Original Research Article

COMPARATIVE STUDIES ON ANTI-INFLAMMATORY, ANTIOXIDANT and ANTIMUTAGENIC ACTIVITIES of *Crassocephalum crepidioides (Bent)* LEAF COLD AND HOT WATER EXTRACTS

ABSTRACT

AIM*:* To investigate the anti-inflammatory, anti-oxidant and genotoxicity activities of *Crassocephalum crepidioides* leaf.

Study Design: Comparative investigations of the medicinal value and toxicity profile of cold water (CW) and hot water (HW) extracts of *C. crepidioides* leaf.

Place and Duration of Study: Biochemistry and Molecular Biology Department, Obafemi Awolowo University, Ile-Ife. January 2015-October 2016.

Materials and Methods: CW and HW of *C. crepidioides* were analyzed for anti-inflammatory activity via red blood cell membrane stabilization technique and *in vitro* methods using DPPH radical scavenging activity, thiobarbituric acid-reactive substances (TBARS), ferric reducing antioxidant power (FRAP) and inhibition of oxidative haemolysis were employed to evaluate the antioxidant property. *Allium cepa* chromosomal assay was adopted to investigate the genotoxic effect of the extracts. Total flavonoid and phenolic contents of the extracts were estimated spectrophotometrically.

Results: Both extracts stabilized stressed red blood cell membranes with maximum percentage stability of 50.97±0.06 and 90.90±0.02 at 0.5 and 2.0 mg/ml for CW and HW extracts respectively. The CW extract elicited no DPPH radical scavenging (IC_{50} -0.63±0.02 mg/ml) and lipid peroxidation (IC_{50} - 0.32±0.00) activities. HW extract had IC_{50} of 0.29±0.02 and 0.17±0.00 mg/ml for DPPH and lipid peroxidation. CW and HW extracts exhibited FRAP activity of 1186.96±0.01 and 1015.54±0.01 µmol AAE/g respectively. CW extract displayed a weaker protection (29.01±0.01%) against oxidative haemolysis compared to HW extract (68.70 ± 0.00%). CW extract contained higher phenolic contents (2.16±0.03 µmolGAE/g extract) while the HW extract contained higher flavonoids (0.61±0.05 µmolQE/g extract). CW and HW extracts inhibited *A. cepa* root growth to 71.40±0.02 and 59.10±0.02% respectively. *A. cepa* mitotic index was reduced to 8.85±0.01 and 8.67±0.02 for CW and HW extracts as compared with control (26.62%).

Conclusion: The study concluded that consumption of *C. crepidioides* leaf in cooked form has more medicinal values however, both CW and HW extracts are capable of causing cellular damage at high doses.

Keyword: *Crassocephalum crepidioides,* anti-inflammatory, antioxidant, genotoxicity.

INTRODUCTION

Inflammation is a localized defensive response of cells to harmful stimuli such as infection, noxious chemicals and drugs, stress or physical factors, ultra-violet exposure, hypoxia, nutritional imbalance, allergic irritants, genetic factors, and environmental factors (1). Lysosomal membrane stabilization is one of the various models employed to investigate the efficacy of anti-inflammatory drugs or agents (2).

Stabilization of lysosomal membranes 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 extracellular release (3).

Uncontrolled activation of macrophages, production of O_2^-. OH radicals and non-free radical species (H_2O_2) have been reported in many inflammatory disorders such as rheumatoid arthritis, atherosclerosis, cancer, aging etc. The released or generated O_2^- initiates lipid peroxidation resulting in membrane

destruction or damage which then provoke inflammatory response by the production of mediators and chemotactic factors (4).

Oxidative stress can cause cellular damage which if not controlled correctly by the body, could lead to chronic inflammation which has been reported to increase the risk for various cancers (1). This implies that eliminating oxidative stress and inflammation may represent a valid strategy for cancer prevention and therapy. Herbal compounds with antioxidant and anti-inflammatory properties have been shown to possess anti-proliferative activity (5).

Crassocephalum crepidioides (Ebolo in Yoruba of Nigeria) is a succulent herb widely used in many tropical and subtropical regions, but is especially prominent in tropical Africa where the fleshy mucilaginous leaves and stems are eaten as vegetable and also used for medicinal purposes (6). In different parts of the world, the leaves are eaten either cooked or raw (6). Furthermore, *C. crepidioides* have been widely used in the treatments of indigestion, headache, swollen lips, sleeping sickness and epilepsy. Also reported to possess antitumor activity associated with nitric oxide production, acute hepatitis, fever and edema (7).

Since *C. crepidioides* leaf is consumed either cooked or raw as food or medicine, this study was therefore designed to compare the anti-inflammatory, antioxidant and genotoxicity effects of the hot water extract (representing the cooked form) and cold water extract (which represents the raw form) of the vegetable.

2.0 MATERIALS AND METHODS

2.1 Materials

2.1.1 Collection and Identification of Plant Material

Fresh samples of *Crassocephalum credipioides* were collected from a local farm in Ile-Ife and authenticated at IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

2.2 METHODS

2.2.1 Preparation of Extracts

Fresh leaves of *C crepidiodes* (500 g) were rinsed in clean tap water and homogenized with electric blender (Model AK-380B) under room temperature with 1L of distilled water. The homogenate was filtered with filter paper and centrifuged for 10 min at 3000 rpm to obtained cold water extract solution. For hot water extract, fresh leaf of *C. crepidiodes* (500 g) was boiled in distilled water (1 L) for 3 h, allowed to cool and blended with electric blender. The boiled *C. crepidiodes* homogenate was filtered and centrifuged for 10 min at 3000 rpm to obtain hot water extract solution. The cold and hot water solutions were separately lyophilized to afford cold and hot water extracts.

2.3 Biochemical Assays

2.3.1 Assay of Membrane Stabilizing Potential

The membrane stabilizing assay was based on the modified procedure (8). The assay mixture consisted of 1.0 ml hyposaline (0.25% w/v NaCl), phosphate buffer (0.15 M, pH 7.4; 0.5 ml), varying concentrations of the extracts (50, 100, 150, 200, 250 and 300 µg/ml) which were made up to 3.0 ml with normal saline and 2% (v/v) erythrocytes (0.5 ml). The reaction mixture was incubated at 56^{o}C for 30 min and centrifuged at 3500 rpm for 10 min. The absorbance of supernatant was read at 560 nm. The same concentrations (50, 100, 150, 200, 250 and 300 µg/ml) was used for the standard drug (Diclofenac a standard nonsteroidal anti-inflammatory drug). The % membrane stability was calculated according to (8).

2.3.2 Estimation of Flavonoids Concentration

The spectrophotometric method using 10% aluminum chloride reaction test (9) was used for the estimation of total flavonoid contents in the sample extracts. Samples were directly resolved in 70% (v/v) ethanol. The assay mixture consisted of 5% (w/v) $NaNO_2$ (0.3 ml), 10% (w/v) $AlCl_3$ (0.3 ml) and 4% (w/v) NaOH (4 ml). The reaction mixture was incubated at room temperature for 15 min while the absorbance

was read at 500 nm. The total flavonoid content was extrapolated from Quercetin standard curve (1, 2, 3, 4 and 5 µg/ml).

2.3.3 Estimation of Total Phenol

The Folin-Ciocalteu's reagent test using gallic acid standard was employed for the estimation of total phenolic contents (10). The assay mixture consisted of extract (0.5 ml which was adjusted to 1.0 ml with distilled water) and 1.5 ml of Folin-Ciocalteu's reagent (1:10). After incubation at room temperature for 15 min, 1.5 ml of 7% (w/w) Na_2CO_3 was added and incubated at same temperature for one and half hour. Finally, the absorbance was read at 750 nm against reagent blank.

2.3.4 Lipid Peroxidation

The modified thiobarbituric acid reactive species (TBARS) reaction test using 10% (v/v) egg yolk was adopted for lipid peroxidation test (11). The reaction mixture consisted of egg homogenate (0.5 ml), extract (0.1 ml) and copper sulphate (0.05 ml; 70 mM) which was incubated at 25^{o}C for 30 minutes. This was followed by addition of 20% acetic acid (1.5 ml; pH 3.5) and 0.8% (w/v) thiobarbituric acid in 1.1% sodium dodecyl sulphate (1.5 ml) which were heated at 95^{o}C for 1 h. After cooling, butanol (5.0 ml) was added and centrifuged at 3000 rpm for 10 min. The positive control was prepared as same but contained gallic acid in place of the extract. The absorbance of the supernatant was measured at 532 nm. Percentage inhibition of lipid peroxidation was calculated according to (11).

Where C is the absorbance of control and E is the absorbance of the test sample.

2.3.5 Ferric Reducing Antioxidant Power (FRAP)

The reducing power of the extracts was based on the standard method (12). The mixture contained extract (0.5 ml), phosphate buffer (1.25 ml; 0.2 M, pH 6.6) and 1% potassium ferricyanide [$K_3Fe(CN)_6$] (1.25 ml). After incubation at 50^{o}C for 30 minutes, 10% (w/v) trichloroacetic acid (1.25 ml) was added and centrifuged at 3000 rpm for 10 min. The supernatant (0.625 ml) was mixed with distilled water (0.625 ml) and 0.1% $FeCl_3$ (0.125 ml). The absorbance of the mixture was measured at 700 nm against water blank. Ascorbic acid standard (1, 2, 3, 4, 5 µg/ml) was used to obtain the regression curve.

2.3.6 DPPH Radical Assay

The 1, 1- diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity was determined using modified procedure (13). Test samples were prepared by dissolving 5 mg of dry extract in 5 ml of methanol. The assay mixture contained extract (0.5 ml) and DPPH (1.0 ml) which were mixed well and incubated in the dark for 30 min. The blank was prepared and made to contain methanol (0.5 ml) and DPPH (1.0 ml). The absorbance was measured at 517 nm on a visible spectrophotometer. All experiments were performed in triplicate.

2.3.7 Oxidative Haemolysis Assay

The ability of the extract to prevent hydrogen peroxide (H_2O_2)-induced lysis of erythrocytes was determined based on standard method (14). The assay mixture contained varying concentrations (50, 100, 150, 200, 250 and 300 µg/ml) of sample extracts which was made up to 1.5 ml with normal saline. Exactly 1 ml of 10 mM $CuSO_4.5H_2O$, 1.0 ml H_2O_2 (30% v/v) and 2% red blood cell (1 ml) was added and incubated at 37°C for 1 h. The tubes were cooled and centrifuged at 3000 rpm for 10 min. The supernatant was collected and absorbance of released haemoglobin was read at 540 nm against the reagent blank. The positive control was prepared and made to contain rutin as standard. The percentage inhibition of oxidative haemolysis was calculated according to (14).

2.3.8 Root Growth Inhibition Assay: *Allium cepa*

Twenty five healthy onion bulbs were purchased and sundried for two weeks. The onion bulbs were planted in distilled water to initiate rooting for 24 h in the dark. The best rooted 20 bulbs were selected and used for the study (15,16). The bulbs were planted in 0, 0.5, 1.0, 1.5, and 2.0 mg/ml of the cold

water and hot water extracts for 72 h with distilled water as control. After 72 h, the roots were harvested and fixed in acetic acid/ethanol (1:3 v/v) for 24 h and later stored at 4°C. The lengths of five roots from each bulb were measured and the mean root length was calculated. The percentage root growth inhibition [18] was calculated.

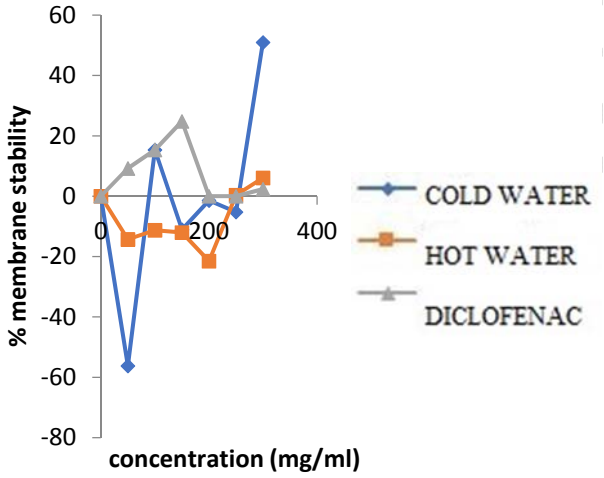
2.3.9 Assay of Genotoxicity Activity in *Allium cepa*

The fixed root (at 4°C) was hydrolyzed in HCl (18% v/v) for 10 min at room temperature. The root tips were squashed on clean slides and stained with FLP-orcein for 15 min (17). The cells were viewed under light microscope at x100 and x400 magnifications. The photomicrographs of the cells were taken at x400 by an Amscope MT microscope camera version 3.0.0.1 attached to the light microscope. The mitotic index was calculated according to (17).

3.0 Results

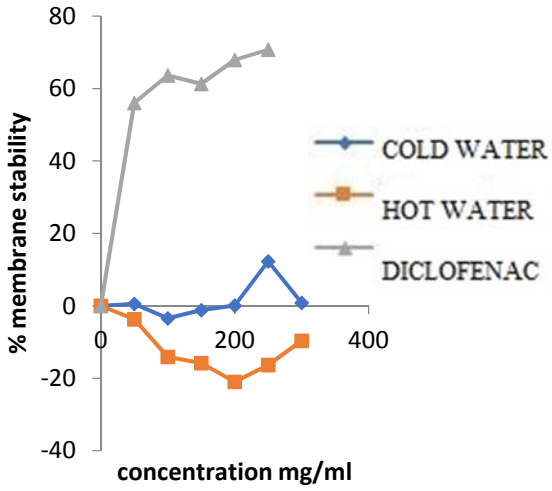
3.1 Membrane Stabilizing Potential of *C. crepidioides* on Bovine Erythrocytes.

The membrane stabilizing activities of the CW and HW extracts of *C. crepidioides* on bovine red blood cells exposed to both heat and hypotonic induced lyses are as shown in Figure 1.0. Both extracts protected stressed erythrocytes but did not compare with Diclofenac (standard anti-inflammatory drug). The percentage stability of the CW extract was concentration dependent with 50.97±0.06, 12.27±0.00, 0.62±0.02 and -0.67±0.01 being the maximum percentage stability at 0.5, 1.0, 1.5 and 2.0 mg/ml respectively while HW extract had 6.06±0.06, -3.71±0.06, 36.27±0.01 and 90.90±0.02% at 0.5, 1.0, 1.5 and 2.0 mg/ml respectively. In contrast, the maximum percentage stability of the Diclofenac standard was 24.81±0.01, 70.76±0.01, 22.72±0.01 and 62.70±0.01 at 0.5, 1.0, 1.5 and 2.0 mg/ml respectively.



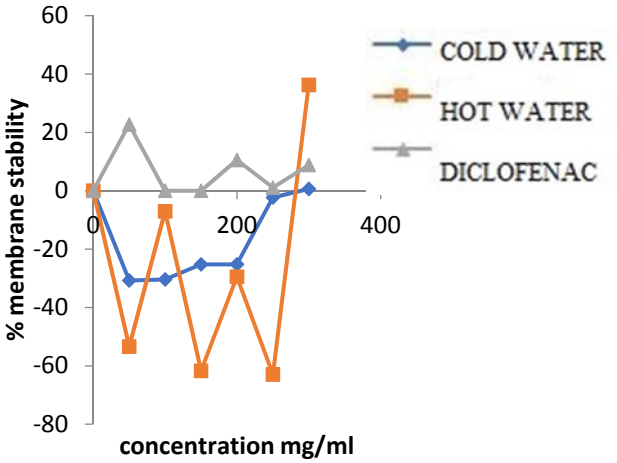
0.5 mg/ml

Figure 1a



1.0 mg/ml

Figure 1b



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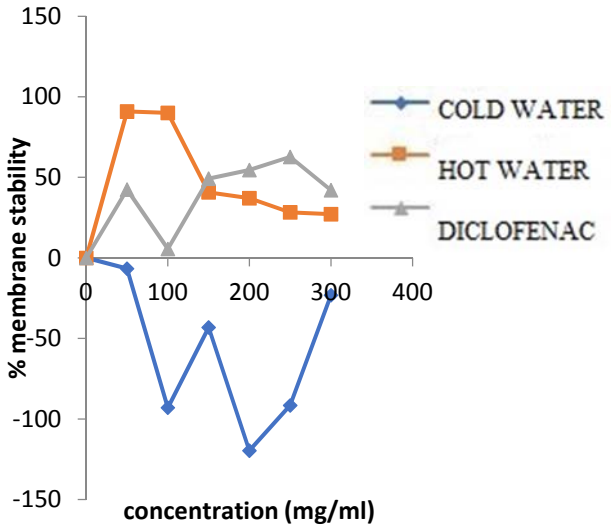


170 1.5 mg/ml 2.0 mg/ml

171 Figure 1c Figure 1d

172 Figure 1a-d: Percentage Membrane Stability Profile of *C. crepidioides* cold water extract, hot water
173 extracts and Diclofenac (Standard drug) at 0.5, 1.0, 1.5 and 2.0 mg/ml. Each value represents the (mean
174 ± SEM).

175

176 **3.2 Antioxidant Assays**

177 **3.2.1 Total Flavonoids and Phenolic Contents**

178 The HW extract had higher flavonoid contents (0.61±0.05 µmol QE/g) than the CW extract (0.44±0.01
179 µmol QE/g) whereas the CW extract had higher phenolic contents (2.16±0.03 µmol GAE/g) than the HW
180 extract (0.94±0.01 µmol GAE/g) (Table 1).

181 **3.2.2 Inhibition of DPPH (1, 1- diphenyl-2-picrylhydrazyl) Radical**

182 The amount of extract required to scavenge 50% of DPPH radicals in a system is often expressed as IC_{50}.
183 The lower the IC_{50} value the higher the antioxidant activity and vice versa. It was observed that CW
184 extract did not exert DPPH scavenging activity while HW extract showed a weak DPPH scavenging
185 activity (IC_{50} = 0.290±0.02 mg/ml) compared to ascorbic acid standard (IC_{50} = 0.01± 0 mg/ml) (Table 1).

186 **3.2.3 FRAP (Ferric Reducing Antioxidant Power)**

187 The FRAP assay measures the amount of the extract which can reduce Fe^{3+} to Fe^{2+} as compared with 1
188 g of ascorbic acid standard. The higher the FRAP value the higher the antioxidant activity. The CW and
189 HW extracts showed reductive capabilities compared to ascorbic acid. CW extract exhibited highest
190 reducing potential (1186.96±0.01 µmol AAE/g) followed by HW extract (1015.54±0.01 µmol AAE/g) and
191 ascorbic acid standard (809.29±0 µmol AAE/g) (Table 1).

192 **3.2.4 Lipid Peroxidation**

193 The HW extract inhibited lipid peroxidation activity with IC_{50} = 0.17± 0 mg/ml and compared with gallic
194 acid (IC_{50} = 2.25±0.01 mg/ml) (Table 1) however, CW extract did not inhibit lipid peroxidation.

195 **3.2.5 Inhibition of Oxidative Haemolysis**

Figure 2.0 showed the percentage inhibition of oxidative haemolysis on bovine red blood cells exposed to H_2O_2-induced lyses. The CW extract inhibited oxidative haemolysis better than HW extract with maximum protection of 68.70±0% at 100 µg/ml. HW extract showed maximum protection at 300 µg/ml with percentage inhibition of 29.01±0.01. Rutin standard produced a better and maximum percentage inhibition of 85.26±0.01 at 150 µg/ml.

Table 1: Total flavonoids, phenolics and antioxidant profiles of *Crassocephalum crepidiodes*

Extracts	Total Phenolics (µmolGAE/g extract	Total Flavonoids (µmoQE/g extract)	DPPH IC_{50} (mg/ml)	FRAP (µmol AAE/g)	Lipid Peroxidation IC_{50} (mg/ ml)
CW	2.16 ±0.03	0.44 ±0.01	-0.63±0.02	1186.96±0.01	-0.32±0.0
HW	0.09±0.01	0.61 ±0.05	029±0.02	1015.54±0.01	0.17±0.00
AA	ND	ND	001±0.00	809.29±0.00	ND
GA	ND	ND	ND	ND	2.25±0.01

CW: Cold Water ; HW: Hot Water ; AA: Ascorbic acid ; QE: Quercetin equivalent; GA: Gallic acid; ND: Not determined. Each value represents the mean ± SEM of 3 readings. Values were considered significant at p≤0.05.

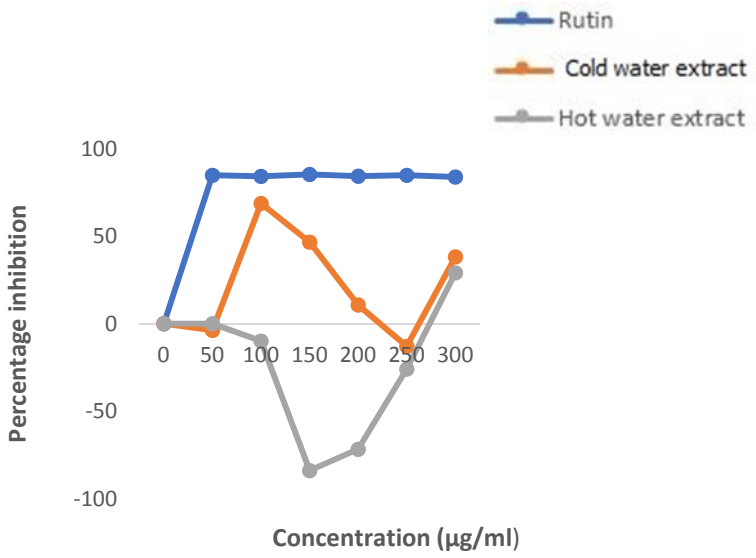


Figure 2.0: Percentage inhibition of H_2O_2-induced oxidative haemolysis of red blood cells by *C. crepidiodes* extracts.

3.8 Cytological Effects of CW and HW Extracts on the Mitosis of *A. cepa*

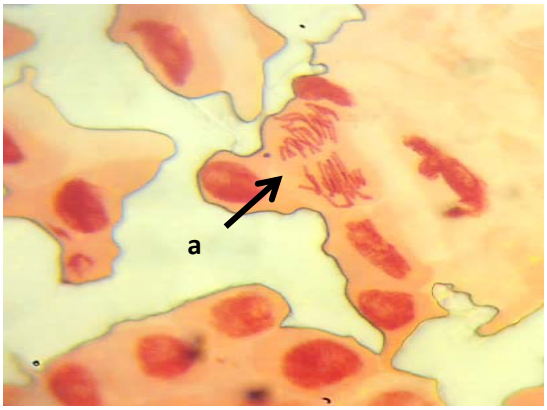
The effects of the cold and hot water extracts on *A. cepa* root-tip cells undergoing mitosis are shown in Tables 2 and 3. The HW extract showed a steady decline in the mitotic index while the CW extract produced no regular pattern. There was a decrease in the mitotic index (MI) with increasing concentration of the extracts (Tables 2 and 3). There were significant reductions (at $p \leq 0.05$) in the *A. cepa* mitotic index for both treated roots; CW extract reduced the mitotic index to 8.11% at 1.5 mg/mL; while the HW extract reduced mitosis index to 7.98% at 2 mg/ml as compared with the control (MI = 21.02%). This showed that cells were actively dividing in control than in treated roots. Some physiological chromosome aberrations were observed at higher concentrations of both extracts such as sticky chromosomes (figures 1 and 2).

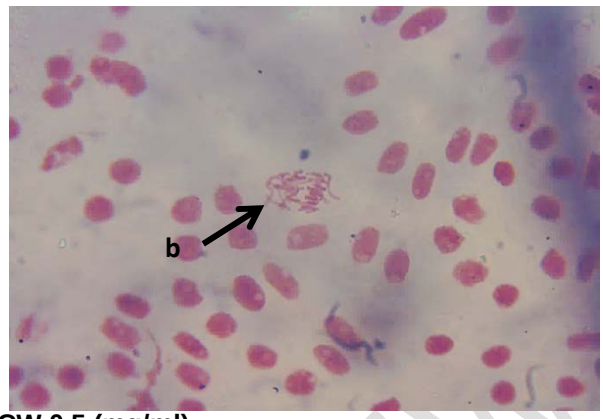
Table 2: Effect of Cold Water Extract on Mitotic Index of *A. cepa* Roots.

Concentration (mg/ml)	Prophase	Metaphase	Anaphase	Telophase	Total Dividing Cells	Total Non-Dividing Cells	Mitotic Index
0	682	059	015	004	760	2855	21.02
0.5	221	008	004	003	236	2232	9.56
1.0	277	039	018	010	344	2442	12.34
1.5	310	028	010	005	353	3987	8.11
2.0	446	025	010	011	492	3802	11.46

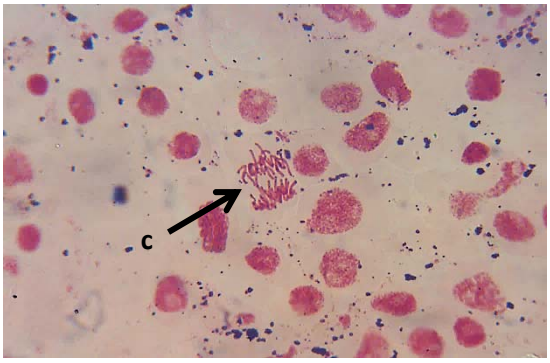
Table 3: Effect of Hot Water Extract on Mitotic Index of *A. cepa* Roots.

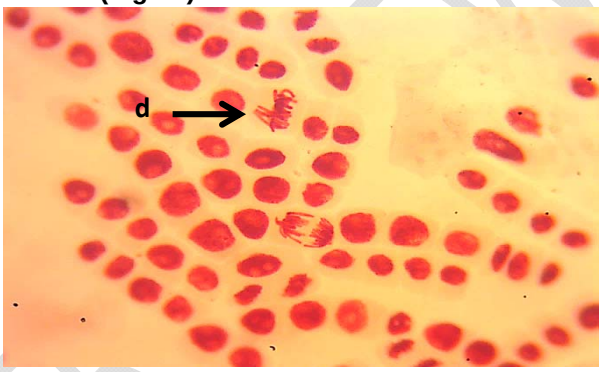
Concentration (mg/ml)	Prophase	Metaphase	Anaphase	Telophase	Total Dividing Cells	Total Non-Dividing Cells	Mitotic Index
0	682	059	015	004	760	2855	21.02
0.5	372	035	017	010	434	2455	15.02
1.0	339	055	024	010	428	3336	11.37
1.5	270	029	007	006	312	3035	9.32
2.0	277	030	008	005	320	3690	7.98



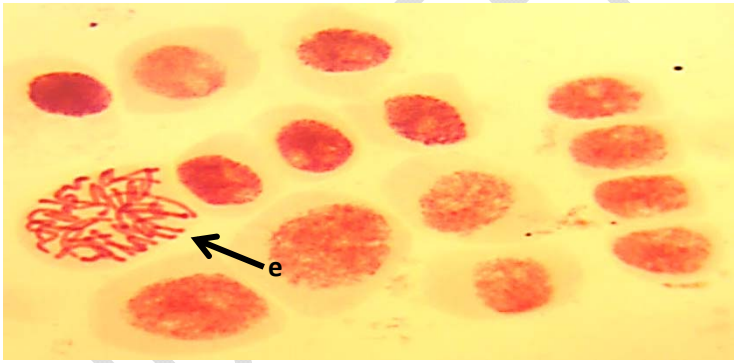


Control **CW 0.5 (mg/ml)**





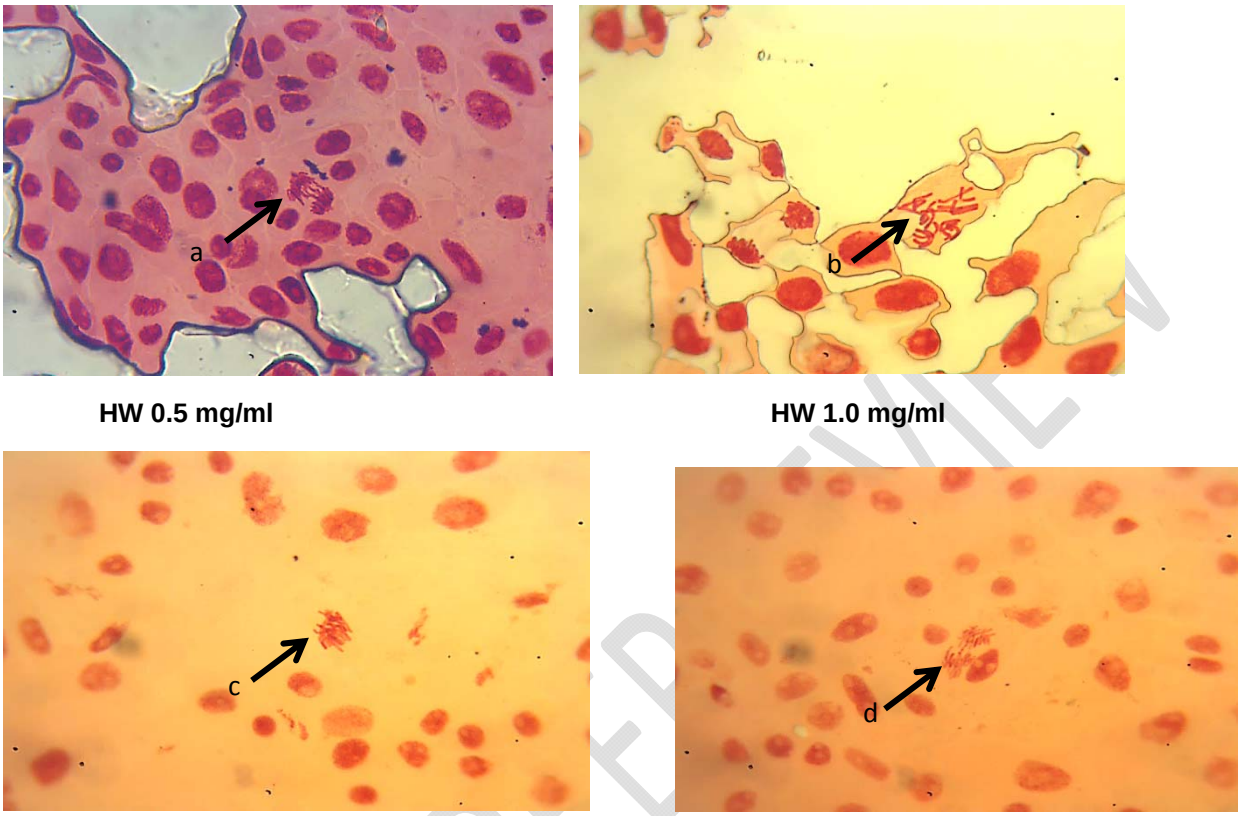
CW 1.0 mg/ml **CW 1.5 mg/ml**



CW 2.0 mg/ml

CW: Juice extract

Figure1: The photomicrograph of *A. cepa* cells undergoing mitosis.
(a) Anaphase (b) Anaphase (c) Anaphase (d) Metaphase (e) metaphase.



HW 0.5 mg/ml **HW 1.0 mg/ml**

HW 1.5 mg/ml **HW 2.0 mg/ml**

HW: Hot water extract

Figure 2: The effects of hot water extract on *A. cepa* chromosomes.

(a) Sticky Anaphase (b) Metaphase (c) Sticky metaphase (d) Sticky anaphase

4.0 DISCUSSION

The study showed that both cold and hot water extracts of *C. crepidioides* leaf protected stressed erythrocytes slightly at lower concentration (1.0 mg/ml) but hot water extract elicited its protection better at higher concentration (2.0 mg/ml) (Figure 1a-d). The ability of the extracts to protect and stabilize the erythrocyte membrane from lyses, suggests the presence of pharmacological compounds effective in the treatment of inflammatory disorder; since one of the mechanisms of inflammation is through the disruptions of cell membrane. It is evident that the mode of protection of the two aqueous extracts is biphasic (not regular). This might be as a result of presence of more than one compounds in the extracts that are antagonistic in effect and thus, interfere with the anti-inflammatory activity of other constituent(s). The mechanism of action of the extract may be by interacting with surface membrane proteins of the erythrocytes or by surface area to volume ratio of the cells which may be brought about by an expansion of membrane or the shrinkage of cells (9).

DPPH is a stable nitrogen-centered free radical that is effectively scavenged by antioxidants. The reaction of DPPH radicals and antioxidants results in the scavenging of radical involving the donation of

hydrogen atom to the free radical which is measured spectrophotometrically (18). The CW extract showed no DPPH radical scavenging activity (IC_{50} = -0.63±0.02 mg/ml). The HW extract however, had an IC_{50} = 0.29±0.02 mg/ml while ascorbic acid (standard) had an IC_{50} = 0.001± 0.00 mg/ml. It could be surmised that the HW extract possesses anti-oxidant property and could be useful in treatment of free radical-pathological damage.

Measurement of the ferric reductive potential revealed ability of the sample extract to transform ferric (Fe^{3+}) to ferrous (Fe^{2+}). Increase in absorbance of the reaction mixture is considered an increase in the reducing power of the sample (19). The CW and HW extracts exhibited better reducing potential than ascorbic acid standard with the CW extract being more active (1186.96±0.01 µmol AAE/g) than HW extract (1015.54±0.01 µmol AAE/g) and ascorbic acid standard (809.29±0.00 µmol AAE/g). The scavenging of oxidants is considered an effective measure to reduce the level of oxidative stress of organisms. It has been reported that intake of vegetables and fruits can help to reduce the risk of many chronic diseases. In fact the healing power of these vegetables and fruits is contained in their antioxidant phytochemicals present in them. Polyphenols, carotenoids and flavonoids are the main kinds of antioxidant phytochemicals, and they contribute the most to the antioxidant properties of foods/plants (19b).

The CW extract had higher phenolic content (2.16±0.03 µmol GAE/g extract) than the HW extract (0.94±0.01 µmol GAE/g extract) which was consistent with FRAP result. There was no significant difference (at $p \leq 0.05$) between the flavonoid contents of the two extracts. Sample extracts with higher total phenolic contents however, have been reported to possess higher FRAP activity (20a). Total phenolic content and total antioxidant activity in phytochemical extracts of different fruits have been reported to have a direct relationship. When the plant extract contains higher total phenolic contents, they tend to possess stronger antioxidant activity (20b).

The roles of medicinal plants in disease prevention or control have been attributed mainly to the anti-oxidant potentials of their constituents usually a wide variety of polyphenolic compounds (20). Flavonoids and phenolic compounds have been reported to exert multiple biological effects which include antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic et cetera (21). In this study, both extracts contained substantial amount of phenolic and flavonoids which attested to the folkloric use of the leaf in the treatment of diseases.

The capacity of the sample extracts to prevent the peroxidation of membrane lipids was assayed by TBARS (malondialdehyde equivalents) method by simply measuring the amounts of malondialdehyde (MDA) that was released. The concentration of MDA in the biochemical assay mixture is an index for measuring oxidative damage. Higher absorbance of MDA signifies higher level of lipid peroxidation and lower capacity of the extract to prevent oxidative damage (20). In this study however, CW *C. crepidioides* leaf extract induced lipid peroxidation (IC_{50} = -0.32± 0.00 mg/ml) while the HW extract inhibited lipid peroxidation instead (0.17± 0.00 mg/ml) and compared favourably with the gallic acid (IC_{50}=2.25±0.01 mg/ml) standard. LPO of cell membranes has been implicated in various pathological conditions such as atherosclerosis, inflammation and liver injury (21). Therefore, the ability of HW extract to inhibit the initiation of lipid peroxidation by scavenging the free radicals that form alkyl peroxyl and alkoxyl radicals (19), is an indication that the extract is probably a suitable source of antioxidant compounds to combat oxidative stress compared to CW extract.

Since the mechanism of free radical damage is diverse and varies from cell to cell, single antioxidant scavenging technique is not sufficient enough to assess the antioxidant potential of any sample material. $CuSO_4$.$5H_2O$ have been substantially reported to react with H_2O_2 to generate hydroxyl radical which can damage the membrane lipids resulting in haemolysis (14). The percentage inhibition of oxidative haemolysis by the cold and hot water extracts of *C. crepidioides* on bovine red blood cells exposed to H_2O_2-induced lyses showed HW extract as better inhibitor of oxidative haemolysis than the CW extracts. The maximum percentage inhibition was obtained with Rutin (85.26±0.01), followed by the hot water extract (68.70±0.00%) while the CW extract had the least percentage inhibition (29.01±0.01).

Toxic effects of drugs or herbal preparations may be investigated by analyzing macroscopic parameters (such as root number and root growth) while genotoxicity and cytotoxicity can be evaluated by investigating the cytological parameters (such as chromosomal aberrations, micronucleus and mitotic index) (22). The result showed growth retardation in onion roots exposed to high

concentration of the *C. crepidioides* extracts. The cold water extract inhibited the root growth to 71.40% at 2.0 mg/ml while the hot water extract inhibited the root growth to 59.10% at 1.5 mg/ml. It has been reported that root growth inhibition over 45% indicates the presence of toxicants (14) having sublethal effects on the test plants (23). Furthermore, inhibition of root growth and the appearance of stunted roots indicate cytotoxicity, while wilting of root explains toxicity. Stunting and wilting of roots occur as a result of suppression of mitotic activity (24).

The cytological study revealed decrease in the mitotic index (MI) with increasing concentration of the extracts. The CW extract reduced the *A. cepa* mitotic index to 8.11% at 1.5 mg/mL (Table 2) as compared with the general control (MI = 21.02%) while the HW extract reduced the mitotic index to 7.98 at 2.0 mg/mL (Table 3) compared with the same control. This showed that more cells were actively dividing in the control than in the treated group. Suppression of mitotic index could be a signal for cell growth inhibition and cell death. The reduction in MI noted in this present study may be due to the inhibition of DNA synthesis or the blocking in the G2 phase of the cell cycle (25).

No chromosomal aberration (CA) was observed in the control group which received only distilled water. However, sticky chromosomes were the predominant aberrations observed in this study. The sticky metaphase and anaphase is an indication that the sample extracts contain toxic substances that are injurious to the chromosomes. Chromosomal stickiness has been shown to be caused by excessive contraction and condensation of chromosomes and partial dissolution of nucleoproteins. Chemical agents capable of inducing chromosomal stickiness are considered very toxic because it is an irreversible process that often leads to cell death (26). Pedro *et al.* and Akinpelu *et al* reported significant decreases in root mean growth and mitotic index of the *A. cepa* grown in different concentrations of *Jatropha gossypiifolia* L. (27) and *Archidium ohioense* (28) extracts which corroborate this present study. Furthermore, members of the *Crassocephalum* genus have been reported to contain toxic alkaloids known as pyrrolizidine alkaloids (PAs). Acute and chronic ingestion of PAs have been reported to cause 10- to 30-fold enlargement of the liver cells (megalocytosis), enlargement of liver nuclei with increasing nuclear chromatin, disruptions of liver cell metabolism with considerable functional losses, irregular mitoses with simultaneous inhibition of mitosis due to DNA blocking (genotoxic), cytoclases and fatty degeneration (29). Therefore, the observed genotoxicity effect of the CW and HW extracts of *Crassocephalum crepidioides* may be linked to the presence of pyrrolizidine alkaloids contamination.

CONCLUSION

The result of this study suggests that the HW extract of *C. crepidioides* leaf exhibit better anti-inflammatory and antioxidant activities compared to CW extract though both elicited significant levels of mitotic inhibition. The study concluded that consumption of *C. crepidioides* leaf in cooked form has more medicinal values, however both CW and HW extracts are capable of causing cellular damage at high doses.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.