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INVITRO ANTI-INFLAMMATORY ACTIVITY OF HYDROMETHANOLIC SEED, FRUIT AND LEAVE EXTRACTS OF *CAPSICUM CHINENSE* (RED PEPPER)

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ABSTRACT

The present investigation was carried out to evaluate the *in vitro* anti-inflammatory potential of *Capsicum chinense*. Hydromethanolic extracts of leave, seed, and fruits of *Capsicum chinense* (Red pepper) was assessed for its *in vitro* anti-inflammatory activity using heat and hypotonic solution induced membrane stabilization activity and protein denaturation assay at different concentration. Diclofenac sodium was used as standard drugs. The results showed a dose dependent ($p<0.05$) RBC membrane stabilization and inhibition of protein denaturation activity. Hydromethanolic leaves extract (400µg/ml) exhibited the highest percent protection of 70.76%, 58.14% and 59.38% against heat and hypotonic solution induced membrane stabilization activity and protein denaturation respectively. From the above study, it was concluded that hydromethanolic seed, fruit and leaves extract of *C. chinense* has significant membrane stabilization property which was comparable to the standard drug Diclofenac. This study provides invitro anti-inflammatory evidence for the use of this plant in the treatment of inflammatory conditions. However, these effects need to be confirmed using *in vivo* models.

KEYWORDS: anti-inflammatory, protein denaturation, membrane stabilization.

INTRODUCTION

The use of plants and plant extracts for medicinal purposes has been going on for thousands of years; it has also form the source of much useful therapy in both herbalism and folk medicine.[1] Traditional medicine has also generated a lot of interest and concern about their

efficacy and safety margin, since 65-70% of the Nigerian population patronizes traditional medicine practitioners in their various forms and methods.[2,3] Chemical compounds with beneficial effects have been isolated and biologically assayed to establish their medicinal activity. Current drugs available such as opoids and non steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorder because of their side effects and potency.[4] Recently, there is a growing interest in the search for other alternatives such medicinal plant. There are a number of anti-inflammatory herbs that could help to achieve similar results without the harmful effect.[5]

Inflammation is a severe response by living tissue to any kind of injury. It is a complex process which is frequently associated with pain, involves occurrences such as increased vascular permeability, increase of protein denaturation and membrane alterations.[6] Recently, fruits and vegetables have been recognized as natural sources of various bioactive compounds.[7] One such vegetable is pepper. Pepper belongs to the genus Capsicum, which is comprised of more than 200 varieties, with *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens* being the main five species.[8] Peppers are consumed worldwide and their importance has increased gradually to place them among the most consumed spice crops in the world.[9] They are usually consumed as food and used as additives in the food industry. They also play a significant role in traditional medicine.

In Nigerian traditional medicine, *C. chinense* commonly known as Tarugu" in Hausa, 'ose' in Igbo and "Atarodo" in Yoruba, is used for the treatment of pain and inflammatory disorders such as arthritis, rheumatism, chronic stomach indigestion, pain reliever etc. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs.[10] The present study was aimed at investigating the invitro anti-inflammatory activities of seeds, fruits and leaves extract of *Capsicum chinense*.

MATERIALS AND METHODS

The plant materials were collected in March, 2014 from Farar dutse village, Aliero Local Government Area, Kebbi State, Nigeria. It was taxonomically identified at the Habarium, Biological Science Department of Kebbi State University of Science and Technology as *Capsicum chinense* with Voucher specimen number 23A. The leaves, fruit and seeds were

separated, dried at room temperature for 7 days and were later grinded with a mortar and pestle to coarse powder.

HYDROMETHANOLIC EXTRACTION

The extraction was done using John *et al.* method with modification.[12] Leaves and fruits (200g each) was weighed into conical flask with 600ml of 70% methanol (180 distilled water and 420 methanol), while 80g of fruits sample was weighed into conical flask with 200ml of 70% methanol (60 distilled water and 140 methanol) for 72hours. The extracts was then filtered using a muslin cloth and dried over a water bath at 40^0C for a week to have slurry concentrate of the extracts (seeds 7.14%, fruits 14% and leaves 21%). The slurry also known as hydromethanolic extract was stored in refrigerator at 4^0C and used for the experiment.

PHYTOCHEMICAL SCREENING

Freshly prepared seed, fruit and leaves extracts of *C. chinense* were tested for the presence of chemical constituents using the standard procedures described by Trease and Evans[12], Harborne[13] and Sofowora.[14]

ANTIINFLAMMATORY STUDIES

Membrane stabilization: Fresh whole human blood (10 mL) was collected and transferred to the heparinized centrifuged tubes. The tubes were centrifuged at 3000 rpm for 10 min and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.[15]

Membrane stabilizing activity (heat induced haemolysis): The reaction mixture contained 2mL hydromethanolic extracts of the plant parts and standard Diclofenac sodium (50-400 µg/mL) and 1 mL of 10% RBCs suspension in five 10mL test tube respectively. Instead of drug only saline was added to the control test tube. The test tubes containing reaction mixture were incubated in a water bath at 56^0C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was measured at 560 nm.[16] Membrane stabilizing activity (in %) was calculated by the following formula.

$$\%\ Inhibition\ of\ haemolysis = 100 \times \frac{Vc - Vt}{Vc}$$

$$\%\ Protection = 100 - [100 \times \frac{Vc - Vt}{Vc}]$$

Where: Vc = absorbance of control ; Vt= absorbance of test.

Hypotonic solution induced haemolysis or membrane stabilizing activity: This test was done according to the method Shinde *et al*. with slight modifications.[16] The test sample consisted of stock 10% RBC suspension mixed with 5ml of hypotonic solution containing extracts preparation ranging from concentration 50-400 µg/ml. The control sample consisted of 10% RBC suspension mixed with hypotonic buffered solution alone. Diclofenac sodium was treated similar to test at 50-400 µg/ml concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

$$\%\ Inhibition\ of\ haemolysis = 100 \times \frac{Vc - Vt}{Vc}$$

$$\%\ Protection = 100 - [100 \times \frac{Vc - Vt}{Vc}]$$

Where: Vc = absorbance of control; Vt= absorbance of test.

Protein denaturation assay: Protein denaturation was performed as described by Elias *et al*. with slight modifications.[17] Test solution consisting of 1ml of hydromethanolic extracts and Diclofenac sodium (standard) ranging from 50-400 µg/ml was mixed with 1ml of fresh egg albumin solution, and 28ml of phosphate saline buffer pH 6.4 in six 250mL beaker while the control contain normal saline instead of plant extract or standard. This was then incubated at 27˚C for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70˚C in a water bath for 10 minutes. After cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\%\ Inhibition = 100 \times [\frac{Vt}{Vc} - 1]$$

Where, Vt = absorbance of test sample; Vc = absorbance of control.

RESULTS AND DISCUSSION

Phytochemical results of this study showed that *C. chinense* is abundantly rich in flavonoids, alkaloids, phenols and terpenoids (Table1). Many reports have shown that plant flavonoids possess potent anti-inflammatory.[18] Their anti-inflammatory activities are probably due to

their inhibitory effect on enzymes involved in the production of the chemical mediators of inflammation and metabolism of arachidonic acid.[19]

Table 1.Phytochemical analysis of seeds, fruits, and leaves extract of *C. chinense*.

Phytochemical	Seeds	Fruits	Leaves
Alkaloid	+	ND	+
Saponins	++	+	++
Flavonoids	+	+	+
Phenols	+	++	++
Tanins	+	+	++
Terpenoids	+	+	++

+ = presence, ++ = abundance, ND= not detected

The hydromethanolic extracts of seed, fruits and leaves of *C. chinense* were studied for *in vitro* anti-inflammatory activity by Human Red Blood Cell membrane stabilization method (Figure 1). The extracts showed significant anti-inflammatory activity in a concentration dependent manner. Leave and seed extracts at a concentration of 400µg/ml showed maximum protection of HRBC (70.76% and 67.69%, respectively) which was comparable to diclofenac sodium % protection of 80.

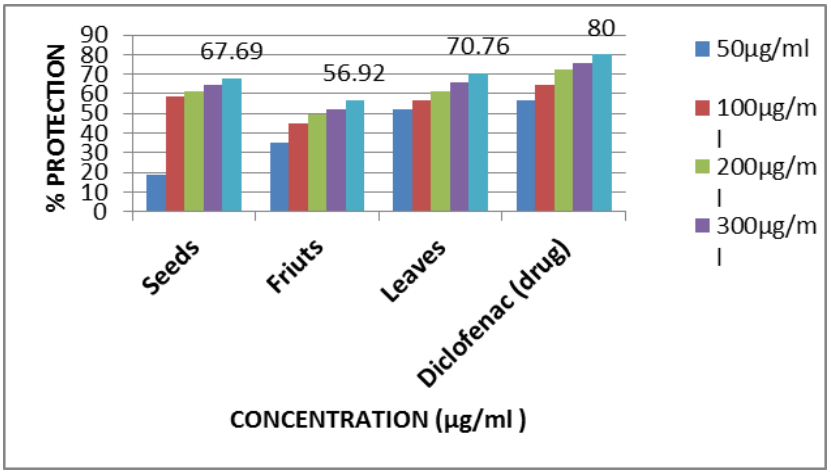


Figure 1: Anti-inflammatory Effects Of *C. Chinense* Extracts On heat-induced heamolysis.

The erythrocyte membrane is analogous to the lysosomal membrane[20] and its stabilization implies that the extract may as well stabilize lysosomal membranes. Exposure of red blood cells (RBCs) to injurious substances such as hypotonic medium and heat results in the lysis of the membranes, accompanied by haemolysis and oxidation of haemoglobin.[21] Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and

proteases, which cause further tissue inflammation and damage upon extra cellular release.[22] The extracts exhibited membrane stabilization effect by inhibiting heat-induced lyses of erythrocyte membrane.

Leaves, seed and fruits of *C. chinense* extracts at concentrations of 50–400 µg/ml also protected human erythrocyte membrane against lysis induced by hypotonic solution and heat (Figure 2). The haemolytic effect of hypotonic solution is related to excessive accumulation of fluid within the cell resulting in the rupturing of its membrane. The inhibition of hypotonicity-induced red blood cell membrane lysis was taken as a measure of the mechanism of anti-inflammatory activity of *C. chinense.* Leave extract at a concentration of 400µg/ml showed maximum protection of HRBC of 58.14% which was not comparable to diclofenac sodium having % protection of 80. Membrane stabilization leads to the prevention of leakage of serum protein and fluids into the tissues during a period of increased permeability caused by inflammatory mediators.[23] *C. chinense* perhaps stabilized the red blood cell membrane by preventing the release of lytic enzymes and active mediators of inflammation.

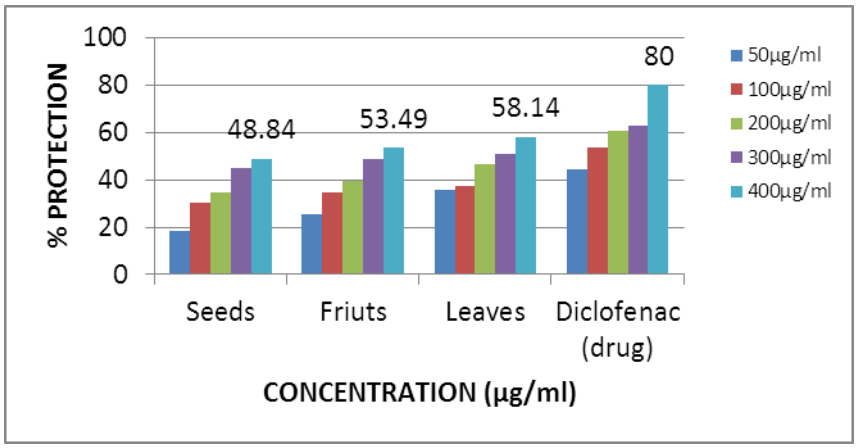
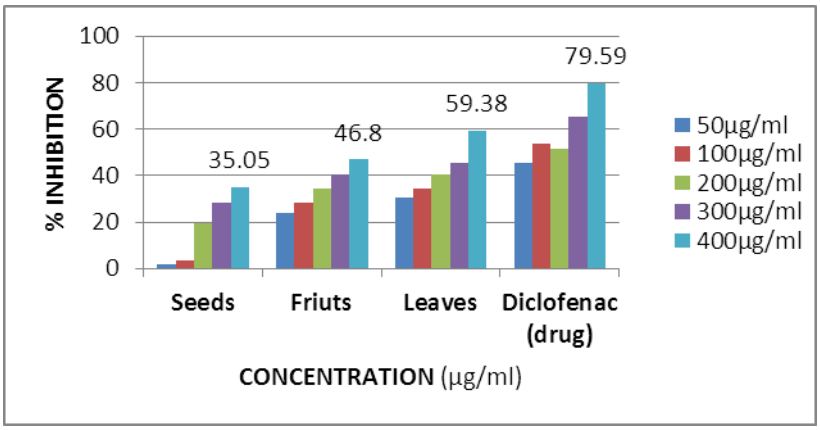


Figure 2: Anti-inflammatory effects of *C. chinense* extracts on hypotonicity-induced heamolysis.

The ability of extracts to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation at a concentration dependent manner (Figure 3). Seeds, fruits and leaves extract at 400µg/ml exhibited maximum inhibition of 35.05%, 46.80% and 59.38% respectively which was not comparable to the standard drug having % inhibition of 79.



Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. The inflammatory drugs (salicylic acid, diclofenac etc) have shown dose dependent ability to thermally induced protein denaturation.[24] Similar results were observed from many reports from plant extracts.[25]

CONCLUSION

In conclusion, present study revealed the invitro anti-inflammatory activity of hydro-methanolic extracts of *C. chinense*. The presence of flavonoids and related phenols may be responsible for these activities. Further investigations are required to isolate, characterize active component of these extract and to confirm their mechanism of action.

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