

## Comparative Studies on Anti-Inflammatory, Antioxidant and Antimutagenic Activities of *Crassocephalum crepidioides* (Bent) Leaf Cold and Hot Water Extracts

B. A. Akinpelu<sup>1</sup>, A. Godwin<sup>1\*</sup>, T. Gbadegesin<sup>1</sup>, N. Ajakaye<sup>1</sup>, S. E. Omotosho<sup>1</sup>,  
S. O. Azeez<sup>2</sup>, M. Oziegbe<sup>2</sup> and O. O. Oyedapo<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Molecular Biology, Obafemi Awolowo University, Ile-Ife, Nigeria.

<sup>2</sup>Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

### Authors' contributions

This work was carried out in collaboration among all authors. Authors BAA and OOO designed the study. Author AG managed the literature searches, analyses of the study and performed the spectroscopy analysis including the first draft of the manuscript. Author SOA handled the cytological study. Author MO handled the photomicrographs. Authors TG and NA assisted in the analyses. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AFSJ/2019/v9i130000

#### Editor(s):

(1) Dr. Nelson Pérez Guerra, Professor, Department of Analytical and Food Chemistry, Faculty of Food Science and Technology, Ourense Campus, University of Vigo, Spain.

#### Reviewers:

- (1) Esther Faboro, Bowen University, Nigeria.  
(2) Javier Espinosa, Universidad Nacional Autónoma de México, México.  
(3) Oshim, Ifeanyi Onyema, Nnamdi Azikiwe University, Nigeria.  
(4) Nnadiukwu, Anthony Tochukwu, University of Port Harcourt, Nigeria.  
Complete Peer review History: <http://www.sdiarticle3.com/review-history/48607>

Received 12 February 2019

Accepted 23 April 2019

Published 30 April 2019

Original Research Article

### 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

\*Corresponding author: Email: anyimgodwin2015@gmail.com;

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  $50.97 \pm 0.06$  and  $90.90 \pm 0.02$  at 0.5 and 2.0 mg/ml for CW and HW extracts respectively. The CW extract elicited no DPPH radical scavenging ( $IC_{50}$   $-0.63 \pm 0.02$  mg/ml) and lipid peroxidation ( $IC_{50}$   $-0.32 \pm 0.00$ ) activities. HW extract had  $IC_{50}$  of  $0.29 \pm 0.02$  and  $0.17 \pm 0.00$  mg/ml for DPPH and lipid peroxidation. CW and HW extracts exhibited FRAP activity of  $1186.96 \pm 0.01$  and  $1015.54 \pm 0.01$   $\mu$ mol AAE/g respectively. CW extract displayed a weaker protection ( $29.01 \pm 0.01\%$ ) against oxidative haemolysis compared to HW extract ( $68.70 \pm 0.00\%$ ). CW extract contained higher phenolic contents ( $2.16 \pm 0.03$   $\mu$ molGAE/g extract) while the HW extract contained higher flavonoids ( $0.61 \pm 0.05$   $\mu$ molQE/g extract). CW and HW extracts inhibited *A. cepa* root growth to  $71.40 \pm 0.02$  and  $59.10 \pm 0.02\%$  respectively. *A. cepa* mitotic index was reduced to  $8.85 \pm 0.01$  and  $8.67 \pm 0.02$  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.

**Keywords:** *Crassocephalum crepidioides*; anti-inflammatory; antioxidant; genotoxicity.

## 1. 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. MATERIALS AND METHODS

### 2.1 Materials

#### 2.1.1 Collection and identification of plant material

Fresh samples of *Crassocephalum crepidioides* 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 obtain 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°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 Oyedapo et al. [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<sub>2</sub> (0.3 ml), 10% (w/v) AlCl<sub>3</sub> (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<sub>2</sub>CO<sub>3</sub> 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°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°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 Gulcin et al. [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<sub>3</sub>Fe(CN)<sub>6</sub>] (1.25 ml). After incubation at 50°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<sub>3</sub> (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<sub>2</sub>O<sub>2</sub>)-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<sub>4</sub>·5H<sub>2</sub>O, 1.0 ml H<sub>2</sub>O<sub>2</sub> (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 [17] 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 [18]. 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. 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 Fig. 1. 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.

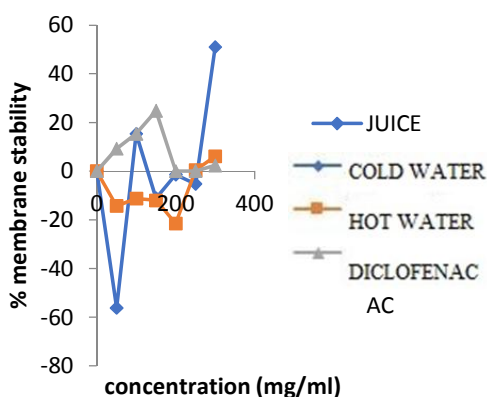
### 3.2 Antioxidant Assays

#### 3.2.1 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).

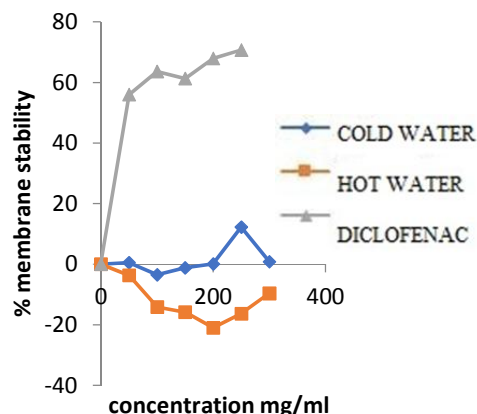
#### 3.2.2 Inhibition of DPPH (1, 1- diphenyl-2-picrylhydrazyl) radical

The amount of extract required to scavenge 50% of DPPH radicals in a system is often expressed as IC<sub>50</sub>. The lower the IC<sub>50</sub> 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<sub>50</sub> = 0.290±0.02 mg/ml) compared to ascorbic acid standard (IC<sub>50</sub> = 0.01± 0 mg/ml) (Table 1).



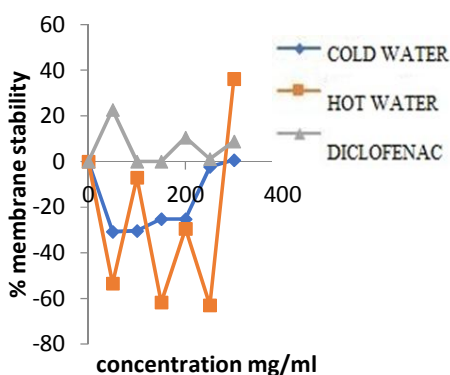
0.5 mg/ml

Fig. 1a.



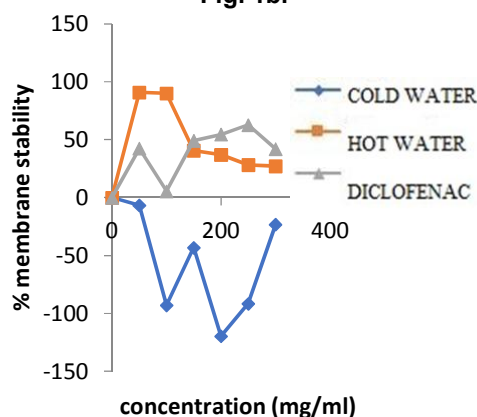
1.0 mg/ml

Fig. 1b.



1.5 mg/ml

Fig. 1c.



2.0 mg/ml

Fig. 1d.

Fig. 1a-d. 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)

Table 1. Total flavonoids, phenolics and antioxidant profiles of *Crassocephalum crepidioides*

Extracts	Total phenolics (µmolGAE/g extract)	Total flavonoids (µmoQE/g extract)	DPPH IC <sub>50</sub> (mg/ml)	FRAP (µmol AAE/g)	Lipid peroxidation IC <sub>50</sub> (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

### 3.2.3 FRAP (Ferric Reducing Antioxidant Power)

The FRAP assay measures the amount of the extract which can reduce Fe<sup>3+</sup> to Fe<sup>2+</sup> 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 µmol AAE/g) (Table 1).

### 3.2.4 Lipid peroxidation

The HW extract inhibited lipid peroxidation activity with IC<sub>50</sub> = 0.17± 0 mg/ml and compared with gallic acid (IC<sub>50</sub> = 2.25±0.01 mg/ml) (Table 1) however, CW extract did not inhibit lipid peroxidation.

### 3.2.5 Inhibition of oxidative haemolysis

Fig. 2 showed the percentage inhibition of oxidative haemolysis on bovine red blood cells exposed to H<sub>2</sub>O<sub>2</sub>-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.

### 3.3 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≤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

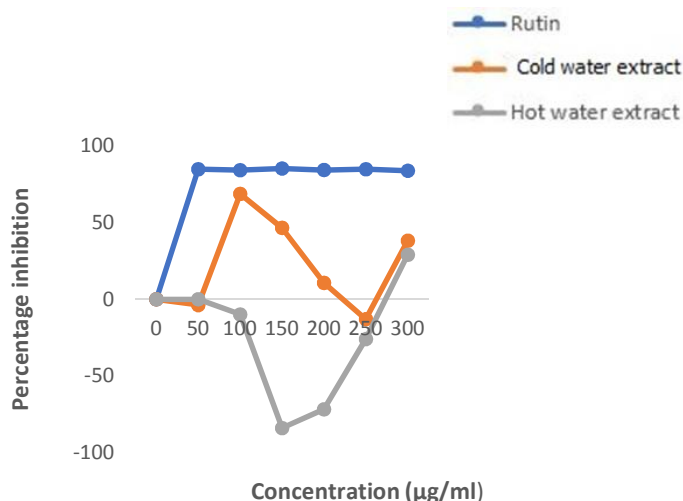


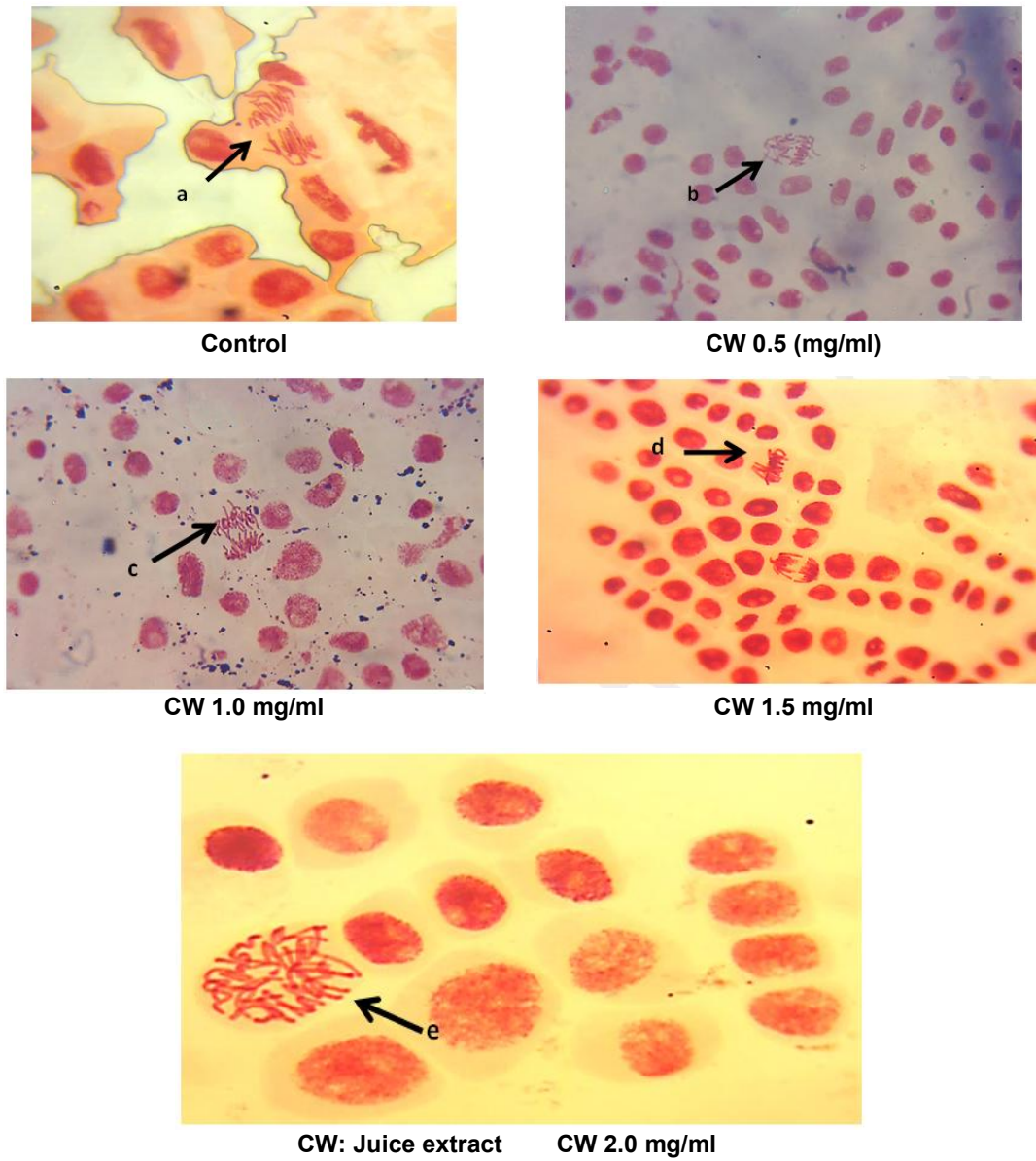
Fig. 2. Percentage inhibition of H<sub>2</sub>O<sub>2</sub>-induced oxidative haemolysis of red blood cells by *C. crepidiodes* extracts

Table 2. Effect of cold water extract on mitotic index of *A. cepa* roots

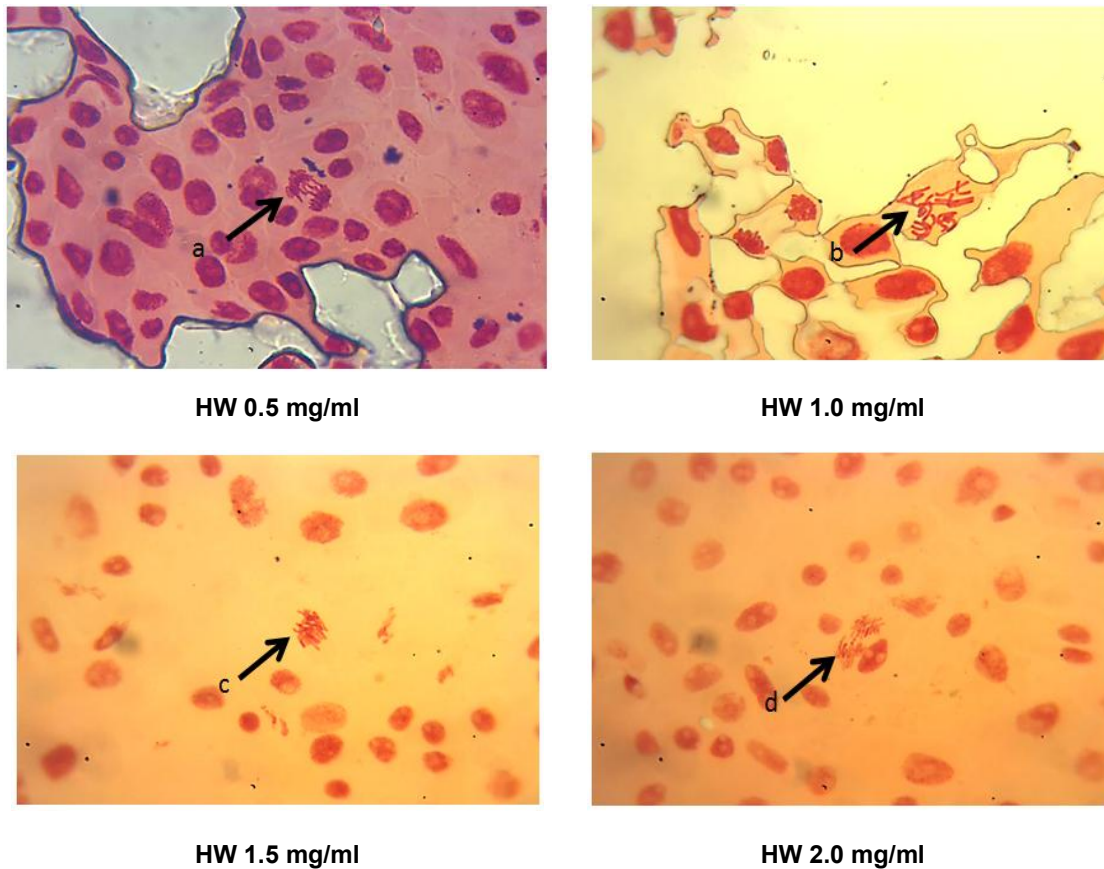
Concentration (mg/ml)	Prophase	Metaphase	Anaphase	Telophase	Total dividing cells	Total non-dividing cells	Mitotic index
0	682	059	015	004	760	2855	21.02
0.5	221	008	004	003	236	2232	9.56
1.0	277	039	018	010	344	2442	12.34
1.5	310	028	010	005	353	3987	8.11
2.0	446	025	010	011	492	3802	11.46

**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



**Fig. 1. The photomicrograph of *A. cepa* cells undergoing mitosis (a) Anaphase (b) Anaphase (c) Anaphase (d) Metaphase (e) metaphase**



**Fig. 2. The effects of hot water extract on *A. cepa* chromosomes**  
 (a) Sticky Anaphase (b) Metaphase (c) Sticky metaphase (d) Sticky anaphase

dividing in control than in treated roots. Some physiological chromosome aberrations were observed at higher concentrations of both extracts such as sticky chromosomes (Figs. 1 and 2).

#### 4. 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) (Fig. 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 ( $IC_{50} = -0.63 \pm 0.02$  mg/ml). The HW extract however, had an  $IC_{50} = 0.29 \pm 0.02$  mg/ml while ascorbic acid (standard) had an  $IC_{50} = 0.001 \pm 0.00$  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 ( $\text{Fe}^{3+}$ ) to ferrous ( $\text{Fe}^{2+}$ ). 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 ( $1186.96 \pm 0.01 \mu\text{mol AAE/g}$ ) than HW extract ( $1015.54 \pm 0.01 \mu\text{mol AAE/g}$ ) and ascorbic acid standard ( $809.29 \pm 0.00 \mu\text{mol AAE/g}$ ). 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 [20].

The CW extract had higher phenolic content ( $2.16 \pm 0.03 \mu\text{mol GAE/g extract}$ ) than the HW extract ( $0.94 \pm 0.01 \mu\text{mol GAE/g extract}$ ) 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 [21]. 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 [22].

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 [23]. 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 [24]. 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 [23]. In this study however, CW *C. crepidioides* leaf extract induced lipid peroxidation ( $\text{IC}_{50} = -0.32 \pm 0.00 \text{ mg/ml}$ ) while the HW extract inhibited lipid peroxidation instead ( $0.17 \pm 0.00 \text{ mg/ml}$ ) and compared favourably with the gallic acid ( $\text{IC}_{50} = 2.25 \pm 0.01 \text{ mg/ml}$ ) standard. LPO of cell membranes has been implicated in various pathological conditions such as atherosclerosis, inflammation and liver injury [24]. Therefore, the ability of HW extract to inhibit the initiation of lipid peroxidation by scavenging the free radicals that form alkyl peroxy and alkoxy 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.  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  have been substantially reported to react with  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$ -induced lyses showed HW extract as better inhibitor of oxidative haemolysis than the CW extracts. The maximum percentage inhibition was obtained with Rutin ( $85.26 \pm 0.01$ ), followed by the hot water extract ( $68.70 \pm 0.00\%$ ) while the CW extract had the least percentage inhibition ( $29.01 \pm 0.01$ ).

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) [25]. 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 [26]. Furthermore, inhibition of root growth and the appearance of stunted roots indicate cytotoxicity, while wilting of roots explains toxicity. Stunting and wilting of roots occur as a result of suppression of mitotic activity [27].

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 [28].

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 [29]. 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 gossypifolia* L. [30] and *Archidium ohioense* [31] 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 [32]. Therefore, the observed genotoxicity effect of the

CW and HW extracts of *Crassocephalum crepidioides* may be linked to the presence of pyrrolizidine alkaloids contamination.

## 5. 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.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## 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, 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: Birkhauser 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. Jamilu Y, Yaro AH, Abubakar MS, Hussaini IM, Anuka JA. Studies on anticonvulsant activity of fractions of hydro-alcoholic root bark extract of *Carissa edulis* (vahl). Nig. Journ. Pharm. Sci. 2007;6(2): 61–66.
  10. 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.
  11. Gulcin GI, Beydemirs S, Elemastas M, Kfrevioglu OI. Comparism of antioxidant activity of Clove (*Eugenia caryophyllata* Thumb) buds and lavender (*Lavander stoechas* L.). Food Chemistry. 2003;87: 393-400.
  12. 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.
  13. Malterud KE, Rydland KM. Inhibitors of 15-lipoxygenase from orange peel. Journal of Agricultural and Food Chemistry. 2000;48: 5576–5580.
  14. 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.
  15. 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.
  16. Fiskesjo G. The Allium test as a standard in environmental monitoring. Hereditas. 1985;102:99–112.
  17. 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.
  18. 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.
  19. Ganpathy S, Chandrashekhar VM, Chitme HR, Lakashmi NM. Free radical scavenging activity of *gossypin* and *nevadensin*. Indian J Pharmacol. 2007;39(6):281-83.
  20. Yu-Jie Z, Ren-You G, Sha Li, Yue Z, An-Na L, Dong-Ping X, Hua-Bin L. Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules. 2015;20:21138–21156.
  21. 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.
  22. Sun J, Chu YF, Wu XZ, Liu RH. Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem. 2002;50:7449–7454.
  23. 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.
  24. Akinwunmi KF, Oyedapo OO. Evaluation of antioxidant potentials of *Monodora myristica* (Gaertn) dunel seeds. African Journal of Food Science. 2013;7(9):317-324.
  25. Namita S, Somia. *Allium cepa* root chromosomal aberration assay: A review. Indian J. Pharm. Biol. Res. 2013;1(3):105-119.
  26. Wierzbicka M. The effect of lead on the cell cycle in the root meristem of *Allium cepa* L. Protoplasma. 1997;207:186-194.
  27. Grant WF. Chromosome aberration assays in Allium. A Report of U.S. Environmental Protection Agency Gene-Tox Program. Mutation Research. 1982;99:273-91.
  28. 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.
  29. 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.

30. Pedro MA, Silvany SA, Maria AM, Ana MB, Ana CB. Genotoxic potential of the latex from cotton-leaf physicnut (*Jatropha gossypifolia* L.). Genetics and Molecular Biology. 2015;38(1):93-100.
31. Akinpelu BA, Godwin A, Aderogba MA, Makinde AM, Azeez SO, Oziegbe M. Evaluation of anti-inflammatory and genotoxicity potentials of the fractions of *Archidium ohioense* (Schimp. ex Mull) extract. Ife Journal of Science. 2018;20(3): 487-495.
32. Roeder E. Medicinal plants in Europe containing pyrrolizidine alkaloids. Pharmazie. 1995;50:2.

---

© 2019 Akinpelu et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle3.com/review-history/48607>