p < 0.01$) of inhibition respectively one and two hours after induction of inflammation.

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.

Effect of *P. msolo* extract on Carrageenan-induced paw oedema: The effect of the crude extract of P. msolo in carrageenan-induced inflammation is presented on table 3. The plant extract reduced the paw oedema all over the experimentation, with a maximal anti-inflammatory effect of 56.41 % and 58.97 %, 30 min after carrageenan injection at 50 and 100 mg/kg, respectively. Indomethacin used as reference drug showed a significant inhibition during the 6 hours of experimentation. This study was designed to verify the analgesic and anti-inflammatory effect of herranone, spinasterol and the stem bark extract of *Pachystela msolo* in rats and mice. Although *P. msolo* is used traditionally for various devices, there is no significant literature of the traditional use of this plant in phytomedicine. 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 tests, it was observed that the extract, herranone, and spinasterol possessed analgesic effects against both models. In acetic acid induced writhing test, the intraperitoneal administering of agents that irritate serous membranes provokes a stereotyped behaviour in mice, which is characterized by abdominal contractions, movements of the body and twisting of the dorso-abdominal muscles. It is the typical pain generated indirectly via endogenous mediators, a suck as bradykinin, serotonin and capsaicin, which stimulate peripheral nociceptive neurons. The releases of arachidonic acid metabolites via COX and PGs biosynthesis are involved (Meddah et *al*, 2013). *P. msolo*,herranone, and spinasterol reduced acetic acid induced writhing in mice. This result suggests that, the extract from*P. msolo*, along the tested product may act by inhibiting PGs synthesis because the nociceptive mechanism of abdominal writhing induced by acetic acid involves the release of arachidonic acid metabolites via COX. But we cannot determine with this test if the activity of the extract and the tested products is of central or peripheral origin. 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 (**Sayyah et *al*, 2004**). Substance P, bradykinin, nitric oxide and prostaglandins are involved in the inflammatory phase (**Garcia et *al*, 2004**). The extract of *P. msolo*, herranone, spinasterol and the co-administered product 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. Carrageenan-induced paw oedema, an *in vivo* model of inflammation, has also been characterized as a biphasic event (**Vinegar et *al*, 196**9) Histamine, bradykinin, and 5-hydroxytryptamine (5-HT) are released in the first phase of oedema (0-1 h). In the second phase (1–6 h), TNF-α, IL-1β, cyclooxygenase (COX-2), and prostaglandin (PGs) are produced more actively **(Chang et *al*, 2012)**. It is well known that the expression of COX-2 is maximal at the late phase of carrageenan-induced paw oedema, which could subsequently increase prostaglandin levels in inflammatory reactions (**Seibert et *al*, 1994**). In this study, *P. msolo* (200 mg/kg) and indomethacin (10 mg/kg) showed significant anti-inflammatory effect on carrageenan-induced mouse paw oedema during the 6 h of experimentation. In contrast after 1h, the effect of *P. msolo* decline compared to that of indomethacin. According to the hypothesis that up to 1h of inflammation induced by carrageenan, TNF-α, IL-1β, COX and PGs is produced more actively. In this phase, the activity of *P. msolo* decline. We can postulate that the activity of *P. msolo* is reduced in the presence of TNF-α, IL-1β, COX and PGs, and was higher in the presence Histamine, bradykinin, and 5-HT. In contrast, NSAIDs, such as indomethacin, seem to suppress only the second phase (**Bogdan, 2001**). Dextran is known to induce serotonin and histamines liberation from mast cells (**Rowley and Beneditt, 1956**).The plant extract (200 mg/kg) and the co-administered product (herranone and spinasterol, 1 mg/kg each;i.p) 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 co-administered products 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. Conclusion

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 cyclooxygenase, serotonin, histamine, and prostaglandins 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 phyto-medicine in painful disorders.

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

Treatments	Doses (mg/kg)	0.5(h)	1(h)
Distwater	10	0.15±0.01	0.17±0.02
Indomethacin	10	0.05±0.01**	0.04±0.01**
P. msolo	50	0.06±0.02**	0.12±0.02*
P. msolo	100	0.06±0.02**	0.10±0.02*
P. msolo	200	0.08±0.01**	0.09±0.01**

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

Treatments	Doses (mg/kg)	2(h)	3(h)
Distwater	10	0.33± 0.02	0.44±0.02
Indomethacin	10	0.16±0.01**	0.18±0.01**
P. msolo	50	0.32±0.03	0.37±0.02*
P. msolo	100	0.23±0.01**	0.32±0.03*
P. msolo	200	0.22±0.02**	0.27±0.01**

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

Treatments	Doses (mg/kg)	4(h)	5(h)
Distwater	10	0.42± 0.03	0.44±0.03
Indomethacin	10	0.18±0.03*	0.21±0.01**
P. msolo	50	0.42±0.03	0.38±0.03
P. msolo	100	0.39±0.04	0.37±0.06
P. msolo	200	0.26±0.01**	0.29±0.02**

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

Treatments	Doses (mg/kg)	6(h)
Distwater	10	0.38±0.02
Indomethacin	10	0.17±0.02**
P. msolo	50	0.38±0.01
P. msolo	100	0.35±0.03
P. msolo	200	0.25±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.

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