



## Analgesic and anti-inflammatory activities of *Zea mays* leaves

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### ARTICLE INFO

**Type:** Original Research

**Topic:** Medicinal Plants

**Received:** April 19<sup>th</sup> 2015

**Accepted:** February 20<sup>th</sup> 2016

### Key words:

- ✓ *Zea mays*
- ✓ Anti-inflammatory
- ✓ Analgesic
- ✓ GC-MS

### ABSTRACT

**Background & Aim:** *Zea mays* leaf used traditionally in Ibibio ethno medicine in Southern Nigeria to treat malaria and other inflammatory diseases. These anti-inflammatory and analgesic properties were evaluated against experimentally induced inflammation and pains using standard models.

**Experimental:** The effects of ethanol leaf extract of *Zea mays* (170 -510 mg/kg) on experimentally induced inflammation and pain were studied in rats using Carrageenin, egg albumin and xylene-induced inflammation as well as acetic acid-induced writhing and thermal induced pain models. The GCMS analysis of the most active fraction was carried out.

**Results:** The extract (170 -520 mg/kg) inhibited inflammation and pains caused by different phlogistic agents used in a dose dependent fashion. The various degrees of inhibitions were statistically significant ( $p < 0.05, 0.01, 0.001$ ), though incomparable to that of the standard drugs used (ASA and indomethacin). The GC-MS revealed the present of polyunsaturated fatty acids and phenolic compounds which may be responsible for the observed activities of the extract.

**Recommended applications/industries:** The present study demonstrates that leaf extract of *Zea mays* might to be useful for the treatment of inflammation and pains.

## 1. Introduction

*Zea mays* L. (Family- Poaceae) known as maize or corn, is an annual grass plant cultivated for human consumption and rearing of animals. It was introduced to Nigeria in the 16th century (Osagie and Eka, 1998). It is tall with strong erect stalks and a fibrous root system. The plant has long narrow leaves that are spaced alternately on opposite side of the stem and bears ears that are enclosed in modified leaves known as husks

(Simmonds, 1979). Besides its nutritive values, maize grains, leaves, cornsilks, stalk, and inflorescence are also used in ethnomedicine for the treatment of several ailments. The corn silk is used as an antidiabetic or diuretic, and decoction of the silk is consumed for the treatment of urinary troubles and gallstones (Foster and Duke, 1990; Gill, 1992; Abo et al., 2008). The ash of the cob is used for the treatment of cough (Gill, 1992) as well as inflammatory diseases. The husks are used in the treatment of pains and arthritis (Owoyele et al., 2010). It is also taken as warm tea for the treatment of malaria in

Ibibio traditional medicine. Biological activities reported on the leaf extract include; anticancer (Balasubramanian and Padma, 2013; Balasubramanian *et al.*, 2014), antioxidant (Balasubramanian and Padma, 2012) and antioxidative stress (Balasubramanian *et al.*, 2015) activities. Anti-inflammatory and analgesic activities have been reported on the husk extract (Owoyele *et al.*, 2010). Eight phenolic compounds (gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, femlic acid, rutin, resveratrol, and kaempferol) have also been detected in ethanol husk extract of *Zea mays* (Dong *et al.*, 2014). Information on the biological activities of the leaf extract is scarce. We report in this study the anti-inflammatory and analgesic activities of the leaf extract to confirm its uses Ibibio ethno medicine.

## 2. Materials and Methods

### 2.1. Plants collection

The plant material *Zea mays* (leaves) were collected in a farmland in Uyo area, Akwa Ibom State, Nigeria in May, 2015. The plant was identified and authenticated by Dr. Margaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Department of Pharmacognosy and Natural Medicine Herbarium.

### 2.2. Extraction

The plant parts were washed and shade-dried for two weeks. The dried plants' materials were reduced to powder using mortar and pestle. The powdered material was soaked in 50% ethanol. The liquid filtrate was concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator and stored in a refrigerator at -4°C.

### 2.3. Phytochemical Screening

Phytochemical screening of the crude extract was carried out employing standard procedures and tests (Trease and Evans, 1989, Sofowora, 1993), to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

### 2.4. Animals

Albino Swiss mice (20 – 25g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. The animals were divided into 5 groups of 6 mice in each all the models studied. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics committee, University of Uyo.

### 2.5. Determination of median lethal dose (LD<sub>50</sub>)

The median lethal dose (LD<sub>50</sub>) of the extract was estimated using albino mice by intraperitoneal (i.p) route using the method of Lorke (1983). This involved intraperitoneal administration of different doses of the extract (1000 -5000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD<sub>50</sub> was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$$LD_{50} = \sqrt{ab}$$

### 2.6. Evaluation of anti-inflammatory activity of the extract

#### 2.6.1. Carrageenin – induced mice hind paw oedema.

Adult albino male mice were used after 24 hours fast and deprived of water only during experiment. Inflammation of the hind paw was induced by injection of 0.1 ml of freshly prepared carrageenin suspension in normal saline into the sub planar surface of the hind paw. The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent. The increase in paw circumference post administration of phlogistic agent was adopted as the parameter for measuring inflammation (Winter *et al.*, 1962; Akah and Nwambie, 1994; Ekpendu *et al.*, 1994, Besra *et al.*, 1996; Nwafor *et al.*, 2010). The difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent was used to assess inflammation (Hess and Milonig, 1992). The leaf extract (170, 340 and 510 mg/kg i.p) was administered to various groups of 6 mice each, 1 h before inducing inflammation. Control mice received carrageenin while reference group received ASA (100

mg/kg). The average (mean) oedema was assessed by measuring with vernier calipers. Average inflammation/oedema ( $C_t - C_0$ ) was calculated for each dose (Oriowo, 1982; Akah and Njike, 1990).

#### 2.6.2. Egg-albumin induced inflammation.

Inflammation was induced in mice by the injection of egg albumin (0.1ml, 1% in normal saline) into the sub planar tissue of the right hind paw (Akah and Nwambie, 1994; Okokon and Nwafor, 2010). The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 hrs after the administration of the phlogistic agent. The leaf extract (170, 340 and 510 mg/kg i.p) and ASA (100 mg/kg orally) were administered to groups (n=6) of 24 h fasted mice 1 h before the induction of inflammation. Control group received 10 ml/kg of distilled water orally. Edema (inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs post administration of the phlogistic agent (Hess and Milonig, 1972). The average (mean) edema was assessed by measuring with vernier calipers. Average inflammation/oedema ( $C_t - C_0$ ) was calculated for each dose (Oriowo, 1982; Akah and Njike, 1990).

**2.6.3 Xylene – induced ear oedema.** Inflammation was induced in mice by topical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act for 15 mins. *Zea mays* leaf extract (170, 340 and 510 mg/kg i.p), dexamethasone (4 mg/kg) and distilled water (0.2 ml/kg) were orally administered to various groups (n=6) of mice 1 h before the induction of inflammation. The animals were sacrificed under light anaesthesia and the left ears cut off. The difference between the ear weights was taken as the oedema induced by the xylene (Tjolsen *et al.*, 1992; Okokon and Nwafor, 2010).

### 2.7. Evaluation of analgesic potential of the extract

**2.7.1. Acetic acid induced writhing in mice.** Writhings (abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hindlimbs) resulting from intraperitoneal (i.p) injection of 2% acetic acid, was induced according to the procedure described by Santos *et al.* (1994), Correa *et al.* (1996) and Nwafor *et al.* (2010). The animals were divided into 5 groups of 6 mice per group. Group 1 served as negative control and received 10 ml/kg of

normal saline, while groups 2, 3 and 4 were pre-treated with 170, 340 and 510 mg/kg doses of *Z. mays* leaf extract intraperitoneally, and group 5 received 100 mg/kg of acetyl salicylic acid. After 30 minutes, 0.2 ml of 2% acetic acid was administered intraperitoneally (i.p). The number of writhing movements was counted for 30 minutes. Antinociception (analgesia) was expressed as the reduction of the number of abdominal constrictions between control animals and mice pretreated with extracts.

**2.7.2. Thermally induced pain in mice.** The effect of extract on hot plate induced pain was investigated in adult mice. The hot plate was used to measure the response latencies according to the method of Vaz *et al.*, (1996) and Okokon and Nwafor, (2010). In these experiments, the hot plate was maintained at  $45 \pm 1^\circ\text{C}$ , each animal was 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 was recorded as the index of response latency. An automatic 30-second cut-off was used to prevent tissue damage. The animals were randomly divided into 5 groups of 6 mice each and fasted for 24 hours but allowed access to water. Group 1 animal served as negative control and received 10 ml/kg of normal saline. Groups 2, 3 and 4 were pre-treated intraperitoneally with 170, 340 and 510 mg/kg doses of *Z. mays* leaf extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 minutes prior to the placement on the hot plate.

### 2.8. Gas chromatography-Mass spectrometry analysis

Quantitative and qualitative data were determined by GC and GC-MS, respectively. 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  $\mu\text{m}$  df, coated with 5 % diphenyl-95 % polydimethylsiloxane, operated with the following oven temperature programme:  $50^\circ\text{C}$ , held for 1 min, rising at  $3^\circ\text{C}/\text{min}$  to  $250^\circ\text{C}$ , held for 5 min, rising at  $2^\circ\text{C}/\text{min}$  to  $280^\circ\text{C}$ , held for 3 min; injection temperature and volume,  $250^\circ\text{C}$  and 1.0  $\mu\text{l}$ , 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,  $280^\circ\text{C}$ ; hydrogen, flow rate, 50 ml/min; air flow rate, 400 ml/min; make-up ( $\text{H}_2/\text{air}$ ), flow rate, 50

ml/min; 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 0C. 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.

**2.8.1. Identification of the compounds.** The identification of components present in the active fraction of the plants' extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley and Nist Libraries, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literatures (Adams, 2001; Setzer *et al.*, 2007).

### 2.9. Statistical analysis and data evaluation

Data obtained from this work were analyzed statistically using Students't-test and ANOVA (One-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means were considered significant at 1% and 5% level of significance i.e.  $P \leq 0.01$  and  $0.05$ .

## 3. Results and discussion

The phytochemical screening of the ethanol leaf extract of *Zea mays* revealed the presence of alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids.

The median lethal dose ( $LD_{50}$ ) was calculated to be 1732.05 mg/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

The effect of ethanol extract of *Zea mays* leaf on carragenin-induced oedema is as shown in Table 1. The extract (170 - 510 mg/kg) exerted a significant ( $P < 0.05 - 0.001$ ) anti-inflammatory effect in a dose -dependent manner. The activity of the highest dose was incomparable to that of the standard drug, ASA, 100 mg/kg (Table 1a and 1b).

Administration of leaf extract of *Zea mays* (170 - 510 mg/kg) on egg albumin - induced oedema in mice caused a significant ( $p < 0.05 - 0.001$ ) dose-dependent anti-inflammatory effect against oedema caused by egg albumin. The effect was incomparable to that of standard drug, ASA (100 mg/kg) (Table 2a and 2b).

Anti-inflammatory effect of crude leaf extract of *Zea mays* against xylene-induced ear oedema in mice is shown in Table 3. The extract exerted a dose-dependent anti-inflammatory effects that were significant ( $P < 0.05 - 0.01$ ) but incomparable to that of the standard drug, dexamethasone (4.0 mg/kg).

The administration of *Z. mays* extract (170, 340 and 510 mg/kg) demonstrated a dose-dependent reduction in acetic acid-induced writhing in mice. The reductions were statistically significant ( $p < 0.05 - 0.001$ ) relative to control and the effect of the highest dose (510 mg/kg) at 30 minute was more than that of the standard drug, ASA (Table 4).

The extract (170, 340 and 510 mg/kg) exhibited a dose - dependent effect on thermally-induced pain in mice. This inhibitions were statistically significant ( $p < 0.05$ ) only at the highest dose of the extract (510 mg/kg) relative to the control but incomparable to that of the standard drug, ASA (100mg/kg) (Table 5).

The GC-MS analysis of the ethyl acetate fraction of *Zea mays* leaf revealed the presence of bioactive compounds with major and minor ones as represented in Table 6.

*Zea mays* leaf is used traditionally for the treatment of various illnesses such as malarial fever, pains and inflammatory conditions. In this study, the ethanol leaf extract was evaluated for analgesic and anti-inflammatory activities using various experimental models.

In the carragenin-induced oedema, the extract (170 - 510 mg/kg) was observed to have exerted a weak effect at the early stage of inflammation (1-2 hr) indicating weak effect probably on histamine, serotonin and kinnins that are involved in the early stage of carragenin-induced oedema (Vane and Booting, 1987). The extract further caused prominent reduction of the later stage of the oedema suggesting its ability to inhibit prostaglandin which is known to mediate the second phase of carragenin induced inflammation (Vane and Booting, 1987).

**Table 1a.** Effect of *Zea mays* leaf extract on carrageenin- induced oedema in rats.

Treatment/ dose (mg/kg)	Time intervals (h)						
	0	0.5	1	2	3	4	5
Control	2.18 ± 0.04	3.13 ± 0.05	3.13 ± 0.01	3.10 ± 0.06	3.04 ± 0.07	2.97 ± 0.01	2.88 ± 0.06
Extract 170	2.44 ± 0.06	3.72 ± 0.12	3.40 ± 0.07	3.26 ± 0.07	3.36 ± 0.28	3.17 ± 0.20	3.05 ± 0.20
340	2.55 ± 0.06	3.70 ± 0.11	3.55 ± 0.07 <sup>a</sup>	3.48 ± 0.14	3.40 ± 0.10	3.18 ± 0.04	3.04 ± 0.13
510	2.45 ± 0.10	3.39 ± 0.10	3.21 ± 0.03 <sup>a</sup>	3.33 ± 0.11	3.08 ± 0.08	2.86 ± 0.09	2.78 ± 0.11
ASA 100	2.21 ± 0.07	2.89 ± 0.04	2.59 ± 0.05 <sup>b</sup>	2.75 ± 0.02 <sup>a</sup>	2.60 ± 0.02 <sup>a</sup>	2.57 ± 0.08	2.51 ± 0.09

Data are expressed as mean ± SEM. Values were considered significant at <sup>a</sup>p<0.05; <sup>b</sup>p< 0.01 when compared to control. n = 6.

**Table 1b.** Effect of *Zea mays* leaf extract on carrageenin induced oedema in rats.

Treatment/ Dose (mg/kg)	Average inflammation/oedema (mm)					
	0.5h	1h	2h	3h	4h	5h
Control	0.95 ± 0.01	0.95 ± 0.01	0.92 ± 0.01	0.86 ± 0.01	0.79 ± 0.01	0.70 ± 0.01
Extract 170	1.27 ± 0.18	0.96 ± 0.13	0.82 ± 0.22	0.92 ± 0.01	0.73 ± 0.25	0.61 ± 0.25
340	1.11 ± 0.07	0.96 ± 0.07	0.89 ± 0.10	0.81 ± 0.10	0.62 ± 0.01 <sup>c</sup>	0.53 ± 0.25 <sup>c</sup>
510	0.90 ± 0.40	0.73 ± 0.03	0.85 ± 0.01	0.60 ± 0.04 <sup>c</sup>	0.41 ± 0.07 <sup>c</sup>	0.33 ± 0.15 <sup>c</sup>
ASA 100	0.68 ± 0.01	0.38 ± 0.01 <sup>c</sup>	0.54 ± 0.01 <sup>c</sup>	0.39 ± 0.01 <sup>c</sup>	0.36 ± 0.01 <sup>c</sup>	0.30 ± 0.01 <sup>c</sup>

Data are expressed as mean ± SEM. Values were considered significant at <sup>c</sup>p < 0.001 when compared to control. n = 6.

**Table 2a.** Effect of *Zea mays* leaf extract on egg- albumin induced oedema in mice.

Treatment/ Dose (mg/kg)	Time intervals (h)						
	0	0.5	1	2	3	4	5
Control	2.65 ± 0.07	3.55 ± 0.25	3.50 ± 0.06	3.45 ± 0.11	3.41 ± 0.07	3.35 ± 0.07	3.25 ± 0.06
Extract 170	2.44 ± 0.37	3.43 ± 0.09	3.09 ± 0.07	3.01 ± 0.07	2.94 ± 0.08	2.87 ± 0.01	2.78 ± 0.04
340	2.38 ± 0.03	3.22 ± 0.12	2.89 ± 0.11 <sup>a</sup>	2.82 ± 0.08 <sup>c</sup>	2.70 ± 0.06 <sup>c</sup>	2.63 ± 0.06 <sup>c</sup>	2.58 ± 0.03 <sup>c</sup>
510	2.31 ± 0.02	3.30 ± 0.09	2.71 ± 0.03 <sup>a</sup>	2.72 ± 0.07 <sup>c</sup>	2.62 ± 0.06 <sup>c</sup>	2.62 ± 0.03 <sup>b</sup>	2.51 ± 0.02 <sup>c</sup>
ASA 100	2.61 ± 0.06	2.99 ± 0.16 <sup>a</sup>	2.89 ± 0.02 <sup>a</sup>	2.84 ± 0.02 <sup>c</sup>	2.76 ± 0.03 <sup>c</sup>	2.64 ± 0.05 <sup>b</sup>	2.64 ± 0.05 <sup>c</sup>

Data are expressed as mean ± SEM. Values were considered significant at <sup>a</sup>p<0.05; <sup>b</sup>p< 0.01, <sup>c</sup>p< 0.001 when compared to control. n = 6.

**Table 2b.** Effect of *Zea mays* leaf extract on egg- albumin induced oedema in rats.

Treatment/ Dose (mg/kg)	Average inflammation/oedema (mm)					
	0.5h	1h	2h	3h	4h	5h
Control	0.90 ± 0.01	0.85 ± 0.01	0.80 ± 0.01	0.76 ± 0.01	0.70 ± 0.14	0.60 ± 0.10
Extract 170	0.99 ± 0.9	0.65 ± 0.07	0.55 ± 0.09 <sup>c</sup>	0.50 ± 0.11 <sup>c</sup>	0.43 ± 0.05 <sup>c</sup>	0.33 ± 0.07 <sup>c</sup>
340	0.83 ± 0.10	0.55 ± 0.09 <sup>c</sup>	0.50 ± 0.07 <sup>c</sup>	0.32 ± 0.06 <sup>c</sup>	0.23 ± 0.06 <sup>c</sup>	0.20 ± 0.03 <sup>c</sup>
510	0.99 ± 0.08	0.39 ± 0.04 <sup>c</sup>	0.41 ± 0.07 <sup>c</sup>	0.31 ± 0.05 <sup>c</sup>	0.30 ± 0.02 <sup>c</sup>	0.20 ± 0.02 <sup>c</sup>
ASA 100	0.38 ± 0.01 <sup>c</sup>	0.28 ± 0.01 <sup>c</sup>	0.23 ± 0.01 <sup>c</sup>	0.15 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>c</sup>	0.03 ± 0.01 <sup>c</sup>

**Table 3.** Effect of *Zea mays* leaf 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 (normal saline) 0.2ml	0.098 ± 0.01	0.043 ± 0.00	(127.90) 0.055 ± 0.00	-
Extract 170	0.090 ± 0.01	0.043 ± 0.00	(109.30) 0.047 ± 0.00 <sup>NS</sup>	14.54

340	0.080 ± 0.01	0.050 ± 0.01	(60.0)	45.45
510	0.056 ± 0.01	0.043 ± 0.01	0.030 ± 0.01 <sup>b</sup> (30.23)	76.36
Dexamethasone 4.0	0.043 ± 0.01	0.036 ± 0.01	0.013 ± 0.01 <sup>c</sup> (19.44)	87.27
			0.007 ± 0.00 <sup>c</sup>	

Figures in parenthesis indicate % increase in ear weight. Significant at <sup>a</sup>p < 0.01, <sup>b</sup>p < 0.001 when compared with control. n = 6.

**Table 4.** Effect of *Zea mays* leaf extract on acetic acid induced writhing in mice.

Treatment/ Dose (mg/kg)	Time intervals (h)						
	5	10	15	20	25	30	TOTAL
Control	6.66 ± 0.88	11.66 ± 1.20	24.66 ± 1.85	17.0 ± 0.57	13.00 ± 1.15	11.0 ± 1.00	83.98 ± 6.65
Extract 170	6.33 ± 0.66	5.00 ± 1.15 <sup>a</sup>	18.33 ± 1.45 <sup>a</sup>	14.00 ± 2.00	12.33 ± 0.88	10.66 ± 0.66	66.65 ± 6.80 <sup>a</sup>
340	4.00 ± 0.57	4.33 ± 1.85 <sup>b</sup>	13.66 ± 1.33 <sup>b</sup>	12.00 ± 1.00 <sup>a</sup>	7.33 ± 1.20 <sup>a</sup>	7.66 ± 0.85 <sup>a</sup>	48.98 ± 6.80 <sup>c</sup>
510	3.00 ± 0.57 <sup>a</sup>	4.00 ± 0.57 <sup>b</sup>	7.00 ± 1.00 <sup>c</sup>	8.66 ± 0.33 <sup>b</sup>	5.33 ± 0.66 <sup>b</sup>	6.00 ± 0.57 <sup>a</sup>	21.32 ± 3.70 <sup>c</sup>
ASA 100	4.00 ± 0.57	6.00 ± 0.57 <sup>a</sup>	8.33 ± 0.88 <sup>c</sup>	8.66 ± 0.33 <sup>b</sup>	7.66 ± 0.66 <sup>a</sup>	4.00 ± 0.57 <sup>b</sup>	38.65 ± 3.58 <sup>c</sup>

Data are expressed as mean ± SEM. Significant at <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.001 when compared to control n = 6.

However, ASA (100 mg/kg) a prototype NSAID, a cyclooxygenase inhibitor whose mechanism of action involves inhibition of prostaglandin, produced a considerable inhibition of the paw swelling induced by carragenin injection.

**Table 5.** Effect of *Zea mays* Leaf extract on hot plate test

Group	Dose mg/kg	Reaction time (sec) (mean ± SEM)	% inhibition
Control	-	4.92 ± 0.23	
<i>Z. mays</i>	170	5.33 ± 0.12	8.33
	340	6.59 ± 0.32	33.94
	510	12.62 ± 0.85 <sup>a</sup>	156.50
ASA	100	29.53 ± 3.48 <sup>c</sup>	500.20

Data are expressed as mean ± SEM. Significant at <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.001 when compared to control. n = 6.

The extract also inhibited egg albumin-induced oedema demonstrating that it can inhibit inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin (Nwafor *et al.*, 2007). However, ASA, a cyclooxygenase inhibitor reduced significantly oedema produced by egg albumin.

The extract exerted a significant (P<0.01) inhibition of xylene -induced ear oedema at higher doses of the extract, suggesting the inhibition of phospholipase A<sub>2</sub> which is involve in the pathophysiology of inflammation due to xylene (Lin *et al.*, 1992). However, dexamethasone, a steroid antiinflammatory agent

produced significant reduction in the mean right ear weight of positive control rats indicating an inhibition of PLA<sub>2</sub>.

The extract significantly reduced acetic acid-induced writhing and delayed the reaction time of animals (mice) to thermally induced pain. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas *et al.*, 1984; Nwafor *et al.*, 2007), and in part through local peritoneal receptors from peritoneal fluid concentration of PGE<sub>2</sub> and PGF<sub>2</sub>α (Deraedt *et al.*, 1980; Bentley *et al.*, 1983). The acetic acid-induced abdominal writhing is a visceral pain model in which the processor releases arachidonic acid via cyclooxygenase, and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Franzotti *et al.*, 2002). It is used to distinguish between central and peripheral pain. These results suggest that the extract may be exerting its action partly through the lipoxygenase and/or cyclooxygenase system.

The study also shows that the extract significantly delayed the reaction time of thermally- induced (hot plate) test. This model is selective for centrally acting analgesics and indicates narcotic involvement (Turner, 1995) with opioid receptors.

Phytochemical screening of the leaf extract revealed the presence of alkaloids, cardiac glycosides, tannins, saponins, terpenes and flavonoids and GC-MS analysis of ethyl acetate fraction showed the presence of pharmacological active compounds such as 2,3 dihydro benzofuran and polyunsaturated fatty acids such as 9-

Octadecenoic acid (Z)-, 2- hydroxyethyl ester, Hexadecanoic acid, ethyl ester and Hexadecanoic acid, methyl ester which have been implicated in the anti-inflammatory activity of plants (Ledon *et al.*, 2003; Kumar, 2010; Raja *et al.*, 2011).

Moreso, anti-inflammatory activities of plants have been linked to antioxidant potentials (Sokeng *et al.*, 2013). The plant extract have been revealed by GCMS to contain phenolic compounds with antioxidant potentials such as p-Hydroxycinnamic acid, ethyl ester and, Phenol, 2,6-dimethoxy-4-(2-propenyl)- (Alamed *et al.*, 2009). Also Triterpene-fatty acid esters and free fatty acids including long chain C16-C20 unsaturated fatty acids have been suggested to be responsible for the anti-inflammatory activity of plants (Li *et al.*, 2004).

Flavonoids are known anti-inflammatory compounds acting through inhibition of the cyclooxygenase pathway (Liang *et al.*, 1999). Some flavonoids are reported to block both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at relatively high concentrations, while at lower concentrations they only block lipoxygenase pathway (Carlo *et al.*, 1999). Some flavonoids exert their antinociception via opioid receptor activation activity (Suh *et al.*, 1996; Rajendran *et al.*, 2000; Otuki *et al.*, 2005). Flavonoids also exhibit inhibitory effects against phospholipase A2 and phospholipase C (Middleton *et al.*, 2000), and cyclooxygenase and/or lipoxygenase pathways (Robak *et al.*, 1998).

Triterpenes have been implicated in anti-inflammatory activity of plants (Huss *et al.*, 2002; Suh *et al.*, 1998) and reports on their analgesic activities have also been published (Liu, 1995; Krogh *et al.*, 1999; Tapondjou *et al.*, 2003; Maia *et al.*, 2006). Ursolic acid is a selective inhibitor of cyclooxygenase-2 (Ringbom *et al.*, 1998). Oleanolic acid is known to exert its analgesic action through an opioid mechanism, and possibly, a modulatory influence on vanilloid receptors (Maia *et al.*, 2006).

The anti-inflammatory and analgesic activities demonstrated by the leaf extract in this study may in part be due to its phytochemical constituents which maybe acting through antioxidant action and other mechanisms.

In conclusion, the results of this study demonstrated that *Zea mays* leaf possess anti-inflammatory and analgesic properties. Further investigation is being

advocated especially in elucidating cellular mechanisms and establishing structural components of the active ingredients with a view of standardizing them.

#### 4. Conclusion

The present study demonstrates that leaf extract of *Zea mays* might to be useful for the treatment of inflammation and pains.

#### 5. Acknowledgement

Authors are grateful to Messrs Aniefiok Ukpong and Nsikan Malachy of Department of Pharmacology and Toxicology, University of Uyo, Uyo, for their technical assistance.

#### 6. References

- Abo, K.A., Fred-Jaiyesimi, A.A., Jaiyesimi, A.E.A. 2008. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *J Ethnopharmacol.*, IS: 67-71.
- Adams, R.P. 2001. Identification of Essential oils by Gas Chromatography Quadrupole Mass Spectrometry. Allured Publishing Corporation, Carol Stream, USA
- Ahmed, M., Sadhu, S.K., Datta, B.K., Kunu, J.K., Bachar, S.C. 1997. Preliminary studies on the antiinflammatory, analgesic and diuretic activity of stagninol, a sesquiterpene isolated from *Persicaria stagnina*. *Pharmazie.*, 52(6):472-475.
- Akah, P.A., Njike, H.A. 1990. Some pharmacological effects of rhizome aqueous extract of *Anchomanes diformis*. *Fitoterapia.*, 60: 368-370.
- Akah, P.A., Nwanbie, A. 1994. Evaluation of Nigerian traditional medicines plants used for rheumatic (inflammatory) disorder. *Journal of Ethnopharmacology.*, 42: 179 – 182.
- Alamed, J., Chaiyasit, W., McClements, D.J., Decker, E.A. 2009. Relationships between free radical scavenging and antioxidant activity in foods. *Journal of Agricultural and Food Chemistry.*, 57: 2969-2976.
- Amico-Roxas, M., Caruso, A., Trombadore, S., Scifo, R., Scapagnime, U. 1984. Gangliosides antinociceptive effects in rodents. *Archives of*

- International Pharmacodynamics and Therapeutics.*, 272: 103-117.
- Balasubramanian, K., Padma, P.R. 2013. Anticancer activity of *Zea mays* leaf extracts on oxidative stress-induced Hep2 Cells. *J Acupuncture and Meridian Studies.*, 6(3):149-158.
- Balasubramanian, K., Padma, P.R. 2012. Screening of Antioxidant Properties of *Zea mays* Leaves at different time periods of growth. *Journal of Pharmacy Research.*, 5(8):4034-4037.
- Balasubramanian, K., Vidhya, A., Thiruselvi, M., Sudhadevi, M. and Padma, P.R. 2014. *Zea mays* leaf extracts exhibits anticancer property and enhance the chemotherapeutic action of etoposide in cancer cells. *Indo American Journal of Pharmaceutical Research.*, 4(03): 1530 - 1539.
- Balasubramanian, K., Jincy, P.A., Padma, P.R. 2015. Influence of methanolic extract of *Zea mays* leaves against CCL<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> induced oxidative stress in *Drosophila melanogaster*. *Indo American Journal of Pharmaceutical Research.*, 5(01): 566-577.
- Bentley, G.A., Newton, S.H., Starr, J. 1983. Studies on the antinociceptive action of agonist drugs and their interaction with opioid mechanisms. *British Journal of Pharmacy.*, 79: 125 - 134.
- Besra, S.E., Sharma, R.M., Gomes, A. 1996. Antiinflammatory effect of petroleum ether extract of leaves of *Litchi Chinensis*. Caertn (sapindaceae). *Journal of Ethnopharmacology.*, 54:1-6.
- Carlo Di, G., Mascolo, N., Izzo, A.A., Capasso, F. 1999. Flavonoids, old and new aspects of a class of natural therapeutic drugs. *Life Science.*, 65: 337-353.
- Correa, C.R., Kyle, D.J., Chakravarty, S., Calixto, J.B. 1996. Antinociceptive profile or the pseudopeptide  $\beta_2$  bradykinin receptors antagonist NPC 18688 in mice. *British Journal of Pharmacology.*, 117: 552-556.
- Deraedt, R., Jougney, S., Falhout, M. 1980. Release of Prostaglandin E and F in an algogenic reaction and its inhibition. *European Journal of Pharmacy.*, 51: 17-24.
- Dong, J., Cai, L., Zhu, X., Huang, X., Yin, T., Fang, H., Ding, Z. 2014. Antioxidant Activities and Phenolic Compounds of Cornhusk, Corncob and *Stigma Maydis*. *J. Braz. Chem. Soc.*, 25(11): 1956-1964.
- Ekpendu, T.O., Akah, P.A., Adesomoju, A.A., Okogun, J.I. 1994. Antinflammatory and antimicrobial activities of *Mitracarpus scaber* extracts. *International Journal of Pharmacology.*, 32:191-195.
- Foster S, Duke JA. 1990. Field Guide 10 Medical Plants: Eastern and Central North America. Houghton MifAin, Boston.
- Gill LS. 1992. Ethnomedical Uses of Plants in Nigeria. Uniben Press, Benin, Nigeria, p. 249.
- Franzotti EM, Santos CVF, Rodrigues HMSL, Mourao RHV, Andrade MR, Antonioli AR. 2002. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. *Journal of Ethnopharmacology.*, 72: 273-278.
- Hess SM, Milonig RC. 1972. Inflammation In: Lepow LH, Ward PS. (Eds). Inflammation, Mechanism and control. Academic Press, New-York, USA. pp.1-2.
- Huss U, Ringbom T, Perera P, Bohlin L, Vasange M. 2002. Screening of ubiquitous plant constituents for COX-2 inhibition with a scintillation proximity based assay. *Journal Natural Products.*, 65: 1517-1521.
- Krogh R, Kroth R, Berti C, Madereira AO, Souza MM, Cechinel-Filho V, Delle-Monache F, Yunes RA. 1999. Isolation and identification of compounds with antinociceptive action from *Ipomoea pes-caprae*. *Pharmazie.*, 54: 464 – 466.
- Kumar PP, Kumaravel S, Lalitha C. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. *Afr J Biochem Res.*, 4 (7): 191-195.
- Liang YC, Huang YT, Tsau SH, Lin-Shiau SY, Chen CF, Lin JK. 1999. Suppression of inducible cyclooxygenase and inducible nitric acid synthase by apigenin and related flavonoid in mouse macrophages. *Carcinogenesis.*, 20: 1945-52.
- Ledón N, Casacó A, Rodríguez V, Cruz J, González R, Tolón Z, Cano M, Rojas E. 2003. Anti-Inflammatory and Analgesic Effects of a Mixture of Fatty acids isolated and purified from sugar cane wax oil. *Planta Medica.*, 69(4): 367-369
- Li, R.W., Leach, D.N., Myers, P., Leach, G.J., Lin, G.D., Brushett, D.J., Waterman, P.G. 2004. Anti-inflammatory activity, cytotoxicity and active compounds of *Tinospora smilacina* Benth. *Phytotherapy Reserch.*, 18: 78-83.



- Lin, L.L., Lin, A.Y., Knopt, J.L. 1992. Cytosolic phospholipase A<sub>2</sub> is coupled to hormonally regulated release of arachidonic acid. *Proceeding of National Academy of Science, U. S. A.*, 89: 6147-6157.
- Liu, J. 1995. Pharmacology of oleanolic acid and ursolic acid. *Journal of Ethnopharmacology.*, 49: 57- 68.
- Lorke, D. 1983. A new approach to practical acute toxicity testing. *Archives of Toxicology.*, 54:275-286.
- Maia, J.L., Lima-Junior, R.C., David, J.P., David, J.M., Santos, F.A., Rao, V.S. 2006. Oleanolic acid, a pentacyclic triterpene attenuates the mustard oil induced colonic nociception in mice. *Biological and Pharmaceutical Bulletin.*, 29: 82–85.
- Middleton, E Jr, Kandaswami, C., Theoharides, T.C. 2000. The effects of plant flavonoids on mammalian cells: Implications for inflammation, heart disease, and cancer. *Pharmacological Reviews.*, 52: 673–751.
- Nwafor, P.A., Jacks, T.W., Ekanem, A.U. 2007. Analgesic and anti-inflammatory effects of methanolic extract of *Pausinystalia mecroceras* stem bark in rodents. *Journal of Pharmacology.*, 3:86-90.
- Nwafor, P.A., Nwajiobi, N., Uko, I.E., Obot, J.S. 2010. Analgesic and anti-inflammatory activities of an ethanol extract of *Smilax krausiana* leaf in mice. *African Journal of Biomedical and Research.*, 13: 141 -148.
- Osagie, A.U., Eka, O.U.1998. Nutritional quality of plant foods. Post Harvest Research Unit, University of Benin, Benin, Nigeria, pp 34-41.
- Okokon, J.E., Nwafor, P.A. 2010. Antiinflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus*. *Pakistan Journal of Pharmaceutical sciences.*, 23(4): 383 - 390.
- Owoyele, B.V., Ngedu, M.N., Olaniran, S.O., Onasanwo, S.A., Oguntoye, S.O., Sanya, J.O., Oyeleke, S.A., Ibadapo, A.J., Soladoyel, A.O. 2010. Analgesic and anti-inflammatory effects of aqueous extract of *Zea mays* husk in male wistar rats. *Journal of Medicinal Food.*, 13 (2):343-347.
- Oriowo, M.A. 1982. Anti-inflammatory activity of piperonyl,-4-acrylic isobutyl amide, an extractive from *Zanthoxylum zanthoxyloids*. *Planta Medica.*, 44: 54 - 56
- Otuki, M.F., Ferreira, J., Lima, F.V., Meyre-Silva, C., Malheiros, Â., Muller, L.A., Cani, G.S., Santos, A.R.S., Yunes, R.A., Calixto, J.B. 2005. Antinociceptive properties of mixture of  $\alpha$ -amyryn and  $\beta$ -amyryn triterpenes: Evidence for participation of protein kinase C and protein kinase A pathways. *Journal of Pharmacology and Experimental Therapeutics.*, 313: 310–318.
- Raja Rajeswari, N., Rama Lakshmi, S., Muthuchelian, K. 2011. GC-MS Analysis of bioactive components from the ethanolic leaf extract of *Canthium dicoccum* (Gaertn.) Teijsm & Binn. *Journal of Chemical and Pharmaceutical Research.*, 3(3):792-798
- Rajendran, N.N., Thirugnanasambandam, P., Viswanathan, S., Parvathavarthini, S., Ramaswamy, S. 2000. Antinociceptive pattern of flavone and its mechanism as tested by formalin assay. *Indian Journal of Experimental Biologiocal.*, 38: 182–185.
- Ringbom, T., Segura, L., Noreen, Y., Perera, P., Bohlin, L. 1998. Ursolic acid from *Plantago major*, a selective inhibitor of cyclooxygenase-2 catalysed prostaglandin biosynthesis. *Journal of Natural Products.*, 61: 1212–1215.
- Robak, J., Shridi, F., Wolbis, M., Krolikowska, M. 1998. Screening of the influence of flavonoids on lipoxygenase and cyclooxygenase activity, as well as on nonenzymic lipid oxidation. *Polish Journal of Pharmacology and Pharmacy.*, 40: 451–458.
- Sokeng, S.D., Koubé, J., Dongmo, F., Sonnhaffouo, S., Nkono, B.L.N.Y., Taiwé, G.S., Cherrah, Y., Kamtchouing, P. 2013. Acute and chronic anti-inflammatory effects of the aqueous extract of *Acacia nilotica* (L.) Del. (Fabaceae) pods. *Academia Journal of Medicinal Plants.*, 1: 1-5.
- Santos, A.R., Cechinel Filho, V., Niero, R., Viana, A.M., Moreno, F.N., Campos, M.M., Yunes, R.A., Calixto, J.B. 1994. Analgesic effects of callus culture from selected species of *Phyllanthus*. *Journal of Pharmacy and Pharmacology.*, 46 : 755 – 759.
- Setzer, W.N., Stokes, S.L., Penton, A.F., Takaku, S., Haber, W.A., Hansell, E., Caffrey, C.R., McKerrow, J.H. 2007. Cruzain Inhibitory activity of leaf essential oils of neotropical lauraceae and essential oil components. *Nat Prod Comm.*, 2(12): 1203–1210.
- Simmonds, N.W. 1979. Evolution of Crop Plants. Longman. London. pp. 128-129.

- Sofowora, A. 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd edn, Spectrum Book Ltd, Ibadan, Nigeria.
- Suh, H.W., Song, D.K., Son, K.H., Wie, M.B., Lee, K.H., Jung, K.Y., Do, J.C., Kim, Y.H. 1996. Antinociceptive mechanisms of dipsacus saponin C administered intracerebroventricularly in the mouse. *General Pharmacology.*, 27: 1167–1172.
- Suh, N., Honda, T., Finaly, H.J., Barchowsky, A., Williams, C., Benoit, N.E. 1998. Novel triterpenoids suppress inducible nitric oxide synthase (iNOS) and inducible cyclooxygenase (COX-2) in mouse macrophages. *Cancer Resesearch.*, 58: 717-723.
- Trease, G.E., Evans, W. C. 1989. Pharmacognosy, 13<sup>th</sup> ed. Bailliere Tindal, London.
- Tapondjou, L.A., Lontsi, D., Sondengam, B.L., Choi, J., Lee, K.T., Jung, H.J., Park, H.J. 2003. In vivo antinociceptive and antiinflammatory effect of the two triterpenes, ursolic acid and 23-hydroxyursolic acid, from *Cussonia bancoensis*. *Archives of Pharmacal Research.*, 26: 143–146.
- Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., Hole, K. 1992. The formalin test: An evaluation of the method. *Pain.*, 51:5-17.
- Turner, R.A. 1995. Screening methods in Pharmacology. Vol 1. Academic Press. New York. Pp. 85- 106.
- Vane, T., Booting, R. 1987. Inflammation and mechanism of action of anti- inflammatory drugs. *FASSEB J.*, 1:89-96.
- Vaz, Z.R., Cechinel, V., Yunes, R.A., Calixto, J.B. 1996. Antinociceptive action of 2-(4-bromobenzoyl)-3-methyl-4-6-dimethoxy bezofuran, a novel xanthoxylene derivative of chemical and thermal models of nociception in mice. *Journal of Pharmacy and Experimental Therapeutics.*, 278: 304 - 312.
- Winter, C.A., Riskey, E.A., Nuss, G.W. 1962. Carrageenin-induced oedema in hind paw of the rats as an assay of anti-inflammatory drugs. *Proceeding of Society for Experimental Biology Medicine.*, 111:544 -547.