

ANALGESIC POTENTIAL OF CERTAIN TRADITIONAL AFRICAN HERBAL EXTRACTS IN HIGH FAT DIET-MANIPULATED HYPERGLYCAEMIC RATS

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ABSTRACT

In this study, an assessment of the analgesic potential of *Zea mays* L., *Nauclea latifolia* Sm leaf; leaf and stalk of *Manibot esculenta* Crantz was made. Pain was induced by 0.6% acetic acid in high fat diet-manipulated, alloxan-induced hyperglycaemic rats. Paracetamol® (65 mg/kgBW) served as positive control, while olive oil was used as negative control. Test groups were administered ethyl acetate extracts of the traditional African herbal extracts (TAHE) at a dose of 50 mg/kgBW. All interventions were administered as a single dose by oral gavage. Analgesic activity was measured by counting the percentage of writhing movements as a measure of alleviation of visceral pain by each intervention. The results showed that ethyl acetate extract of *Zea mays* L. and *Nauclea latifolia* Smith leaf, *Manibot esculenta* Crantz leaf and stalk had peripheral analgesic activities of 53.27 %, 55.71%, 69.02% and 60.73% respectively as compared to Paracetamol® (57.32%).

INTRODUCTION

Analgesia is an ill-defined, unpleasant sensation, usually evoked by external and internal noxious stimuli. Analgesics are drugs that selectively relieve pain by acting on the CNS or on peripheral pain mechanisms, without significantly altering consciousness (Dey *et al.*, 2010). Analgesics relieve pain as a symptom without affecting its cause (Hasan *et al.*, 2009). Paracetamol®, otherwise called acetaminophen has potent analgesic and antipyretic actions. It has a peripheral rather than a central site for the analgesic action (Lim *et al.*, 1964). Pain and inflammation are associated with pathology of various clinical conditions like arthritis, cancer, and vascular diseases (Weitzmann and Gordan, 1990). In various traditional medicinal systems a number of natural products are used to relieve the symptoms of pain and tropical African continent is a repository of vast medicinal plants.

The analgesic action of most NSAIDs is due to inhibition of PG formation at peripheral sites and this has been demonstrated using mouse or rat abdominal constriction tests. These tests involve the production of “writhing” or “stretching” along the abdominal wall by an *i. p* injection of dilute acetic acid, acetylcholine, or phenylbenzoquinone (Collier *et al.*, 1968). Acetaminophen inhibited the abdominal constriction response to *i. p* administration of

acetic acid (Sewell *et al.*, 1984) and acetylcholine (Ferrari *et al.*, 1990) in mice. Analgesic oral doses of acetaminophen also attenuated the *ex vivo* synthesis of brain PGE₂ in a dose-related manner (Ferrari *et al.*, 1990), thus providing evidence for a central component of the analgesic action of this NSAID. It is highly likely that acetaminophen inhibits the synthesis of PGs by competing with arachidonic acid for the active site on the COX enzyme (Botting, 2000). This research work aims to study the analgesic effects of the ethyl acetate extracts of *Zea mays* L. and *Nauclea latifolia* Smith leaf as well as *Manibot esculenta* Crantz leaf and leaf stalk in pain management, using high fat diet-manipulated, alloxan-induced hyperglycaemic rats.

MATERIALS AND METHODS

Plant Materials

Zea mays leaf as well as *Manibot esculenta* leaf and stalk were collected from a local farm near Osun State University, Oke-Baale, Osogbo, Osun State, Nigeria; while *Nauclea latifolia* leaf was collected at the New Saw Mill area Ogbomosho, Oyo State, Nigeria. They were identified at the herbarium of the Department of Plant Biology, University of Ilorin, Kwara State, Nigeria (Voucher Numbers UIH001/681, UIH002/1094 and UIH004/506, respectively). The individual plant leaves and cassava stalks were air-dried for 3 to 4 weeks. They were then pulverized to powder. Dry

powder (100 g) of each sample was soaked in 1L ethyl acetate and left for 48 h. The mixture was sieved using a filter paper, then the resulting filtrates were concentrated using rotary evaporator.

Experimental Animals

Wistar rats were purchased at the Animal House, Department of Anatomy, University of Ibadan, Ibadan, Oyo State, Nigeria. They weighed 120-180 g. The rats were housed in a standard environmental condition of temperature ($30 \pm 1^\circ\text{C}$), humidity ($60 \pm 0.2\%$) and 12 h light and 12 h dark cycle. They were fed *ad libitum* with a formulation of high energy diet made from normal pellet diet powdered, mixed with sucrose, lard, vitamin-mineral mix and NaCl.

Reagents

Ethyl acetate, Glacial acetic acid, Distilled water, Alloxan monohydrate (Sigma Aldrich, Germany), Sodium chloride (Sigma Aldrich, Germany), and other chemicals and reagents were of analytical grade.

Qualitative Phytochemical Screening (Sofowora, 1993)

Test for Tannins

Few drops of ferric chloride were added to 2 mL of extract. Dark green colour was obtained indicating the presence of tannins.

Test for Flavonoids

Few drops of aqueous sodium hydroxide solution were added to 2 mL extract. Black colour was observed confirming the presence of flavonoid.

Test for Saponins

Extract (2 mL) was shaken for 2 min to see if there is frothing. Frothing was obtained, indicating the presence of saponins.

Test for Alkaloids

Extract (1 mL) was dissolved in 2 mL of 0.1% of HCl in a test tube and 2 drops of Mayer's reagent was added. Cream colour was observed indicating the presence of alkaloids.

Determination of Total Phenolic Contents

The amount of total phenolics in extract was

determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi (1965) with slight modification using tannic acid as a standard. Briefly, 1.0 mL of extract solution (5 mg/mL) was added in a 100 mL volumetric flask that contained about 60 mL distilled water. Then, 5.0 mL of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 1-8 min, 15.0 mL Na_2CO_3 (20 %) was added and the volume was made up to 100 mL using distilled water. The mixture was allowed to stand for 2 hr with intermittent shaking. The absorbance was measured at 760 nm using a UV-Vis Spectrophotometer (Jenway 6100, Dunmow, Essex, UK). The total phenolic content was determined as milligrams of tannic acid equivalent (TAE) using an equation obtained from the standard tannic acid calibration graph.

$$\text{Total Phenolic Content} = \frac{\text{Absorbance reading for test}}{\text{Absorbance reading for standard}} \times \text{Standard concentration}$$

Total Monomeric Anthocyanin by the pH Differential Method

The total monomeric anthocyanin content was determined by the pH-differential method as described by Giusti and Wrolstad (2001), with slight modifications. Briefly, 0.1 mg of extract dissolved in 10 mL DMSO was prepared as stock. The appropriate dilution factor for the sample was determined by diluting with 0.025M Potassium Chloride buffer (pH 1.0) at 700 nm (the absorbance was less than 1.2). The final volume of the sample was divided by the initial volume to obtain the dilution factor. Two dilutions of the sample were prepared, one with Potassium Chloride buffer (pH 1.0) and the other with 0.4 M Sodium Acetate buffer (pH 4.5). These dilutions were allowed to equilibrate for 15 min. The absorbance of each dilution was measured at 700 nm (to correct for haze) against a blank cell filled with DMSO. The pigment content was calculated as cyanidin-3-glucoside equivalent (C3GE), where $\text{MW} = 449.2$ and $\epsilon = 26,900$.

$$\text{Absorbance of the diluted sample (A)} = (A_{700})_{\text{pH } 1.0} - (A_{700})_{\text{pH } 4.5}$$

$$\text{Monomeric anthocyanin pigment (mg/l)} = (A \times \text{MW} \times \text{DF} \times 1000) \div \epsilon \times \text{Pathlength of cuvette}$$

Induction of Hyperglycaemia

Rats were subjected to diet manipulation with high fat diet for 2 months. Thereafter, they were fasted for 16 hours but allowed access to water *ad libitum* prior to intraperitoneal administration of alloxan dissolved in 0.9 % saline at 50 mg/kg BW. Hyperglycaemia was confirmed in the alloxan-treated rats by measuring the fasting blood glucose concentration 72 hours after alloxan injection. The high fat diet was prepared by adding 20 % Sucrose, 10 % Lard, 6 % Vitamin-Mineral Mix, 0.1 % NaCl to Normal Pellet Diet (Srinivasan *et al.*, 2005).

Induction of Pain

Glacial acetic acid (0.6% w/v) was given by intraperitoneal injection (Koster, 1958) in dosages of 5 mL/kg BW to induce pain. The resulting pain was inferred from the writhing movement which was monitored for the first 5 minutes before interventions were given. The animals were then

monitored for intervals of 5 minutes for up to 1hr, yielding cumulative total writhing per hour.

Twenty-four diabetic rats were allocated to four intervention groups of four rats per group.

Group 1 animals were administered Paracetamol® at dose 65 mg/kgBW and served as positive control (the dosages for human adults using a conversion factor of 0.026). Group 2 animals were administered ethyl acetate extract of *Zea mays* leaf at dose 50 mg/kgBW. Group 3 animals were administered ethyl acetate extract of *Nauclea latifolia* leaf at dose 50 mg/kgBW. Group 4 animals were administered ethyl acetate extract of *Manihot esculenta* leaf at dose 50 mg/kgBW. Group 5 animals were administered ethyl acetate extract of *Manihot esculenta* leaf stalk. Group 6 animals were administered olive oil and served as the negative control.

All interventions were administered on a given day as a single dose by gavage.

RESULTS

Table 1: Quantitative Analysis of Monomeric Anthocyanin and Total Phenolic Contents of Tested Traditional African Herbal Extracts.

Quantification	Ethyl acetate extract of <i>Zea mays</i> leaf	Ethyl acetate extract of <i>Nauclea latifolia</i> leaf	Ethyl acetate extract of <i>Manihot esculenta</i> leaf	Ethyl acetate extract of <i>Manihot esculenta</i> stalk
Monomeric Anthocyanin Content (mg C3GE/l)	0.814 ± 0.004	1.128 ± 0.002 ^a	0.918 ± 0.034 ^{a, b}	1.102 ± 0.023 ^{a, b, c}
Total Phenolic Content (mg TAE/g DW)	22.25 ± 0.396	36.27 ± 0.834 ^a	23.17 ± 1.541	28.40 ± 0.354 ^a

Qualitative Phytochemical Screening

Table 2: Presence of Phytochemicals in Ethyl Acetate Extracts of Tested Traditional African Herbal Extracts

Phytochemical	EtOAc <i>Me</i> leaf	EtOAc <i>Me</i> stalk	EtOAc <i>Zm</i> leaf	EtOAc <i>N</i> /leaf
Tanins	+	+	+	+
Flavonoids	+	+	+	+
Saponins	+	+	+	+
Alkaloids	+	+	+	+

In the study, all test animals (n = 24) that were used did not suffer any lethal effect or death except two in the group administered ethyl acetate extract of *Zea mays* leaf that experienced torticollis, which

might have resulted from toxic effects. The number of writhings of all test animals and the mean cumulative total of writhings in the intervention groups are presented in Table 3.

Table 3: Number of Writhings in Diabetic Rats after Intervention and Induction of Pain by 0.6% Acetic acid.

Intervention Groups	Cumulative number of writhing in diabetic rats (5 minutes/session)					Mean ± SD
	1 st	2 nd	3 rd	4 th	5 th	
Olive Oil (Negative Control)	50	72	68	94	126	82±29.16
Paracetamol (Positive Control)	30	37	24	39	45	35.0±8.16*
Ethyl acetate extract of <i>Zea mays</i> leaf	16	14	11	10	9	12.00±2.90*, a
Ethyl acetate extract of <i>Nauclea latifolia</i>	15	10	7	8	11	10.20±3.10*, a
Ethyl acetate extract of <i>Manihot esculenta</i> leaf	22	29	18	30	28	25.40±5.18*, a, b, c
Ethyl acetate extract of <i>Manihot esculenta</i> stalk	27	25	34	32	43	32.2±7.05*, b, c

It can be seen that there was a reduction in the cumulative total of writhings in the Paracetamol® group, and ethyl acetate extracts of all tested traditional African herbal plants in comparison with the negative control group that received olive oil. Significant reductions in cumulative total writhings were observed for ethyl acetate extracts of *Zea mays* and *Nauclea latifolia*. This indicates that Paracetamol®, and all tested leaf extracts were effective in reducing the number of writhing movements as a response to pain induced by intraperitoneal injection of 0.6% acetic acid. The more significant reductions in writhing observed for *Zea mays* and *Nauclea latifolia* may be due to the presence of other monomeric anthocyanins in the crude extracts.

The analgesic power of Paracetamol®, ethyl acetate extracts on the writhing reflex induced by intraperitoneal acetic acid administration is presented in Table 4. Analgesic power was

calculated on the basis of the inhibitory capacity of the interventions on the writhing movements of the experimental animals (Hendershot and Forsaith, 1959), as follows;

$$\% \text{ writhing protection} = 100 - ((E/C) \times 100)$$

where E = Cumulative total writhing in the experimental animals after intervention.

C = Mean Cumulative total of writhing in negative control.

A medication is said to have analgesic activity if it is capable of effecting a reduction of > 50% in the number of writhings of the experimental animals as compared to the control group (Hendershot and Forsaith, 1959). To determine the analgesic power, the percentage reduction in cumulative total number of writhings is calculated against the negative control group. The calculated percentage is termed the analgesic power against the pain induced by acetic – acid stimulation of the experimental animals.

Table 4: Percentage of Writhing Protection of Paracetamol and Ethyl Acetate Extracts of *Manibot esculenta* Stalk and Leaf, *Zea mays* and *Nauclea esculenta* leaf.

Intervention Groups	Writhing protection (%) after intervention, (5 minutes/session)					Mean \pm SD
	1 st	2 nd	3 rd	4 th	5 th	
Paracetamol (Positive Control)	63.41	54.88	70.73	52.44	45.12	57.32 \pm 9.94
Ethyl acetate extract of <i>Zea mays</i> leaf	58.01	46.77	65.33	52.44	43.77	53.27 \pm 3.30*
Ethyl acetate extract of <i>Nauclea latifolia</i>	59.36	52.18	70.73	55.14	41.07	55.71 \pm 3.28*, a
Ethyl acetate extract of <i>Manibot esculenta</i> leaf	73.17	64.63	78.05	63.41	65.85	69.02 \pm 6.32*, a, b
Ethyl acetate extract of <i>Manibot esculenta</i> stalk	67.07	69.51	58.54	60.98	47.56	60.73 \pm 8.61*, a, b, c

The analgesic power of the negative control is not calculated; it is assumed as 0% as it has been used as the divisor in the calculation of the analgesic power.

There was a significant difference in percentage writhing protection in the Paracetamol® group and ethyl acetate extract groups. Ethyl acetate extracts of *Zea mays*, *Nauclea latifolia*, *Manibot esculenta* leaf, and *Manibot esculenta* stalk groups at all dosages showed high percentage writhing protection against glacial acetic acid (53%, 56%, 69%, 61% respectively) as compared to Paracetamol® group (57%) at $p < 0.05$. The calculation of the analgesic power with the percentage of writhings in the negative control group (olive oil) as divisor showed that Paracetamol and the ethyl acetate extracts of *Zea mays*, *Nauclea latifolia* at their given dosages did not differ significantly in analgesic power ($p > 0.05$). The analgesic power of ethyl acetate extracts of *Manibot esculenta* leaf and stalk were higher than the value observed for Paracetamol®.

DISCUSSION

From Table 4, it is clear that there was an increase in the percentage of writhing protection at dosages of the 50 mg/kgBW ethyl acetate extracts of *Manibot esculenta* leaf and stalk and a bit less in Paracetamol® at 60mg/kgBW. The analgesic power was highest for ethyl acetate extract of *Manibot esculenta* leaf (50mg/kgBW). The analgesic power of ethyl acetate extracts of *Zea mays* and

Nauclea latifolia leaf at 50mg/kgBW was significantly lower as compared to the other extract test groups, but were not significantly different from that of Paracetamol® at 65mg/kgBW. The analgesic power of ethyl acetate extracts of *Manibot esculenta* leaf and stalk were significantly different higher than that of Paracetamol® ($p > 0.05$). Therefore it may be concluded that ethyl acetate extracts of *Zea mays* and *Nauclea latifolia* leaf had analgesic power equivalent to that of Paracetamol® at a dosage of 65 mg/kgBW; while *Manibot esculenta* stalk and leaf at doses 50 mg/kgBW respectively had analgesic power surpassing that of Paracetamol® at same dosage. An analgesic activity study of ethanolic extract of *Manibot esculenta* leaf at varying doses in mice yielded a similar result (Miladiah *et al.*, 2011). Another study on the anti-inflammatory property of aqueous extract of *Manibot esculenta* leaf in rats yielded similar results, being significantly higher than that of Indomethacin at a dosage of 10 mg/kgBW (Afolabi *et al.*, 2008).

From the foregoing, it can be concluded that ethyl acetate extracts of *Zea mays*, *Nauclea esculenta*, *Manibot esculenta* leaf and stalk presumably contain analgesic and anti-inflammatory bioactive principles. Cassava leaf contain flavonoids (Isnatin *et al.*, 2011) and as flavonoids belong to phenolic acids, nearly all flavonoid compounds possess analgesic activity due to their actions on the inhibition of prostaglandin synthesis and consequent decreased stimulation of

nociceptors or pain-receptors. Flavonoids are thought to interact with the cyclooxygenase system so as to interfere with arachidonic acid synthesis and inhibit the production of prostaglandins (Purwantini *et al.*, 2007).

A growing body of evidence suggests that anthocyanins and anthocyanidins may possess analgesic properties in addition to neuroprotective and anti-inflammatory activities (Korte *et al.*, 2009). In addition to these, the analgesic effect may also be due to the presence of other antioxidants in *Manibot esculenta* e.g α - carotenes and vitamin C (Fasuyi, 2005). Antioxidants are capable of neutralizing the free radicals released by phagocytes in response to cellular injury, so as to suppress the inflammatory response caused by these free radicals, leading to a decreased pain response (Raji *et al.*, 2002). *Manibot esculenta* leaf and stalk contain anthocyanins, which play an important role as antioxidants (Byamukama *et al.*, 2009), and have been demonstrated to be able to prevent alcohol-induced hepatotoxicity (Boby & Indira, 2004). Other substances suspected of playing a role in the analgesic activity are the saponins, which are known to have analgesic and anti-inflammatory property, and eliminate pain without affecting cell viability (Borgi *et al.*, 2008). In addition to the saponin, the analgesic effect may also be due to the presence of other antioxidants in the *Zea mays* plant e.g vitamin C (Somchit *et al.*, 2005).

CONCLUSION

The ethyl acetate solvent used in this study extracts semipolar active compounds, thus it is probable that the analgesic substances are soluble in polar solvents, but to test this suggestion requires further study. As a preliminary study, the present work has employed a simple physiological method to assess the analgesic power of the crude extracts as well as screen them for their analgesic potential.

Ethyl acetate extracts of *Manibot esculenta* leaf and stalk as shown by this study exert analgesic effects on pain induced by 0.6% acetic-acid in alloxan-induced hyperglycaemia in rats. However, further studies are necessary to ascertain exactly the active constituents of these traditional African herbal extracts that are responsible for their analgesic

properties.

Statistical Analysis

Percentages of writhing protection were analyzed by means of a one-way ANOVA, at a significant level of $p < 0.05$.

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