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 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 spectrophometrically.

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 is capable of causing cellular damage at high doses.

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

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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).

Excessive activation of phagocytes, production of O_2. OH radicals as well as non-free radical species (H_2O_2) which are capable of damaging tissues either directly or indirectly 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 chemostatic 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. Compounds with antioxidant and anti-inflammatory properties have been shown to possess anti-proliferative activity (5).

C. crepidioides is a succulent herb used widespread 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 for medical purposes (6)). In Sierra Leone, leaves are also popular and are made into sauce with groundnut paste while it is eaten as salad green, either cooked or raw in Australia. *C. crepidioides* have been reported in the treatments of indigestion and headache, swollen lips, sleeping sickness, epilepsy, also 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 for medicinal purposes, this study was designed to compare antioxidant, anti-inflammatory and genotoxic effects of the cold and hot water extracts 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 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 with 1L of distilled water. The homogenate was filtered with a double layered cheese cloth 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 with a double layered cheese cloth 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 contained hyposaline (1.0 ml), phosphate buffer (0.15 M, pH 7.4; 0.5 ml), varying concentrations of the extracts (0-300 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 percentage membrane stability was calculated as:

$$\% \text{ membrane stability} = 100 - \frac{\text{Abs of test drug - Abs of drug control}}{\text{Abs of blood control}} \times 100$$

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 contents were extrapolated from the Quercetin standard curve.

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 as:

% Inhibition = [(1-E)/C] x 100

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 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 (0-300 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. Then 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 as:

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% Inhibition = [(Abs of blank – Abs of test) / Abs of blank] x 100.

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^{o}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 as: Inhibition (%) = [1- (sample/ control)] x 100.

2.3.9 Assay of Genotoxicity Activity in *Allium cepa*

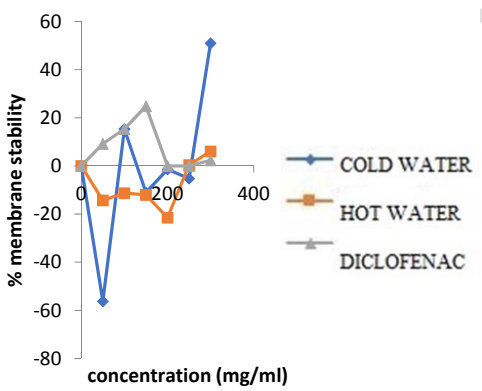
The fixed root (at 4^{o}C) was hydrolyzed in HCl (18% v/v) for 10 min at room temperature. The root tips were squashed on clean slides and stained with FLP-orcein for 15 min (17). The cells were viewed under light microscope at x100 and x400 magnifications. The photomicrographs of the cells were taken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

$$Mitotic\ index = (Number\ of\ dividing\ cell/Total\ numberof\ cells)\ \times 100\%$$

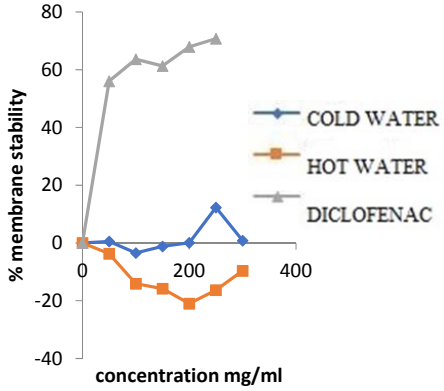
3.0 Results

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

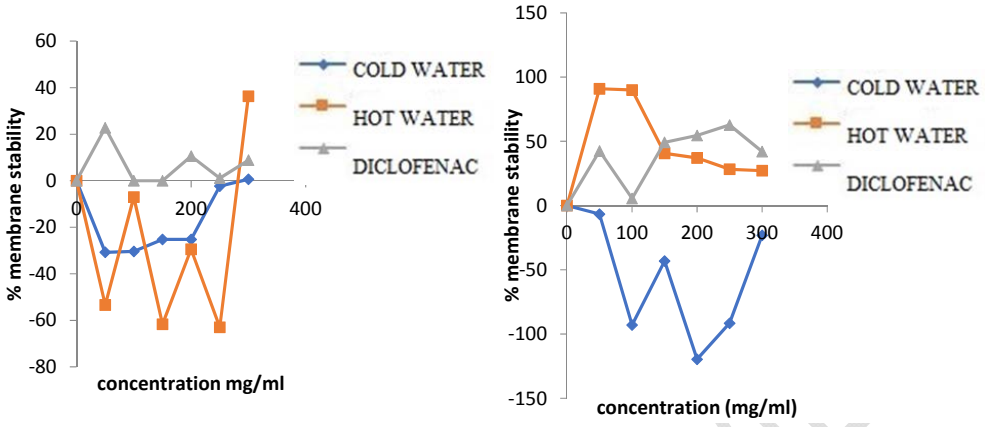
The membrane stabilizing activities of the CW and HW extracts of *C. crepidioides* on bovine red blood cells exposed to both heat and hypotonic induced lyses are as shown in Figure 1.0. Both extracts protected stressed erythrocytes but did not compare with Diclofenac (standard anti-inflammatory drug). 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



1.0 mg/ml



1.5 mg/ml 2.0 mg/ml

Figure 1.0: Percentage Membrane Stability Profile of *C. crepidioides* cold water extract, hot water extracts and Diclofenac (Standard drug) at 0.5, 1.0, 1.5 and 2.0 mg/ml. Each value represents the mean ± SEM of 3 readings.

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3.2 Total Flavonoids and Phenolic Contents

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

3.3 Inhibition of DPPH (1, 1- diphenyl-2-picrylhydrazyl) Radical

The amount of extract required to scavenge 50% of DPPH radicals in a system is often expressed as IC_{50}. The lower the IC_{50} value the higher the antioxidant activity and vice versa. It was observed that CW extract did not exert DPPH scavenging activity while HW extract showed a weak DPPH scavenging activity (IC_{50} = 0.290±0.02 mg/ml) compared to ascorbic acid standard (IC_{50} = 0.01± 0.00 mg/ml) (Table 1).

3.4 FRAP (Ferric Reducing Antioxidant Power)

The FRAP assay measures the amount of the extract which can reduce Fe^{3+} to Fe^{2+} as compared with 1 g of ascorbic acid standard. The higher the FRAP value the higher the antioxidant activity. The CW and HW extracts showed reductive capabilities compared to ascorbic acid. CW extract exhibited highest reducing potential (1186.96±0.01 µmol AAE/g) followed by HW extract (1015.54±0.01 µmol AAE/g) and ascorbic acid standard (809.29±0.00 µmol AAE/g) (Table 1).

3.5 Lipid Peroxidation

The HW extract inhibited lipid peroxidation activity with IC_{50} = 0.17± 0.00 mg/ml and compared with gallic acid (IC_{50} = 2.25±0.01 mg/ml) (Table 1) however, CW extract did not inhibit lipid peroxidation.

3.6 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±0.00% 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.

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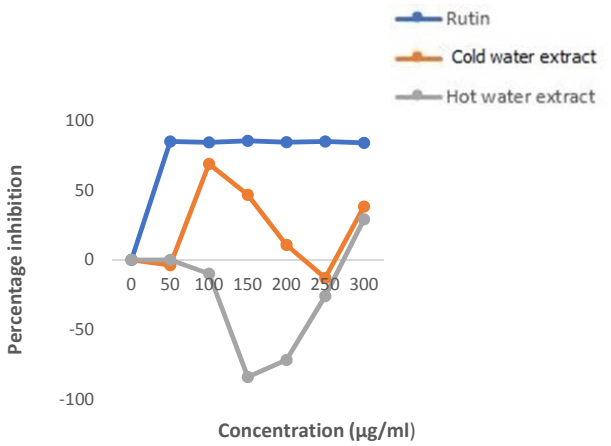


Figure 2.0: Percentage inhibition of H_2O_2-induced oxidative haemolysis of red blood cells by *C. crepidiodes* extracts.

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 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).

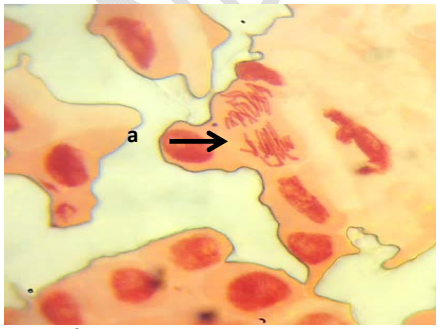
Comment [H7]: Significant reduction at what P-value?

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

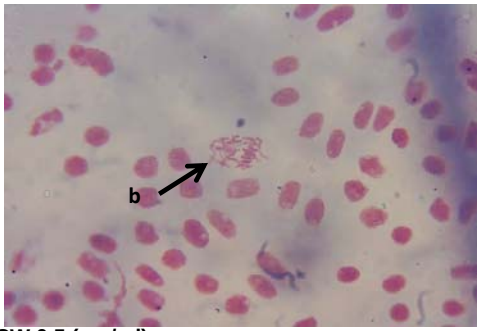
Concentration (mg/ml)	Prophase	Metaphase	Anaphase	Telophase	Total Dividing Cells	Total Non-Dividing Cells	Mitotic Index
0	682	059	015	004	760	2855	21.02
0.5	221	008	004	003	236	2232	9.56
1.0	277	039	018	010	344	2442	12.34
1.5	310	028	010	005	353	3987	8.11
2.0	446	025	010	011	492	3802	11.46

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



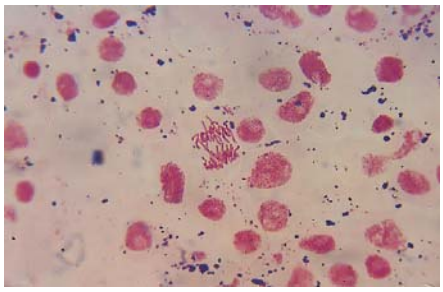
Control

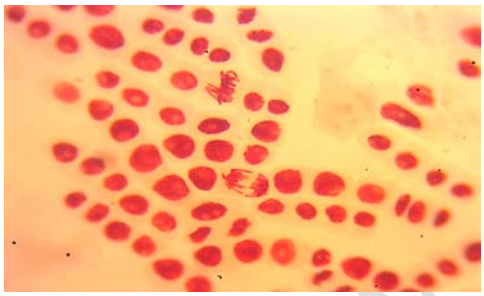


CW 0.5 (mg/ml)

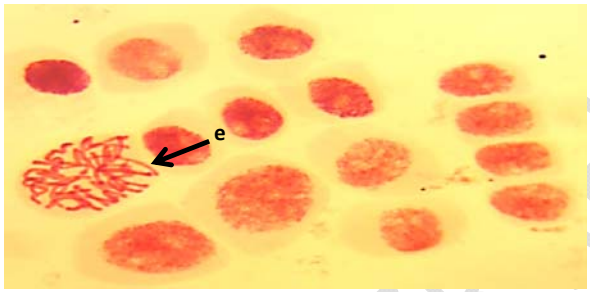
c

d





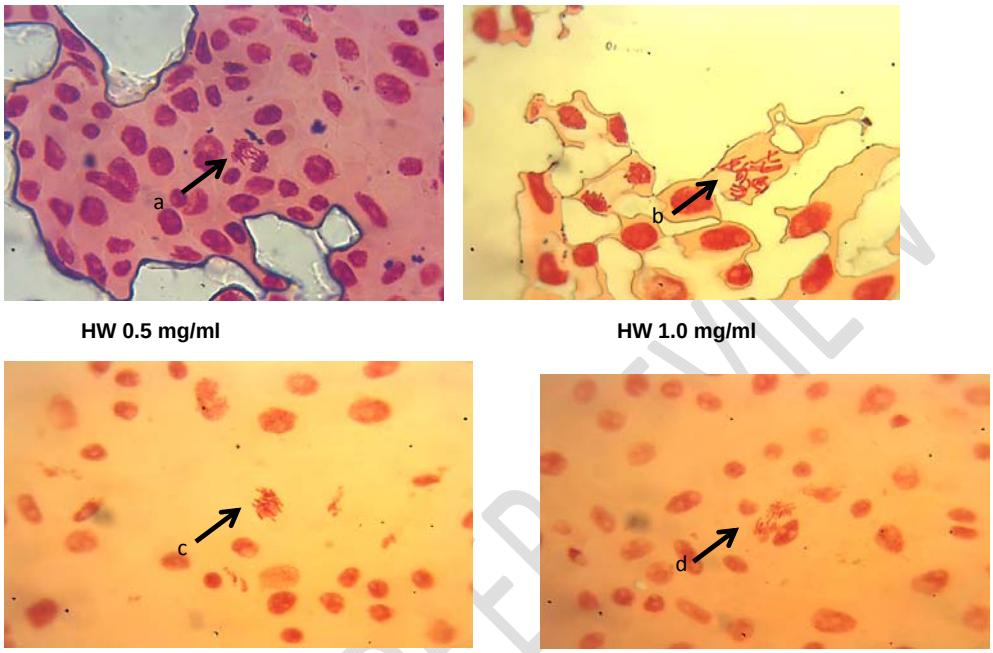
CW 1.0 mg/ml **CW 1.5 mg/ml**



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 juice

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

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

4.0 DISCUSSION

The studies 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). These extracts probably exhibited membrane stabilization effect by inhibiting hypotonicity-induced lyses of erythrocyte membrane which is analogous to the lysosomal membrane (8).

DPPH is a stable nitrogen-centered free radical which is effectively scavenged by antioxidants. The reaction between the DPPH radicals and antioxidant molecules from the extracts often results in the scavenging of the radical by simply donating hydrogen atom to the free radical. Such interaction causes a change in absorbance of DPPH radical which is often measured at 517 nm. The reduction of the DPPH radicals by the antioxidants often results in discoloration from purple to yellow (18). The *C. crepidioides* cold water extract showed no DPPH radical scavenging activity (IC_{50} = -0.63±0.02 mg/ml). The hot water extract, however, had an IC_{50} = 0.29±0.02 mg/ml while ascorbic acid (standard) had an IC_{50} = 0.001± 0.00

Comment [H8]: Study or studies?

mg/ml. It could be surmised that the hot water extract exhibits free radicals scavenging property and probably 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+}) - ferrous (Fe^{2+}). Increase in absorbance of the reaction mixture is considered an increase in the reducing power of the sample (19). The cold and hot water extracts of *C. crepidioides* leaf exhibits better reducing potential than ascorbic acid with the cold water extract being more active (1186.96±0.01 µmol AAE/g) than hot water extract (1015.54±0.01 µmol AAE/g) and ascorbic acid standard (809.29±0.00 µmol AAE/g).

The CW extract had higher phenolic content (2.16±0.03 µmol GAE/g extract) than the HWextract (0.94±0.01 µmol GAE/g extract) which was consistent with FRAP result. There was no significant difference between the flavonoid contents of the two extracts. Sample extracts with higher total phenolic contents however, have been reported to show higher FRAP activity (20).

Comment [H9]: Space bar

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 etc. (21). In this study, both extracts contained substantial amount of phenolic and flavonoids which attested to the folkloric use of the leaf treatment of disease.

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. MDA (which is a secondary product of the oxidation of membrane lipids) reacts with two molecules of TBA, to form a pink-red chromogen with an absorbance maximum at 532 nm. The concentration of MDA in the assay mixture becomes the index of measuring the oxidative damage. The higher the absorbance of MDA, the higher the level of the lipid breakdown and the lower the capacity of the sample extract to curb oxidative damage (20). In this study however, CW *C. crepidioides* leaf extract showed no anti-lipid peroxidation activity (IC_{50} = -0.32± 0.00 mg/ml) while the hot water extract anti-lipid peroxidation activity (0.17± 0.00 mg/ml) compared favourably with gallic acid (IC_{50} =2.25±0.01 mg/ml). LPO of cell membranes has been implicated in various pathological conditions such as atherosclerosis, inflammation and liver injury (21). Therefore, the ability of the HW extract to inhibit the initiation of lipid peroxidation by scavenging the free radicals that form alkyl peroxyl and alkoxyl radicals (19), is a potential 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 juice 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 hot water 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).

CONCLUSION

The result of this study suggests that the hot water extract of *C. crepidioides* leaf exhibit better anti-inflammatory and antioxidant activities compared to cold water extract though both had a very slight degree of mitotic index inhibition. The study concluded that consumption of *C. crepidioides* leaf in cooked form has more medicinal values however, both is capable of causing cellular damage at high doses.

REFERENCES

1. Iwalewa EO, McGaw LJ, Naidoo V, Eloff JN. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology. 2007;6(25):2868-2885.
2. Oyedapo OO, Sab FC and Olagunju JA. Bioactivity of fresh leaves of *Lantana camara*. Biomedical Letters. 1999;59:175-183.
3. Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. Phytother. Res. 1997;11:152-154.
4. Lewis DA. In “Antiinflammatory Drugs from Plants and Manne Sources”, *Basel:Bikhauser Vileg*. 1989; 135.
5. Bagora B, Imae HN, Charlemagne G, Roger N, Albert Y, Laurent M, Gilles F, Jean-Baptiste N, Jean-Marc A, Jacques S. Chemical Composition, Antioxidant, Anti-Inflammatory and Anti-proliferative Activities of Essential Oils of Plants from Burkina Faso. PLOS ONE 2014; 9(3).
6. Grubben GH. Crassocephalum crepidioides (Benth.) S. Moore. In: Plant Resources of Tropical Africa 2, Vegetables. PROTA Foundation Wageningen, Netherlands/Backhuys Publishers, Netherlands; 2004.
7. Kongsareree, P, Prabpai S, Sriubolmas N, Vongvein C, Wiyakrutta S. Antimalarial dihydroisocoumarins produced by Geotrichumsp; an endophytic fungus of *Crassocephalum crepidioides*. Journal of Natural Products. 2003; 66(5):709-711.
8. Oyedapo OO, Akinpelu BA, Akinwunmi KF, Adeyinka MO, Sipeolu FO. Red blood cell membrane stabilizing potentials of extracts of Lantana camara and its fractions. International Journal of Plant Physiology and Biochemistry. (2010); 2(4): 46-51.
9. Singleton VL, Orthofer R, Lamuela RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology. 1999; 299:152-178.
10. Gulcin GI, Beydemirs S, Elemastas M, Kfrevioglu OI. Comparism of antioxidant activity of Clove (*Eugenia caryophylata* Thumb) buds and lavender (Lavander stoechas L.). Food chemistry. 2003; 87: 393-400.
11. Masao H, Yang XW, Miyashiro H, Nabma T. Inhibitory effects of monomeric and dimeric phenyl propanoids from mice on lipid peroxidation *in vivo* and *in vitro*. Phytotherapy Research. 1993;7:395-401.
12. Malterud KE, Rydland KM. Inhibitors of 15-lipoxygenase from orange peel. Journal of Agricultural and Food Chemistry. 2000; 48:5576–5580.

13. Konaté K, Souza A. In vitro Antioxidant, Lipoxigenase and Xanthine Oxidase Inhibitory Activities of fractions from *Cienfuegosia digitata* Cav., *Sida alba* L. and *Sida acuta* Burn F. (Malvaceae). Pakistan Journal of Biological Sciences. 2010;13(22):1092-1098.
14. Amzal H, Aloni K, Tok S, Errachidi A, Charof R, Cherrah Y, Benjouad A. Protective effect of saponins from Argania spinosa against free radical induced oxidative haemolysis. Fitoterapia. 2008; 79: 337-344.
15. Fiskesjo G. The Allium test as a standard in environmental monitoring. Hereditas. 1985; 102:99–112.
16. Rank J, Nielson MH. A modified Allium test as a tool in the screening of the genotoxicity of complex mixture. Hereditas. 1993; 118:49-53.
17. Adegbite AE, Olorode O. Karyotype studies of three species of *Aspilia Thouar* (Heliantheae-Asetraceae) in Nigeria. Plant Scientific Research and Communication. 2002;3: 11-26.
18. Ganpathy S, Chandrashekhar VM, Chitme HR, Lakashmi NM. Free radical scavenging activity of *gossypin* and *nevadensin*. Indian J Pharmacol. 2007; 39(6):281-83
19. Priyanka P, Junaid N, Gagandeep C, Kalia AN. *In vitro* antioxidant potential of *Jasminum mesnyi* Hance (Leaves) extracts. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2011; 2(1):348-357.
20. Carlos LC, Mohammed E, Natalia P, Julio A. Antioxidant and cardioprotective activities of phenolic extracts from fruits of Chilean blackberry *Aristotelia chilensis* (Elaeocarpaceae), Maqui. Food Chemistry. 2008;107: 820–829.
21. Akinwunmi KF, Oyedapo OO. Evaluation of antioxidant potentials of Monodora myristica (Gaertn) dunel seeds. African Journal of Food Science. 2013; 7(9):317-324.
22. Namita S, Somia. *Allium cepa* root chromosomal aberration assay: A review. Indian J. Pharm. Biol. Res. 2013; 1(3):105-119.
23. Wierzbicka M. The effect of lead on the cell cycle in the root meristem of *Allium cepa* L. Protoplasma. 1997; 207:186-194.
24. Grant WF. Chromosome aberration assays in Allium. A report of U.S. Environmental Protection Agency Gene- Tox Program. Mutation Research. 1982; 99:273-91.
25. Sudhakar R, Gowda N, Venu G. Mitotic abnormalities induced by silk dyeing industry effluents in the cells of *Allium cepa*. Cytologia. 2001; 66:235-239.
26. Rencuzogullari E, Kayraldiz A, Ila HB, Cakmak T, Topaktas M. The cytogenetic effects of sodium metabisulfite, a food preservative in root tip cells of *Allium cepa* L. Turkish Journal of Biology. 2001; 25:361-370.

COMPETING INTERESTS

Authors have declared that no competing interests exist.