PROXIMATE COMPOSITION, ANTI-INFLAMMATORY AND EFFECTS OF AQUEOUS EXTRACT OF *Dennettiatripetala* FRUIT ON INDOMETHACINE AND ETHANOL INDUCED ULCER IN ALBINO RATS

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Abstract: The proximate composition and the effect of *Dennettiatripetala* aqueous fruit extract on platelet aggregation and erythrocyte membrane stability were assessed, the effect of oral administration of the extract on indomethacin and ethanol in HCl induced ulcer were also investigated in Albino Wistarrats. Results indicated that the extract contains 43.69 13.00, 5.50, 13.91, 17.40 and 6.50 percent of carbohydrates, crude fibre, ash content, protein, moisture and fat respectively. The extract treatment reduced the viscosityof platelet aggregation hence of the blood and increased transmission of the solution. Treatment with the extract, significantly ($p<0.05$) inhibited indomethacin induced ulceration in a dose related manner but non-significantly ($p>0.05$) inhibited ethanol inHCl induced ulceration. The percentage inhibition of the extract at 200 mg/kg b.w was comparable to cimetidine. This is an indication that aqueous fruit extract of *Dennettiatripetala* has anti-inflammatory and anti-ulcerogenic effect.

Keywork: Anti-inflammatory, anti-ulcerogenic, cimetidine, *Dennettiatripetala*, indomethacin, proximate composition

Introduction

Dennettiatripetala G. popularly known as pepper fruits belongs to the family of *Annonaceae*. It is a well known Nigerian spicy medicinal plant and a common ethno medicinal plant in West Africa. It appears red when ripe and green in its unripe form. The fruit extract have been shown (Gafar and Itodo, 2011) to be active against *Saccharomyces cerevisae*, *Candida* species, *Crytococeus* speciesand also as an anti-fungal agent (Oyemitan, 2009). It serves as an insecticide against Aedesaegypt mosquitoes and as bio-insecticides for the control of the rice weevil *Sitopuluszeamais.* The peppery fruits of *Dennettiatripetala* are addedto the diet of expectant and post-partum women, because it is claimed (Okwu *et al.*, 2005) that spices and herbs aid uterine contraction.

Adedayo *et al.* (2010) has reported that *Dennettiatripetala* fruit contains essential oil, phenolic acid, alkaloids, ethyl acetate, flavonoids, tannins and glycosides. Because the fruits produce unique peppery effect when chewed (Block *et al.*, 2007) they are used as masticators. This peppery spicy taste as observed by Daniel and Clement (2008), serves as a mild stimulant to the consumers. Recent communication by Timothy and Okere (2008) recorded that eating *Dennettiatripetala* fruits aid the intra ocular pressure (IOP) hence limiting the onset of glaucoma. Oyemitan *et al.* (2008) also reported that the essential oil of *Dennettiatripetala* has anti-nociceptive and anti-inflammatory effect in rodent.

This present study is aimed at determining the proximate composition, anti-inflammatory and anti-ulcerogenic effect of the aqueous fruit extract of *Dennettiatripetala* on Albino Wistarrats.

Materials and Methods

Materials

Ripe *Dennettiatripetala* fruits were purchased from Anyigba central market in April 2015, in Dekina Local Government Area of Kogi State. They were rinsed, dried at room temperature and pulverized with Creson high speed milling machine into a coarse powder.

Animals

The animals used in this study were forty (40) male WistarAlbino rats (130 – 140 g) which were purchased from the Animal House of the Department of Biochemistry, Kogi State University Anyigba, Kogi State, Nigeria.

Blood samples

A: Blood samples were collected via cardiac puncture into heparinized centrifuge tubes and spun at 1000 rpm for 5 min to separate the plasma from the cells.

B: 9 ml quantity of blood was drawn from an adult female human.

Chemicals

Sodium chloride, calcium chloride and trisodium citrate were obtained from British Drug House (BDH).

Methods

Proximate Composition

Proximate composition was carried out by the method described by AOAC (2007).

Platelet aggregatory activity and activity on erythrocyte membrane was carried out by a modified method of Nwodo (1981).

Experimental design for platelet aggregatory activity:

The effect of the extract on platelet aggregatory activity was carried out by a modified method of Nwodo (1981). Nine milliliters (9 ml) of blood sample was drawn by vein-puncture from an adult female who had not taken drugs of any kind for two weeks. The blood was gently and carefully transferred into a centrifuge tube containing 1 ml of 3.8% trisodium citrate and centrifuged at 3000xg for 10 min. The supernatant was used as the platelet rich plasma (PRP). Reaction medium (3.0 ml) containing 2.7 ml of normal saline and 0.2 ml PRP was used as the control.

Induction of aggregation was by addition of 0.1 ml 4 m $MCaCl_2$. The absorbance of the medium was monitored at 520 nm for six minutes at room temperature using a spectrophotometer. Accordingly, three different reaction media were made in which different increasing concentration of the extract were added and their absorbance monitored. The order of addition is shown in Table 1.

Table 1: Reaction media for induction of platelet aggregation

Tubes	Normal saline (ml)	Extract (ml)	$CaCl_2$ (ml)	PRP (ml)
1	2.1	0.5	0.1	0.2
2	1.7	1.0	0.1	0.2
3	0.7	2.0	0.1	0.2

Media that do not contain PRP but *Dennettiatripetala* aqueous fruit extract were used as blanks for each tube.

Designforthe Activity for Erythrocyte Membrane Stability

The precipitate of the supernatant that was the PRP was used as the human red blood cells (HRBC). The HRBC was re-suspended in 9 ml of normal saline and used for the experiment. The reaction medium (2.1 ml) containing 0.1 ml of HRBC, 1.0 ml normal saline and 1.0 ml of water was used as the control. It was incubated at 37^{o}C for 30 min and centrifuged at 3000 rpm for 10 min. The supernatant was drawn and its absorbance at 418 nm was monitored for 6 min. Appropriate blanks containing the extract without HRBC were used for each tube.

Three different reaction media were further made in which various increasing concentration of the extract were added, they were incubated at 37^{o}C for 3 min and centrifuged at 3000 rpm for 10 min. The supernatants were drawn and the absorbance at 418 nm was monitored for 6 min in the order shown in Table 2.

Table 2: Reaction media for erythrocyte membrane stability

Tubes	HRBC (ml)	Normal saline (ml)	Water (ml)	Extract
1	0.1	0.9	1.0	0.1
2	0.1	0.8	1.0	0.2
3	0.1	0.6	1.0	0.4

Experimental design for indomethacin and ethanol in HCl-induced ulcers

Forty (40) male Albino rats weighing 100-120 g were divided into two sets of four Groups A, B, C and D of five animals each. Group A which is the control received normal saline (5 ml/kg), Groups B, C, and D were served orally with 200, 400 mg/kg of the extract and 32 mg/kg b.w of cimetidine. All the groups were administered ethanol in HCl (25 mg/kg of 0.3M HCl in 60% ethanol) b.w. set 1 and (20 mg/kg) b.w. indomethacin to set 2.

Animals were allowed access to food and clean water, fasted for 18 h after the last dose, the animals were sacrificed and their stomach removed and cut along the greater curvature and rinsed in a stream of water. The lesion on the gastric mucosa were observed with a x10 hand lens and scored using an arbitrary scale (0-4);

where: 0 = no lesion, 0.5 = hyperaemia, 1 = one or two lesion, 2 = severe lesion, 3 = very severe lesions and 4 = mucosa full of lesions.

Statistical analysis

The data obtained were analysed using one way analysis of variance (ANOVA). Results were presented as mean ± standard error of mean (SEM). Differences between means were considered significant at $p<0.05$.

Results and Discussion

Table 3 shows the percentage value of moisture, ash, crude fibre, fat, protein and carbohydrate content to be 17.40, 5.50, 13.00, 6.50, 13.91 and 43.69%, respectively. Table 4 shows time dependent decreases in optical density from 1 minute through 6 minutes. Extract does not cause any occlusion or enhance viscousityof blood. There was a dose dependent decrease in absorbance in the three tubes (Table 5). However, there were no significant differences in absorbance with time. The extract stabilizes the erythrocyte membrane

Table 3: Proximate composition of *Dennettiatripetala* fruits

Moisture content %	Ash content %	Crude fibre %	Fat content %	Protein content %	Carbohydrate content %
17.40±2.73	5.50±1.81	13.00±3.03	6.50±0.63	13.91±4.74	43.69±3.52

Values are express as Mean ± Standard deviation.

Table 4: Effect of *Dennettiatripetala* fruit extract on the absorbance ofcalcium-induced platelet aggregation medium for 6 min

Tubes	Extract (ml)	1 minute	2 minutes	3 minutes	4 minutes	5 minutes	6 minutes
1	0.50	1.38±0.03	1.34±0.02	1.28±0.01	1.22±0.00	1.06±0.02	1.08±0.01
2	1.00	1.58±0.01	1.56±0.00	1.44±0.02	1.37±0.01	1.33±0.04	1.31±0.00
3	2.00	1.88±0.00	1.79±0.01	1.75±0.01	1.68±0.03	1.59±0.05	1.53±0.00

Values are express as Mean±Standard deviation.

Table 5: Effect of *Dennettiatripetala* fruit extract on erythrocyte membrane stability

Tube	Extract (ml)	1 minute	2 minutes	3 minutes	4 minutes	5 minutes	6 minutes
1	0.10	2.5±0.01	2.5±0.00	2.5±0.00	2.5±0.02	2.5±0.01	2.5±0.00
2	0.20	2.1±0.02	2.0±0.01	2.0±0.01	2.1±0.01	2.1±0.01	2.1±0.02
3	0.40	0.8±0.01	0.8±0.03	0.9±0.02	0.8±0.01	0.9±0.02	0.8±0.03

Values are express as Mean±Standard deviation.

Table 6 shows the groups of animals treated with increasing doses (200-400 mg/kg) of the extract, there were significant ($p<0.05$) scalar reductions in the ulcer index originally induced by indomethacin. Thus ulcer index decrease of 1.05, 1.38 and 1.07 in group B, C and D represent percentage protection of 50.48, 66.34, and 60.05%, respectively. In Table 7, extract at 200 mg/kg b.w. gave a percentage protection of 6.50% which is the same with the protection of the standard drug cimetidine. Scalar dose of the extract at 400 mg/kg b.w. gave a percentage protection of 25.49%, respectively.

Table 6: Effect of *Dennettiatripetala* fruit extract on indomethacin induced ulcer

Group	Treatment	Dose (mg/kg)	Ulcer index	% Protection
A	Normal saline	5 ml/kg	2.08 ± 004	0.00
B	Extract	200	1.03 ± 000	50.48
C	Extract	400	0.70 ± 1.47	66.34
D	Cimetidine	32	0.81 ± 0.30	60.05

Table 7: Effect of *Dennettiatripetala* fruit extract on ethanol in HCl induced ulcer

Group	Treatments	Dose (mg/kg)	Ulcer index	% Protection
A	Normal saline	5 ml/kg	2.55 ± 0.21	0.00
B	Extract	200	2.40 ± 0.42	6.50
C	Extract	400	1.90 ± 0.42	25.49
D	Extract	32	2.40 ± 0.71	6.50

Moisture content is among the vital measurement in the processing, preservation and storage of food (Onwukka, 2005). *Dennettiatripetala* fruits is usually stored by users in the dry form when it is out of season, hence long storage will still preserve the shelf life of the fruits as observed in Table 3. The ash content is generally taken to be a measure of mineral content of the original food (Donald, 2007), the low concentration of crude fibre is considered appropriate because it aids absorption of glucose and fat. Even though, Olajidiand Mike (2005) had recorded that its high concentration can cause intestinal irritation, lower digestibility and decreased nutrient usage. The low concentration of crude fibre in *Dennettiatripetala* fruit can enable digestibility in users. The presence of proteins, carbohydrates and lipids which are important biomolecules of the body, indicate its potential as food supplement because they are critical in many physiological repair, blood clotting immune responses and supply of energy (Pamela *et al*., 2005). Their combination with many other sources of protein such as animal protein may result in adequate nutritional value.

The absorbance at 520 nm of the aqueous extract on calcium-induced aggregation increased at different increasing concentration of the extract, when optical density was set at 0.5 nm for 6 min. The extract stabilizes platelet, which implies that it does not enhance viscousity of blood or cause occlusion which is an anti-aggregating activity. Showing anti-aggregating activity even when the PRP was challenged with $CaCl_2$, supports its interference with calcium utilization (Ojogbane *et al*., 2011).

Platelet aggregation is stimulated by thromboxane. Non-Steroidal Anti-inflammatory Drugs (NSAID) block the cyclooxygenase-1-enzyme, inhibiting thromboxane production and thus interfere with normal platelet aggregation. *Dennettiatripetala* fruit extract may play important role in inflammation and allergic responses. There was also concentration dependent decrease in the absorbance at 418 nm in Table 5 at different increasing concentration of the extract which suggest that it stabilizes erythrocyte membrane, confirming its anti-inflammatory property, so it may inhibit the activities of phospholipase A_2, NSAID, and some malarial drugs of mobilizing their substrate (phospholipids and free fatty acids) for the production of inflammatory mediators (Okwu and Uchenna, 2009).

In Table 6 and 7, *Dennettiatripetala* fruit extract protected against ulcer induced by indomethacin and ethanol in HCl. Gastric mucosa damage caused by indomethacin and ethanol in HCl results from the inhibition of prostaglandin synthesis (Hostetteman, 2009) via the arachidonic pathway Karou *et al.* (2011) observed that prostaglandin serve protective function in the stomach, maintaining gastric microcirculation and causing gastric secretion of bicarbonate and mucus. Thus the effect of aqueous extract of *Dennettiatripetala* fruit in this study suggested that it possesses cytoprotective action.

Conclusion

The fruit extract of *Dennettiatripetala* possesses anti-inflammatory and anti-ulcerogenic effects.

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