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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF ETHANOL ROOT EXTRACT OF *Setaria megaphylla* (STEUD) T. DUR AND SCHINZ (POACEAE)

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ABSTRACT

Background: *Setaria megaphylla* (Steud) T. Dur and Schinz (Poaceae), is a medicinal plant used in South-South Nigeria to treat malaria, hemorrhoids, urethritis, inflammation, diabetes, fevers and various pains.[1] While some researches have been carried out to authenticate the uses of the leaves of the plant, very little research has been done on the root and no publication has been found on its root fractions. **Objectives:** This work is therefore designed to authenticate analgesic and anti-inflammatory activities of the roots of *Setaria megaphylla* to ascertain folkloric claims of its medicinal usefulness. **Methodology:** The ethanol extract was also partitioned in n-hexane, dichloromethane, ethyl acetate and n-butanol in the order listed above to obtain fractions. Qualitative data of dichloromethane and n-butanol fractions were determined by gas chromatography mass spectroscopy (GC-MS). The root extract (150, 300, 450 mg/kg) was investigated for analgesic activity against chemical and heat induced pains and anti-inflammatory activity against carrageenan, fresh egg albumin and xylene - induced edema. **Results:** Significant ($p < 0.05 – 0.001$) dose-dependent effects were observed against carrageenan, egg albumin and xylene -induced inflammation and acetic acid -induced writhing, formalin-induced paw licking and hot plate-induced pain in mice. **Conclusions:** The results of this study revealed that the ethanol root extract of *S. megaphylla*

possesses anti-inflammatory and analgesic activities through its phytochemical components. This therefore justifies its ethnomedicinal usage in the treatment of various pain and inflammations.

KEYWORDS: Analgesic, Antiiflammatory, *Setaria megaphylla*, medicinal plant.

1.0 INTRODUCTION

Pain is an ill-defined, unpleasant, sensation that is usually evoked by external or internal noxious stimuli and is usually a warning signal primarily protective in nature that causes discomfort. Analgesics are drugs that selectively relieve pain by acting on the central nervous system or on peripheral pain mechanisms without significantly altering consciousness.[2] Centrally-acting analgesics such as narcotics inhibit both phases equally while peripherally-acting ones such as NSAIDs (aspirin, diclofenac) and steroids (dexamethasone, hydrocortisone) suppress primarily the late phase.[3]

Inflammation is a localized reaction that produces redness, warmth, swelling, pain and loss of function as part of the complex reparative and protective responses of body tissues to harmful stimuli, such as pathogens, damaged cells, auto-immune stimuli, mechanical injury or irritants.[4] Inflammation is a defensive mechanism of the body to remove injurious stimuli as well as initiate the healing process for the tissue and if left unchecked can lead to onset of diseases such as vasomotor rhinorrhea, rheumatoid arthritis and artherosclerosis.[5] Anti-inflammatory and analgesic activities have similar underlying mechanisms but compounds differ in their profile of activity. eg corticosteroids possess anti-inflammatory but not analgesic properties, paracetamol and narcotic analgesics have analgesic but little inflammatory effects while aspirin like all non-steroidal anti-inflammatory drugs (NSAIDs) has both properties. Most NSAIDs have well balanced anti-inflammatory activity, due to inhibition of cyclo-oxygenase and therefore prostaglandin synthetase activity and are used to treat conditions that lead to inflammation, pyrexia and pain of all origin eg rheumatoid conditions, gout, dysmenorrhea, neoplastic diseases and headache.[6]

Setaria megaphylla is a perennial broad-leafed bristle grass, erect, stout and cane-like with very coarse and robust roots usually about 30 cm diameter at the base.[7] It is found along rivers in low lying areas or forests and in dense bushveld where there is plenty of moisture, occurring in tropical and subtropical areas where there is high rainfall.[8]

The plant has anodynal and analgesic properties.[9] In South Africa, Nigeria and in Republic of the Congo it used to treat bruises, headache and pains.[9] It is used in Ivory Coast to treat babies suffering from convulsions or fits of epilepsy, is given in Tanganyika for mental derangement, is sedative on cough and is also indicated for oedema.[9] It has beneficial action on urino-genital troubles, is given in Ivory Coast, Tanganyika and Gabon for amenorrhoea and blennorrhoea and to pregnant women to ease delivery.[9] The root in Ubangi is held to be abortifacient. The leaves are used for anuria, psychosis, debility, neurasthenia and insanity.[10] Leaf extract of *Setaria megaphylla* exhibited significant ($p < 0.05$) anti-inflammatory activity against acute inflammation and also possesses significant ($p < 0.05$) dose dependent analgesic activity against chemical and thermal induced pains.[11] A preliminary phytochemical screening of the ethanolic root extract of *Setaria megaphylla* carried out employing the standard phytochemical procedures revealed the presence of tannins, saponins, flavonoids, terpenes, anthraquinone and cardiac glycoside.[12]

2.0 MATERIALS

2.1 Collection and Identification of Plant Sample

Setaria megaphylla roots were collected from Anwa forest in Uruan, Uruan Local Government Area of Akwa Ibom State, Nigeria. Identification and authentication was done by Dr. Mrs Margaret Bassey, a plant taxonomist in the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen with number FPHUU 221 was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo.

2.2 Animal Stock

Adult Swiss albino mice were obtained from the Animal House of the University of Uyo, Uyo, Akwa Ibom State and were maintained in the University of Uyo Animal House and fed with growers pellet feed with water given *ad libitum.*

3.0 METHODS

3.1 Extraction

The roots of the plant were washed and air-dried to get a constant weight, cut into small pieces and pulverized to powder using pestle and mortar. The powder was divided into two parts. One part (1.5 kg) was macerated in 70% ethanol for 72 hours, while the second part (1.5 kg) was successively and gradiently macerated for 72 hours in 2.5 litres of each of these solvents, `n-hexane, dichloromethane, ethyl acetate and n-butanol in the order listed above. The liquid filtrate of the extract and fractions were concentrated and evaporated to dryness in

-vacuo at 40°C using rotary evaporator and then weighed and stored in a refrigerator at -4°C until used for the proposed experiments.

3.2 Acute Toxicological Study

Acute toxicity study was carried out to determine the median lethal dose (LD_{50}) of the root extract using the Miller and Tainter (1944.[13] method as reported by Okokon, Antia and Udobang (2012).[14] The mice were treated with various doses (1000 - 5000 mg/kg) of the ethanol extract and observed for 24 hours. Physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and deaths were recorded.

3.3 Phytochemical Studies.

i) Qualitative Phytochemical Screening: The ethanol root extract of *Setaria megaphylla* was subjected to phytochemical screening to reveal the presence of chemical constituents in the plant such as saponins, alkaloids, tannins, flavonoids, terpenes and cardiac glycosides using the methods described by Odebiyi and Sofowora (1978).[15] and Trease and Evans (2002).[16]

ii) Phytochemical Analysis: Quantitative data were determined by gas chromatography mass spectroscopy (GC-MS). The fraction was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split / splitless injector. The column used was an DB-5(Optima-5), 30 m, 0.25 mm i.d, 0.25 µm df, coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following oven temperature programme: 50°C, held for 1 minute, rising at 3°C/minute to 250°C, held for 5 minute, rising at 2°C/minute to 280°C, held for 3 minute; injection temperature and volume, 250°C and 1.0 µl, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280°C; hydrogen flow rate, 50 ml/minute; air flow rate, 400 ml/minute; make-up (H2/air), flow rate, 50 ml/minute; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu). Agilent 6890N GC was interfaced with a VG Analytical 70 - 250s double -focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250^{o}C.

The GC was fitted with a 30 m x 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above. The

components present in the various active fractions of the plant extract were identified by comparing the retention times of the GC peaks with standard compounds run under identical conditions and by comparison of retention indices[17] and mass spectra.[18] with those found in the literature and by comparison of mass spectra with those stored in the NIST 107 and NIST 21 and Wiley 229 libraries.

3.4 Evaluation of Analgesic Activity

3.4.1 Acetic acid-induced Writhing

Abdominal constrictions following intraperitoneal injection of 0.1 ml of 3% acetic acid and consisting of the contraction of abdominal muscles together with stretching of hind limbs were used to evaluate the analgesic activity of the extract using the method of Santos *et al.*, (1994[19] and Correa, Kyle, Chakrararty and Calixto (1996).[20] Adult Swiss albino mice were separated into five groups of six mice each and fasted for 24 hours but allowed access to water. Group 1 (control) received 10 ml/kg of distilled water while groups 2, 3 and 4 were respectively pretreated with ethanol extract 150, 300 and 450 mg/kg/i.p. Group 5 received standard drug acetyl salicylic acid (100 mg/kg, i.p). After 30 minutes, acetic acid (0.1 ml) was administered (i.p). The number of writhing movements were counted for 30 minutes. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with the extract.

3.4.2 Formalin-induced Paw Licking

The method of Hunskaar and Hole (1987).[21] and Correa and Calixto (1993).[22] was adopted. 20 µL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (137 mM NaCl, 2.7 mM KCl and 10 mM phosphate buffer and pH 7.4) was injected subcutaneously into the right hind paw of each of the animal. The amount of time each mouse spent licking the injected paw was timed and used as indication of pain. The first of the nociceptive response normally peaks 5 minutes after injection and the second phase 15 - 30 minutes after formalin injection, which represent the neurogenic and inflammatory pain responses respectively.[21] The mice were separated into five groups of six animals each. Group 1 (control) animals received 10 ml/kg distilled water. Groups 2, 3 and 4 were respectively pretreated with 150, 300 and 450 mg/kg of ethanol extract respectively 30 minutes before being challenged with buffered formalin. Group 5 animals received 100 mg/kg of ASA intraperitoneally.

3.4.3 Hot plate-induced Pain

The effect of extract on hot plate-induced pain was investigated in adult Swiss albino mice according to the standard procedure of Vaz, Cechinel, Yunes and Calixto (1996).[23] and Nwafor and Okwuasaba (2003).[24] The hot plate test was used to measure the response latencies and was kept at 45 ± 1^{o}C throughout these experiments. The mice were placed into a glass beaker of 50 cm diameter on the heated surface, and the time(s) between placement and shaking or licking of the paws or jumping were recorded as the index of response latency. An automatic 30 seconds cut-off was used to prevent tissue damage. The mice were divided into five groups of six mice each. All the animals were fasted 24 hours before the experiment but allowed access to water. Group 1 animals (control group) were administered distilled water 10 ml/kg. Groups 2, 3 and 4 were respectively pretreated with 150, 300 and 450 mg/kg of ethanol extract intraperitoneally 30 minutes prior to the placement on the hot plate. Group 5 animals were administered 100 mg/kg of acetyl salicylic acid (i. p).

5 Evaluation of anti-inflammatory activities.

3.5.1 Carrageenin-induced mouse hind paw oedema

Acute inflammation was induced by sub-plantar injection of a phlogistic agent and measured as increase in the mouse hind- paw linear circumference.[25] Adult Swiss albino mice of either sex that were fasted for 24 hours but allowed free access to water except during the experiment, were separated into five groups of six mice per group. 0.1 ml of freshly prepared carrageenan suspension (1%) in normal saline was injected into the sub-plantar surface of the mouse hind-paw to cause inflammation. The linear circumference of the injected paw was measured with vernier calipers in millimeters (mm) before, 30 minutes and then every one hour for 5 hours after the administration of the phlogistic agent. The increase in paw circumference after administration of phlogistic agent as stated above was adopted as the parameter for measuring inflammation.[25][24] The oedema (inflammation) was assessed as the difference in paw circumference between the control and 30 minutes to 5 hours. The ethanol extract 150, 300 and 450 mg/kg were respectively administered intraperitoneally to mice in groups 2, 3 and 4 one hour before inflammation was induced. Group 1 (control group) received 10 ml/kg of distilled water while group 5 (reference group) received acetyl salicylic acid, ASA (100 mg/kg) intraperitoneally (ip).

3.5.2 Fresh Egg Albumin-induced Inflammation

All the adult Swiss albino mice were fasted for 24 hours before the experiment but water was withdrawn during the experiment. Inflammation was induced in mice by the injection of 0.1 ml of fresh egg albumin into the subplantar surface of the right hind paw.[26] Adult albino mice of either sex were separated into five groups of six mice per group. Group 1 served as control and received 10 ml/kg of distilled water. Groups 2, 3 and 4 were respectively administered 150, 300 and 450 mg/kg of the extract (i.p) respectively. Group 5 animals received 100 mg/kg of acetyl salicylic acid (i.p). Each mouse was administered 0.1 ml of fresh egg albumin subcutaneously (s.c) 30 minutes after ethanol extract and drug treatment into the right paw. The linear circumference of the paw was measured before, 30 minutes, then every one hour for 5 hours using vernier calipers. Oedema was assessed as the difference in paw circumference between the control and 1 - 5 hours after administration of the phlogistic agent.[27] Hence, the linear circumference of the injected paw was measured before and 30 minutes to 5 hours following the administration of the phlogistic agent.

3.5.3 Xylene-induced Ear Oedema

Inflammation was induced in adult Swiss albino mice by topical administration of 2 drops of xylene at the inner surface of the right ear for a period of 15 minutes. Mice of either sex were divided into five groups of six mice per group. Group 1 animals received 10 ml/kg of distilled water and served as control. Groups 2, 3 and 4 received 150, 300 and 450 mg/kg of ethanol extract (i.p) respectively. Group 5 animals were administered 4 mg/kg of dexamethasone orally. The animals were fasted for 24 hours before the experiment started. All treatments were given to the mice 30 minutes before the induction of inflammation. They were thereafter sacrificed under light anesthesia and both ears were cut off. The difference between the ear weights were recorded as the oedema induced by the xylene.[28] [29]

3.6 Statistical Analysis

Data obtained from this study was analysed using one way analysis of variance (ANOVA) followed by Tukey-kramer multiple comparison post test. Differences between means were considered significant at 5% level of significance ie $p \leq 0.05$.

4.0 RESULTS

4.1 Determination of Median Lethal Dose (LD_{50}).

From the study, the median lethal dose (LD_{50}) was calculated to be 1500 ± 35 mg/kg. The physical signs of toxicity noted were excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma followed by death.

4.2 1 Phytochemical Screening

The result of the phytochemical screening of *S. megaphylla* root extract revealed that alkaloids, flavonoids, tannins, terpenes, saponins and cardiac glycosides were present.

4. Phytochemical Analysis

The GC-MS analysis of the dichloromethane and n-butanol fractions of *Setaria megaphylla* roots revealed the presence of 14 bioactive compounds in each fraction as shown in Figures 1 and 2 and Tables 1 and 2. While borneol and α –terpineol were found in both fractions, the n-butanol fraction had hexadecanoic acid, cervacrol, linalool, camphor, menthofuran, menthone and α –eudesmol among others and the dichloromethane fraction had such constituents as astaxanthin, terpinen-4-ol, β-cis bergamotene, citronellol and germacrene D. These constituents are mostly monoterpenes and sesquiterpenes.

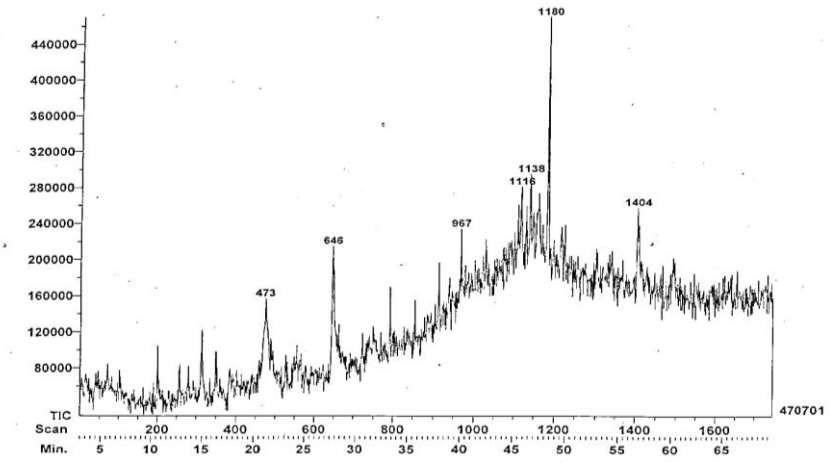

Figure 1: GC-MS spectrum of dichloromethane fraction of *Setaria megaphylla*

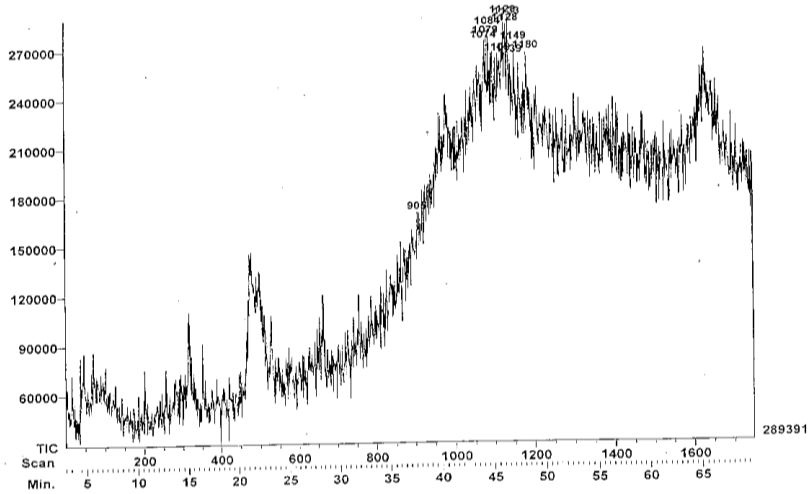

Figure 2: GC-MS spectrum of n-butanol fraction of *Setaria megaphylla.*

Table 1: GC-MS analysis of dichloromethane fraction of *Setaria megaphylla.*

S/No	Name of compound	Mol. Wt	Chemical formula	RI
1.	3-Methyl Benzaldehyde	164	C_8H_8O	200
2.	2,4,6-Trimethyl Octane	156	$C_{11}H_{24}$	310
3.	6-Methyl Tridecane	198	$C_{14}H_{30}$	360
4.	5-Propyl Decane	184	$C_{13}H_{26}O_2$	473
5.	Undecanoic Acid Ethyl Ester	214	$C_{13}H_{26}O_2$	646
6.	1-octen-3-ol	128	$C_{17}H_{36}O$	967
7.	trans-p-Menth-2-en-1-ol	154	$C_{18}H_{36}O_2$	1116
8.	Borneol	154	$C_{10}H_{18}O$	1138
9	Astaxanthin	598	$C_{48}H_{52}O_4$	1179
10.	α-Terpineol	154	$C_{10}H_{18}O$	1180
11.	Terpinen-4-ol	154	$C_{10}H_{18}O$	1185
12.	B-cis Bergamotene	204	$C_{15}H_{24}$	1404
13.	Citronellol sp	156	$C_{10}H_{20}O$	1212
14.	Germacrene D	204	$C_{15}H_{24}$	1484

Table 2: GC-MS analysis of n-butanol fraction of *Setaria megagylla*

S/No	Name of compound	Molecular Weight	Chemical formula	RI
1.	3-Methyl Benzaldehyde	120	C_8H_8O	200
2.	2,4,6-Trimethyl Octane	156	$C_{11}H_{24}$	320
3.	9,10-Secocholesta-5,7,10(19)-Triene,-3,24, 25-Triol (3a',5Z,7E)	416	$C_{27}H_{44}O_3$	350
4.	3-Methyl-Undecane	170	$C_{12}H_{26}$	490
5.	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	660
6.	1,2 -Benzenedicarboxylic acid, Isodecyl Octyl Ester	418	$C_{26}H_{42}O_4$	906
7.	Cervacrol	150	$C_{10}H_{14}O$	1076
8.	Linanlool	154	$C_{10}H_{18}O$	1084
9.	Camphor	152	$C_{10}H_{16}O$	1128
10.	Borneol	154	$C_{10}H_{18}O$	1139

11.	Menthofuran	150	$C_{10}H_{14}O$	1140
12.	Menthone	154	$C_{10}H_{18}O$	1149
13.	α –Terpineol	154	$C_{10}H_{18}O$	1180
14.	α –Eudesmol	222	$C_{15}H_{26}O$	1650

4.3 Anti-inflammatory Activities

4.3.1 Carrageenan-induced Oedema in Mice: The result revealed that the extract (150 - 450 mg/kg) exerted a statistically significant ($p < 0.05 – 0.001$) anti-inflammatory effect in a dose-dependent manner. The effect exhibited was comparable to the standard drug, ASA, 100 mg/kg. (Table 3).

4.3.2 Egg Albumin- induced Oedema in Mice: The extract (150 - 450 mg/kg) exerted a significant ($p < 0.05 - 0.001$) anti-inflammatory effect when compared to control. This effect was dose-dependent and not comparable to that of standard drug, ASA (100 mg/kg). (Table 4).

4.3.3 Xylene-induced Ear Oedema in Mice: The result showed that the extract (150 - 450 mg/kg) exerted a dose-dependent statistically significant anti-inflammatory effect ($p < 0.001$) when compared to control. The exerted effect was observed to be incomparable to that of standard drug, ASA (100 mg/kg) (Table 5).

4.4 Analgesic Activity

4.4.1 Acetic Acid-induced Writhing in Mice: The extract (150 - 450 mg/kg) demonstrated a dose-dependent reduction in acetic acid-induced writhing activity. These reductions were statistically significant ($p < 0.05 - 0.001$) relative to control but weak compared to that of the standard drug, ASA 100 mg/kg. (Table 6).

4.4.2 Formalin-Induced Hind Paw Licking in Mice: Table 7 shows the analgesic effect of root extract of *Setaria megaphylla* on formalin-induced hind paw licking. The extract **(**150 - 450 mg/kg) exerted a dose - dependent inhibition that was statistically significant relative to the control ($p < 0.001$). This observed effect was weak compared to that of the standard drug, ASA. 100 mg/kg.

4.4.3 Hot Plate-induced Pain in Mice: The result showed that pretreatment of mice with the extract (150 - 450 mg/kg), caused a dose-dependent increase in latency of response (analgesic effect). The observed effect was statistically significant ($p < 0.001$) relative to control but was weak compared to the standard drug ASA 100 mg/kg. (Table 8).

Table 3: Effect of extract on carrageenin- induced oedema in mice.

Treatment/ Dose (mg/kg)	Time Intervals (hrs)						
	0	0.5	1	2	3	4	5
Control	0.23 ± 0.01	0.38± 0.01	0.39 ± 0.01	0.40 ± 0.01	0.38 ± 0.01	0.35 ± 0.01	0.32 ± 0.01
Extract 150	0.22 ± 0.01	0.37± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.34 ± 0.01	0.32 ± 0.01	0.29 ± 0.01
Extract 300	0.24 ± 0.01	0.37± 0.01	0.36 ± 0.01	0.34 ± 0.01^{b}	0.32 ± 0.01^{b}	0.30 ± 0.01^{a}	0.28 ± 0.01
Extract 450	0.23 ± 0.01	0.35± 0.01	0.35 ± 0.01	0.32 ± 0.01^{c}	0.29 ± 0.01^{c}	0.27 ± 0.01^{c}	0.26 ± 0.01^{b}
ASA 100	0.24 ± 0.01	0.35± 0.01	0.33 ± 0.01	0.30 ± 0.01^{c}	0.28± 0.01^{c}	0.26 ± 0.01^{c}	0.25 ± 0.01^{c}

Data are expressed as mean ± SEM. Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control. n = 6.

Table 4: Effect of extract on egg- albumin induced oedema in mice.

Treatment/ Dose (mg/kg)	Time Intervals (hrs)						
	0	0.5	1	2	3	4	5
Control	0.24 ± 0.01	0.32± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
Extract 150	0.25 ± 0.01	0.34± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0.28 ± 0.01
Extract 300	0.25 ± 0.01	0.33± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.29 ± 0.01	0.29 ± 0.01^{b}	0.27 ± 0.01
Extract 450	0.23 ± 0.01	0.33± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.28 ± 0.01^{a}	0.27 ± 0.01^{a}	0.26 ± 0.01^{b}
ASA 100	0.24 ± 0.01	0.35±0.01	0.34± 0.01	0.29 ± 0.01^{a}	0.26 ± 0.01^{c}	0.26 ± 0.01^{b}	0.25 ± 0.01^{c}

Data are expressed as mean ± SEM. Significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$when compared to control. n = 6.

Table 5: Effect of extract on xylene-induced ear oedema in mice

Treatment/Dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	% inhibition
Control 0.2ml	0.0782 ± 0.001	0.038 ± 0.001	0.0402 ± 0.001	---
Extract 150	0.0596 ± 0.001	0.0374 ± 0.001	0.0222 ± 0.001^{c}	44.77
Extract 300	0.0514 ± 0.001	0.0384 ± 0.001	0.0130 ± 0.001^{c}	67.66
Extract 450	0.0486 ± 0.001	0.038 ± 0.01	0.0106 ± 0.001^{c}	73.63
Dexamethasone 4.0	0.0452 ± 0.001	0.0372 ± 0.001	0.0080± 0.00^{c}	80.09

Values are expressed as mean ± S.E.M. Significance relative to control: $^{c}p < 0.001$, n = 6.

Table 6: Effect of extract on acetic acid -induced writhing in mice

Treatment/ Dose (mg/kg)	Time intervals (hrs)						
	5	10	15	20	25	30	Total
Control	15.33± 1.76	17.00 ± 1.52	18.66 ± 1.52	13.00± 1.73	13.00 ± 1.00	10.33 ± 1.33	74.32 ± 8.86
Extract 150	7.66±1.20^{c}	11.30 ± 0.88	14.00± 1.00^{a}	7.66 ± 1.20^{a}	6.66 ± 0.20^{b}	5.60 ± 0.32^{a}	52.88 ± 4.80^{c}
Extract 300	1.66±0.33^{c}	5.66 ± 0.30^{c}	4.30 ± 0.76^{c}	4.00 ± 0.24^{b}	4.13 ± 0.33^{c}	4.16. ± 0.39^{b}	23.91 ± 5.05^{c}
Extract 450	0.66±0.16^{c}	4.00±0.37^{c}	4.33 ± 0.88^{c}	4.02 ± 0.36^{c}	3.00 ± 1.15^{c}	3.66 ± 0.34^{c}	19.67 ± 3.26^{c}
ASA 100	0.00^{c}	3.00 ± 1.73^{c}	6.00 ± 0.88^{c}	5.66 ± 0.57^{c}	4.00 ± 1.28^{c}	2.13 ± 0.45^{b}	20.79 ± 4.91^{c}

Data are expressed as mean ± SEM. significant at $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared tocontrol. n = 6.

Table 7: Effect of extract on formalin - induced hind paw licking in mice.

Treatment/ Dose (mg/kg)	Time Intervals (mins)						
	5	10	15	20	25	30	Total
Control	24.33 ± 0.33	22.37± 0.66	20.66± 0.44	14.33± 0.23	13.03± 0.14	10.16 ± 0.16	104.88± 1.96
Extract 150	19.60 ± 0.52	6.66 ± 0.32^{c}	5.31 ± 0.88^{c}	3.32 ± 0.27^{c}	4.50 ± 0.57^{c}	3.33 ± 0.21^{c}	42.72 ± 2.77^{c}
Extract 300	14.14 ± 0.18^{c}	4.00 ± 0.57^{c}	3.00 ± 0.57^{c}	3.66 ± 0.88^{c}	3.14 ± 0.66^{c}	3.00 ± 0.57^{c}	30.94 ± 3.43^{c}
Extract 450	14.12 ± 0.45^{c}	3.10 ± 1.15^{c}	3.33 ± 0.88^{c}	3.00 ± 0.57^{c}	3.32 ± 0.88^{c}	2.31 ± 0.32^{c}	29.18 ± 4.25^{c}
ASA 100	8.00 ± 1.00^{c}	3.66 ± 1.20^{c}	2.00 ± 0.57^{c}	2.13 ± 0.88^{c}	1.11 ± 0.13^{c}	0.39 ± 0.92^{c}	17.29 ± 4.70^{c}

Data are expressed as mean ± SEM. significant at $^{c}p < 0.001$ when compared to control. n =6.

Table 8: Effect of extract on hot plate-induced pain in mice.

Group	Dose mg/kg	Reaction Time (mean ± SEM)	% inhibition
Control	---	4.83 ± 0.20	---
Extract	150	5.79 ± 0.22	19.87
Extract	300	12.39 ± 0.62^{c}	156.52
Extract	450	20.89 ± 0.64^{c}	332.50
ASA	100	22.93 ± 0.51^{c}	374.74

Data are expressed as mean ± SEM. Significant at $^{c}p < 0.001$ When compared to control, n= 6.

5.0 DISCUSSION

Setaria megaphylla root extract (150 – 450 mg/kg) exerted a dose-dependent significant ($p < 0.05$ -0.001) antiinflammatory effect in the three models (carrageenan, fresh egg albumin and xylene-induced oedema) evaluated in this work. ASA (100 mg/kg) a prototype NSAID and cyclooxygenase inhibitor whose mechanism of action involves inhibition of prostaglandin, also caused significant ($p < 0.05$ - 0.001) anti-inflammatory effect in the three models evaluated. The anti inflammmatory effect of the extract was weak when compared to the standard drug acetyl salicyclic acid (100 mg/kg).

Carrageenan-induced inflammation model is a predictive test for mediators of acute inflammation.[30] Development of carragenin-induced oedema consists of two distinct phases, with the first phase (1 hour) involving the release of serotonin and histamine[31], while the second phase (over 1 hour) is mediated by prostaglandins, leucotrienes, the cyclooxygenase products and the continuity between the two phases is provided by kinins.[32] Egg albumin-

induced edema is known to also have a biphasic phase with the early phase due to release of histamine, serotonin and kinins and the late phase mediated by bradykinin, leukotrienes and prostaglandins released from tissue macrophages.[31] Phospholipase A_2 (PLA_2) is involved in the pathophysiology of inflammation due to xylene.[33] PLA_2 catalyzed hydrolysis of membrane phospholipids results in the production of arachidonic acid and a lysophospholipid which are precursors of inflammatory mediators like prostaglandins, leukotrienes and platelet activating factors (PAF).[34] However, dexamethasone, a steroidal anti-inflammatory agent also produced significant reduction in the mean right ear weight of positive control rats indicating an inhibition of PLA_2. The extract could have caused the observed anti-inflammatory effects from one or all of the mechanisms outlined above.

Free radical scavenging agents like flavonoids and terpenes also play a role in inflammatory process by preventing the generation or actions of free radicals. Flavonoids and tannins are also reported to inhibit PG synthesis as part of their anti-inflammatory activity.[35] The monoterpenes borneol, α- terpineol, citronollel, cervacrol, linalool and the terpene astaxanthin identified in this plant are all known to have antioxidant and anti-inflammatory properties that work by inhibition of COX-2 enzyme activity, neutrophil influx and Interleukin (IL) production.[37] [38] Borneol exerts anti-inflammatory effects through fewer intercellular adhesion molecule (ICAM)-1 positive vessels, interleukin (IL)-1β positive cells, TNF-α positive cells and number of neutrophils as well as reducing leukocyte migration.[39][40] α-Terpineol exerts anti-inflammatory activity by inhibition of COX-2 enzyme activity, IL production[37][38] and neutrophil influx.[41] Citronellol has anti-inflammatory effect[42] and has been reported to have significantly ($p< 0.05$) decreased paw edema at least in part, through the spinal cord lamina I inhibition.[43] Cervacrol affects anti-inflammatory activity by suppressing COX-2 thus interfering in the release and/or synthesis of inflammatory mediators, such as the prostanoids[44] and activating peroxisome proliferator-activated receptor (PPAR) gamma.[45] α -Eudesmol has anti-neurogenic inflammation action that may be due to its presynaptic inhibition of the release of neuropeptides calcitonin gene-related peptide (CGRP) and substance P from perivascular trigeminal terminals.[46] The components of this extract could have been responsible for the observed anti-inflammatory activity through any or all of the mechanisms mentioned above.

The extract (150 - 450 mg/kg) produced a statistically significant ($p < 0.05$ - 0.001) dose-dependent reduction in acetic acid-induced writhing and formalin-induced paw licking in

mice and increased the latency of response in the hot plate test. The effects produced were not comparable to that of the standard drug, acetyl salycyclic acid (ASA) 100 mg/kg. Acetic acid writhing test is a chemical method used to induce pain of peripheral origin. The signals are transmitted to the central nervous system in response to pain due to irritation and cause release of mediators such as prostaglandins PGE_2 and $PGE_{2\alpha}$ which contributes to the increased sensitivity to nociceptors.[47] Analgesic activity of a test compound is inferred from decrease in the frequency of writhings.[48] Quintans-Júnior *et al.*, (2011)[49] reported that α-terpineol has antinociceptive effects while De Sousa *et al.*, (2008)[50] reported on the analgesic properties of menthone. Citronellol, a monoterpene possesses analgesic activity probably mediated by inhibition of peripheral mediators, tumour necrosis factor alpha (TNF-a) and NO synthesis, as well as central inhibitory mechanisms via opioid central receptors.[51]

The mechanism of pain of formalin is thought to be biphasic.[28] The first phase, neurogenic (0 -5 minutes), which is chemical stimulation of pain receptors involves the release of Substance P and bradykinin, while the second phase, inflammatory pain (15 -30 minutes), results from sensitization of both peripheral and central neurons and involves release of histamine and prostaglandins.[52] Monoterpenes present in this extract might be responsible for part of its activity. Borneol, a constituent has peripherally and centrally acting analgesic properties[39], Linalool‘s antihyperalgesic and antinociceptive effects are known to induce significant reductions of the acetic acid-induced writhing response, a model of inflammatory pain and the hot plate test, a model of supraspinal analgesia.[53] Linalool’s effect is due to stimulation of the cholinergic, opioidergic and dopaminergic systems, to its local anesthetic activity and to the blockade of N-Methyl-d-aspartate receptors (NMDA).[54] Linalool’s antinociception has also been attributed to an inhibition of glutamatergic transmission[55], or of voltage-gated Ca^{2+} channel[56], a potentiation of GABAergic transmission[57] and a modulation of adenosine A_1 and A_{2A} receptors[58], in addition to nerve conduction inhibition. The phytochemical constituents of this extract may be responsible for its analgesic effect through one or a combination of the mechanisms mentioned above.

The hot plate test is used to evaluate central analgesic activity. ASA induces analgesic effect through activation of opioid receptors[59] and the apparent similarity between the results of extract with standard ASA, indicates that they might work in a same manner to reduce pain sensation. The analgesic activity of *S. megaphylla* extract might be due to the interference of its active principle(s) with the release of pain mediators. Flavonoids are reported to increase

the amount of endogenous serotonin or may interact with 5-HT_{2A} and 5- HT_3 receptors which may be involved in the mechanism of central analgesic activity.[60] Borneol, linalool and terpineol among other components are thought to be responsible for the analgesic qualities of *Artemisia argy*.[61] Borneol exerts peripheral and centrally acting analgesic properties by reducing leukocyte migration[39] and menthol engages in synergistic excitation of γ-aminobutyric acid (GABA) receptors and sodium ion channels resulting in analgesia.[62]

Some components of this extract are reported to possess central acting activity indicating the involvement of narcotic or opioid receptors. This extract therefore has the potential of inhibiting the neurogenic, non-neurogenic and narcotic pains from the results of the analgesic models conducted. These compounds present in this extract may have been responsible for the observed analgesic activity of this extract.

6.0 CONCLUSION

The results of this research work indicates that *Setaria megaphylla* extract through its phytochemical constituents possess analgesic and anti-inflammatory properties and also supports the ethnomedicinal use of the roots of *Seteria megaphylla* in the treatment of hemorrhoids, urethritis, inflammation, fevers and various pains. Further investigation is hereby recommended to demonstrate cellular mechanisms and structural components of the active ingredients of this root extract in order to standardize them.

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