IJABR Vol. 11(1): 53- 65 (2020)

Original article

ANTIOXIDANT PROPERTIES OF HYMENOCARDIA ACIDA STEM BARK, CRATEVA ADANSONII AND PTEROCARPUS ERINACEUS LEAVES

Madaki, F.M*, Kabiru, A.Y, Hamza, R.U., Aina, P.A and Prince, O.

Department of Biochemistry, Federal University of Technology, Minna, Nigeria

Submitted: May, 2020; Accepted: May, 2020; Published: June, 2020

ABSTRACT

Medicinal plants are considered as rich sources of phytoconstituents which may be useful in drug development. In this study the methanol extracts of *Hymenocardia acida* stem bark*, Crateva adansonii and Pterocarpus erinaceus* leaves were screened for their phytochemical constituents and *in vitro* antioxidant properties using standard procedures. Results revealed the presence of flavonoids, phenols, saponins, and alkaloids in all the plants extracts. Quantitative phytochemical determination of the plants extracts showed that the flavonoid content of extracts ranged between 52.62 $\pm$ 0.03 mg/g and 68.31$\pm$ 0.03 mg/g, phenols; 61.33 $\pm$ 0.03 mg/ml and 75.02 $\pm$ 0.04 mg/ml and total phytate content; 3.97$\pm$ 0.02 mg/ml and 9.39 $\pm$ 0.03 mg/ml. The antioxidant activity of the plant extracts is in a dose dependent manner. *H. acida* showed the highest free radical scavenging activity (IC_{50}=128.83 µg/ml), for 1, 1,-diphenyl-2-picrylhydrazyl (DPPH) compared to other extracts and significantly lower (at $P < 0.05$) than the standard (ascorbic acid) being (IC_{50} =101.83 µg/ml). It also has a higher lipid peroxidation inhibition (90.91 $\pm$ 0.05 %) when compared to other extracts and standard. The result of this study showed that methanol extracts of *H. acida,* has significantly higher antioxidant activity at $P < 0.05$ compared to *C. adansonii and P. erinaceus.* The antioxidant activities exhibited by these plants could be due to the presence of phytoconstituents, and could serve as a source of antioxidant.

KEYWORDS: Phytoconstituents, Antioxidants, *Hymenocardia acida, Crateva adansonii* and *Pteriocarpus erinaceus*

***Corresponding author's email address**:nmmadaki@gmail.com

INTRODUCTION

Medicinal plants contain phytoconstituents that may be used for therapeutic purposes and as precursors in drug development [1]. Many of such plants known to be used traditionally to alleviate symptoms of illnesses have been screened and shown to have medicinal values include; *Crateva adansonii, Walthera indica, Hymenocardia acid, Pterocarpus erinaceus* [2, 3, 4]. The medicinal value of these plants lies in their bioactive phytochemical constituents that produce definite physiological action [5]. Phytochemicals are chemical compounds that occur naturally in plants and which may affect health and are not generally considered as essential nutrients [6].

Free radicals have the capacity of attacking the healthy cells of the body, leading to loss of structure and function. Free radicals are very reactive unpaired electron in their valance shell; as a result, they can attack the nearest stable molecule and elicit a chain reaction [7, 8], which stimulate apoptosis; program cell death, and can provoke various cardiovascular, neurological and physiological disorders [9, 10]. Damage to cell caused by these free radicals tends to play a role in aging process and associated degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction [11]. This is because they are useful to the body when moderately produced or strictly regulated. The reactive free radicals produced include hydroxyl radicals, superoxide radicals as well as excited or singlet oxygen [12]. Antioxidants are scavengers of oxidation process, even at relatively small concentration and thus have diverse physiological role in the body [12]. Antioxidant compounds like phenol, polyphenols and flavoniods present in plants inhibit the mechanism that produce free radicals which may leads to degenerative disease [13]. Largely, plants containing phenolic compounds are most powerful natural antioxidants and are highly valued for their antioxidant, anti-ageing anti-microbial effects. Antioxidants have been widely used as ingredients in dietary supplements and exploited to maintain health and prevent oxidative stress-mediated diseases [14, 15, 16].

Several studies on the extracts of *Crateva adansonii, Hymenocardia acida and Pterocarpus erinaceus* have been reported for their various medical uses which include treatment of ear infections, syphilis, jaundice, yellow fevers infections [3, 17, 18, 19, 20]. These plants have also been reported to have anti-microbial, anti-trypanosoma, anti-inflammatory, and anti-sickling properties [21, 22, 17, 23]. This paper evaluated the phytoconstituents and comparative antioxidant activity of *C. adansonii, H. acida and P. erinaceus*.

MATERIALS AND METHODS

Plant Materials

The plant materials were selected on an ethno pharmacological basis. The stem bark of *Hymenocardia acida*, leaves of *Crateva adansonii* and *Pterocarpus erinaceus* were collected in July 2017, from Bosso Local Government area in Niger State, Nigeria. They were identified by a Botanist in the Department of Plant Biology, Federal University of Technology Minna, Nigeria.

Preparation of Crude Extracts

The fresh leaves of *C. adansonii, P. erinaceus* and stem bark of *H. acida* were washed with clean-water and air dried at

room temperature (28° C – 30° C) for one week, pulverized into powder using a grinder mill and stored in an air-tight containers until required. One hundred gram (100g) of the powdered plant sample was extracted under reflux with methanol at 45˚ C for 2 hours. The extract was filtered using muslin cloth and Whatman (No.1) filter paper, and the resulting methanol extract was concentrated in a water bath (40^0C) and the percentage yield determined using the formula below:

$$\text{P (\%)} = \frac{\text{Weight(g)} of\ the\ conc. extract}{weight\ (g) of\ the\ sample\ leaf} X100$$

Where P= percentage yield

The dried extract was stored in a refrigerator until required.

Phytochemical Analysis

Qualitative Screening

Chemical tests were carried out on methanolic extracts of *Crateva adansonii, Hymenocardia acida* and *Pterocarpus erinaceus*, to identify the secondary metabolites using standard procedures as described by [24, 25, 26, 27].

Test for Flavonoids

Exactly 0.1g of the extract was heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 mins. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration indicates the presence of flavanoids [24, 25].

Test for Terpenoids

Exactly 0.2g of each plant methanol leaf extract was mixed with 2ml of chloroform, and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface formed, which shows positive result for the presence of terpenoids [24, 25].

Test for Tannins

Exactly 0.5g of the extract was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added to the filtrate and observed for brownish green or blue black colouration [24, 25].

Test for Cardiac Glycosides (Keller-killani test).

Exactly 0.5g of each plant extract was mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by the addition of 1 ml concentrated sulphuric acid. Brown ring formed at the interface indicated deoxy-sugar characteristics of cardenoloides. A violet ring may appear beneath the brown ring, while in the acetic acid layer; a greenish ring may also form just gradually throughout the thin layer [24, 28].

Test for Reducing Sugars (Benedict Test)

Exactly 0.5 g of the plant extract was mixed thoroughly with 30ml of benedicts reagents and placed in a boiling water bath for 5 minutes. The formation of a brick red precipitate indicates reducing sugar [24, 28].

Test for Alkaloids

Exactly 0.5g of extract was stirred with 5 ml of 1% aqueous HCl in steam bath, few drops of picric acid solution was added to 2 ml of the extract. The formation of reddish brown precipitate was taken as a preliminary evidence for the presence of alkaloids [26, 28].

Test for Anthraquinones (Borntrager's test)

Exactly 0.5g of plant extract was shaken with 5ml chloroform, the chloroform layer was filtered and 0.5ml of 10% ammonia was added to the filtrate. The mixture was shaken thoroughly, the

formation of pink/violet or red, yellow colour in the ammonia phase indicates the presence of anthraquinones [24, 28].

Test for Steroids (Salkowski reaction)

Exactly 0.5g of methanol extract of each plant was mixed with 2ml of chloroform and a few drop of conc. H_2SO_4 was added to form a layer. Reddish colour observed at interphase indicates the presence of steroidal ring.

Test for Saponins

Exactly 2.0g of the extract was boiled in 20ml of distilled water in a test tube of boiling water bath and filtered. 10ml of the filtrate was mixed with 5ml distilled water and was shaken vigorously to the formation of a stable froth. The froth was mixed with drop of olive oil and was shaken vigorously for the formation of emulsion thus a characteristic of saponins [24, 28].

Quantitative Phytochemical Screening of Methanol Extracts of *Hymenocardia acida,* Stem bark*, Crateva adansonii* and *Ptericarpus erinaceus* Leaves.

Determination of Total Phenol

The total phenolic contents of each sample were determined using a Folin–Ciocalteu assay method [29, 30] and the absorbance measured at 765nm. The reaction mixture contained 100µg/ml of plant extracts, 0.5 ml of the Folin–Ciocalteu reagent (1:10v/v), and 1 ml of 10 % sodium carbonate and 7.5 ml of distilled water. After 45 minutes of reaction at ambient temperature, the absorbance at 765 nm was measured using a UV-visible spectrophotometer (Shimadzu UV-1800 series). A calibration curve of various concentration (0.016, 0.008, 0.004, 0.002 and 0.001 mg/ml, r^2= 0.995) of gallic acid as standard was plotted. The results were expressed on a dry weight basis (dw) as mg gallic acid equivalents (GAE), per 100 g of dry sample.

Determination of Total Flavonoid

Total flavonoid was determined using aluminum chloride colorimetric method [31]. Quercetin was used as the standard for the calibration curve. Exactly 100µg/ml of the diluted sample was added into test tube containing 1.5ml of methanol. 0.1ml of 10% $AlCl_3$ solution and 0.1ml sodium acetate ($NaCH_3COO^-$) and 2.8ml of distilled water. After incubation at room temperature for 30min, the absorbance of the reaction mixture was measured at 415nm using spectrophometer (Shimadzu UV-1800 series). The amount of 10% $AlCl_3$ was substituted by the same amount of distilled water in blank.

Determination of Phytic Acid Content

The phytic acid content was determined using a modified indirect colorimetric method of [32]. The method depends on an iron phosphorus ratio of 4:6 and is based on the ability of standard ferric chloride to precipitate phytate in dilute HCI extract of the sample. 5g of the sample was extracted with 20ml of 3% trichloroacetic acid and filtered. 5ml of the filtrate was used for the analysis; the phytate was precipitated as ferric phytate and converted to ferric hydroxide and soluble sodium phytate by adding 5ml of IM NaOH. The precipitate was dissolved with hot 3.2M HNO_3 and the absorbance was taken immediately at 480nm. Preparation of standard curve for phytic acid was done as follows: standard curve of different Fe $(NO_3)_3$ concentrations was plotted against the corresponding absorbance of spectrophotometer to calculate the ferric iron concentration. The phytate phosphorus was calculated from the concentration of ferric iron assuming 4:6 iron: phosphorus molar ratio.

Antioxidant Assay

DPPH Radical Scavenging Activity

DPPH radical scavenging activity was carried out using the method of [33]. To 1.0 ml of 100.0 µM DPPH solution in methanol, equal volume of the test sample in methanol of different concentration (50,100, 150, 200 & 250 µg/ml) was added and incubated in dark for 30 minutes. The change in coloration was observed in terms of absorbance using a spectrophotometer (Shimadzu UV-1800) at 517 nm. 1.0 ml of methanol instead of test sample was added to the control tube. The different concentration of ascorbic acid was used as reference compound. Percentage of inhibition was calculated using the equation below:

$$\text{PI}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of } test}{Absorbance\ of\ control} X100$$

Where PI = Percentage of inhibition

IC_{50} value were calculated using Graph pad prism 5.0.

Determination of Lipid Peroxidation Capacity of the Plant Extracts

Lipid peroxidation activity of plant extracts were evaluated by thiobarbituric acid reactive substances (TBARS) assay following the method of [36]. With slight modifications where ferrous sulfate used to activate lipid peroxidation and butanol was used in place of n-butanol and pyridine solution. The egg yolk homogenate (10% *v*/v, in ultrapure distilled water) was used as lipid rich media. The different concentrations (125–2000 µg/ml) of plant extracts were prepared from stock solution. The plant extract (50 µl) was mixed with egg homogenate (250 µl) in test tube and volume was adjusted up to 500 µl by distilled water. Finally, ferrous sulfate (25 µl, 0.07 M) was added to reaction mixture and incubated at 25 °C for 30 min to induce lipid peroxidation. Following the incubation period, 750 µl of acetic acid (20% *v*/v, pH 3.5), 750 µl of thiobarbituric acid (0.8% *w*/*v* prepared in 1.1% *w*/*v* sodium dodecyl sulfate) and 25 µl of trichloro acetic acid (20% *w*/*v*) were added to reaction mixture and incubated further for 60 mins in boiling water bath. After cooling, 1-butanol (3 ml) was added and was centrifuged (Centifuge 80-2 made by Life assistance U.K) at 940×g for 10 min. The upper organic layer was used to measure the absorbance at 532 nm by UV- visible spectrophotometer (Shimadzu UV-1800 series). Lipid peroxidation inhibition activity was evaluated using the non-linear regression functionality in Graph Pad Prism (dose-response analysis).

Data Analysis: Data were subjected Analysis of Variance (ANOVA) SPSS 21.0, 2013 to compare the mean while ducan multiple range was used to separate the means where significant differences were observed. Data were expressed as mean and Standard Error of Mean (±SEM) of triplicate determinations. Significant was considered at $p < 0.05$.

RESULTS

Table 1 shows the result of percentage yield of extracts expressed in g/100g. It was observed that *Crateva adansonii* had the highest yield, with 23.5 g per 100 g of sample followed by *Pterocarpus erinaceus* with a yield of 16.57 g and *Hymenocardia acida* with 13.68 g per 100g.

Table 2 shows results of qualitative phytochemical screening of methanol extracts of *Hymenocardia acida* stem bark, *Crateva adansonii* and *Pterocarpus erinaceus* leaves. It was observed that flavonoids, tannins, saponins and phenol were present in all the extracts.

Quantitative Phytochemical Analysis of Methanol Extracts of *Hymenocardia acida Stem bark, Crateva adansonii* and *Pterocarpus erinaceus* Leaves

Table 3 shows the quantitative phytochemical contents of the extracts of *Hymenocardia acida, Crateva adansonii* and *Pterocarpus erinaceus* expressed in mg/g. It was observed that *Pterocarpus erinaceus* had higher phytochemical content with phenol and flavonoids content of 62.14 $\pm$ 0.02 and 61.3368.31 $\pm$ 0.03 respectively while *Hymenocardia acida had higher* phytate content of 9.39 $\pm$ 0.03 and *Crateva adansonii had the least content of all the phytochemical tested.*

Table 1: Percentage yield of plant samples

Plant sample	% yield
Hymenocardia acida	13.68
Crateva adansonii	23.5
Pterocarpus erinaceus	16.57

Table 2: Qualitative Phytochemical Constituents of Methanol Extract of *Hymenocardia acida* Stem bark, *Crateva adansonii* and *Pterocarpus erinaceus* Leaves

Compound screened	*Hymenocardia acida*	*Crateva adansonii*	*Pterocarpus erinaceus*
Alkaloids	+	+	_
Flavonoids	+	+	+
Saponins	+	+	+
Phenols	+	+	+
Steroids	+	+	+
Terpernoids	+	+	_
Tannins	+	+	+
Cardiac glycoside	_	+	_

Key: - Not detected + Detected

Table 3 : Quantitative Phytochemical Analyses of Methanol Extracts of *Hymenocardia acida* Stem bark, *Crateva adansonii* and *Pterocarpus erinaceus* Leaves

Compound screened for	*Hymenocardia acida* (mg/g)	*Crateva adansonii* (mg/g)	*Pterocarpus erinaceus (mg/g)*
Phenol	$62.14^{a} \pm 0.02$	$61.33^{a} \pm 0.03$	$75.02^{a} \pm 0.04$
Phytate	$9.39^{b} \pm 0.03$	$3.97^{b} \pm 0.02$	$7.4^{b} \pm 0.05$
Flavonoid	$52.62^{c} \pm 0.03$	$55.48^{c} \pm 0.06$	$68.31^{c} \pm 0.03$

Data are express in the average of triplicate determinations ± S.E.M. Values with different letters are significantly different at $p < 0.05$.

DPPH Antioxidant Activity

The DPPH scavenging activity of methanol extracts of *H. acida* stem bark, *C. adansonii* and *P. erinaceus* leaves (Figure1) showed that *H. acida* methanol stem bark extract had significantly ($p < 0.05$) higher DPPH scavenging activity followed by *P. erinaceus* methanol leaf extract and then methanol leaf extract *C. adansonii* had the least DPPH scavenging activity.

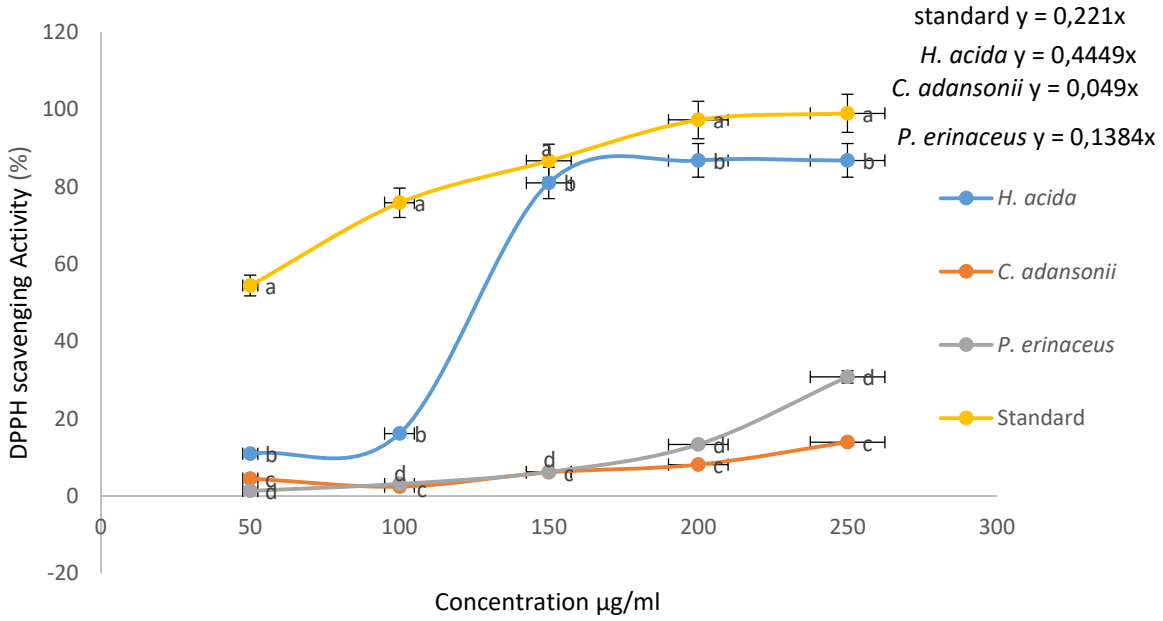

Figure 1: DPPH scavenging activity of methanol extracts of *H. acida* stem bark, *C. adansonii* and *P. erinaceus* leaves.
*Values with different letters are significantly different at $p < 0.05$

Inhibition of Lipid Peroxidation of Extracts

Figure 2 showed that the percentage inhibition of lipid peroxidation of methanol extracts of *H. acida* stem bark was observed to be significantly higher ($P < 0.05$) than standard ascorbic acid and followed by *Crateva adansonii* methanol leaf extract and *Pterocarpus erinaceus* methanol leaf extract had the least percentage inhibition of lipid peroxidation.

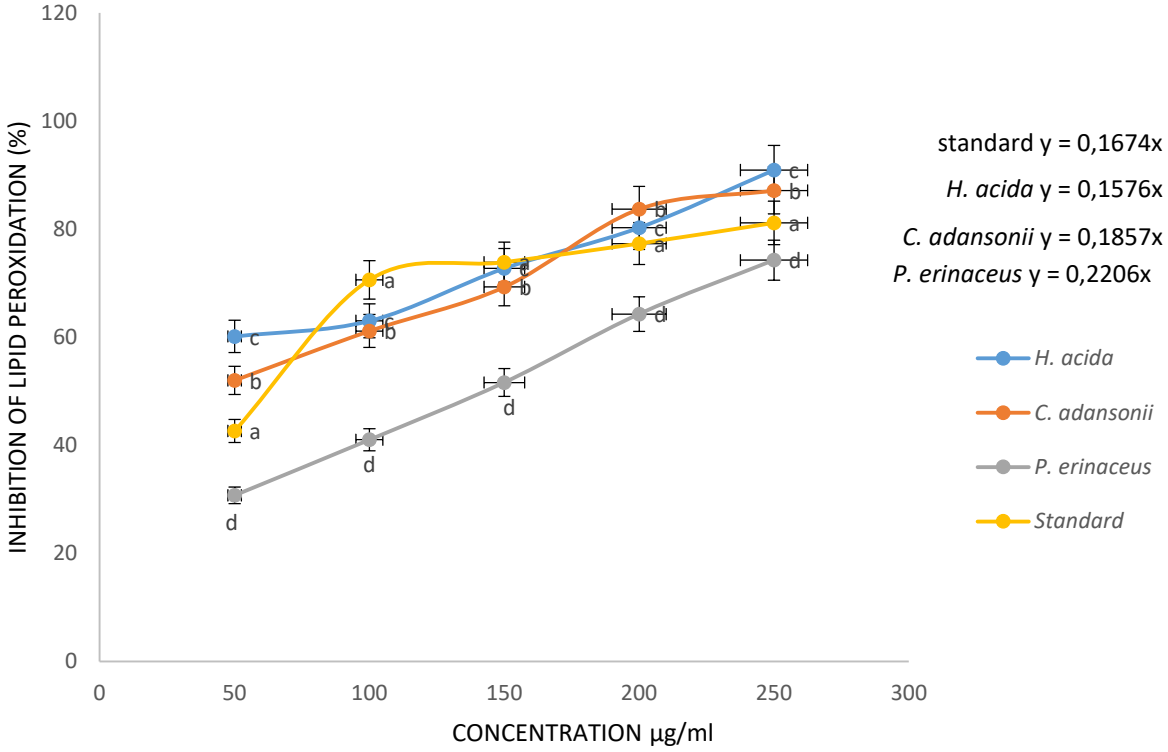

Figure 2: Percentage inhibition of lipid peroxidation methanol extracts of *Hymenocardia acida* stem bark*, Crateva adansonii* and *Pterocarpus erinaceus* leaves

Values with different letters are significantly different at $P < 0.05$

DISCUSSION

The percentage (%) yield result revealed that the methanol extract of *Crateva adansonii* gave the highest yield followed by *Pterocarpus erinaceus* and *Hymenocardia acida* which could be due to plant part variation. Qualitative screening of the extracts revealed the presence of phenols, tannins, flavonoids, and saponins in all the plant extracts, which gives credence to the use of these plants in traditional medicine. Quantitatively, phenol and flavonoids were found to be the most abundant in *Pterocarpus erinaceus* while phytate was found to be highest in *Crateva adansonii.* Flavonoid and phenolic compounds can exert protective mechanism and prevention of heart related diseases through the inhibition of cyclooxygenase and lipooxygenase activities of macrophages [35]. They are free radical scavengers, super antioxidants which prevent oxidative stress and prevent other degenerative

diseases [36]. Phenolic compounds retard lipid peroxidation with their most important role serving as defense against pathogens and therefore may be used in human pathogen control [37]. The antioxidant effect exerted by these extracts may be due to the presence of flavonoids and phenolic contents.

A large number of plants have been shown to contain phytochemicals which are viable sources of antioxidants that acts by upsetting the molecular events involved in the initiation, promotion and progression of degenerative diseases [16]. The free radical scavenging ability of the extracts was determined using DPPH photometric assay. It was found that *Hymenocardia acida* has the highest reductive potential at 50 % inhibition of DPPH scavenging activity (IC_{50}=128.83 µg/ml) compared to other plants and significantly lower than standard (101.83 µg/ml).

The *Hymenocardia acida* stem bark extract showed the highest percentage lipid peroxidation inhibition at all concentrations except at a concentration of 200 µg/ml where *Crateva adansonii* is highest (83.70 $\pm$ 0.05 %). Although this study showed that methanol extracts of *Hymenocardia acida,* has higher antioxidant properties compared to *Crateva adansonii* and *Pteriocarpus erinaceus*, this finding was consistent with [38], who previously reported the strong reductive capacity of phenol-rich extracts. The three plant extracts showed antioxidant activity and could be a source of antioxidants for the prevention of degenerative disease associated with oxidative stress.

CONCLUSION

From the results of the study, it could be concluded that plants with higher phenol and flavonoid content had better *in vitro* antioxidant properties. The demonstrated *in vitro* antioxidant and antilipid peroxidation effects of all the plant extracts; methanol extracts of *Hymenocardia acida, C. adansonii and Pteriocarpus erinaceus* may be the rationale behind some of its folkloric uses and also may be responsible for some of its pharmacological effects.

REFERENCES

1. Rasool, H.A. (2012). 'Medicinal Plants (Importance andUses)'. *Journal of Pharmaceutical Anlytical Acta,* 3: 10-11.

2. Madaki, F. M., Kabiru, A. Y., Ogbadoyi, E.O., Busari, M.B. &Hassan Maishera (2016). Anti-trypanosomal Activity of Methanol Extract of *Chamaecrista mimosoides* leaf in *Trypanosoma brucei* Infected Mice. *World Journal of Pharmacy and Pharmaceutical Sciences*;5(6) 196-208.

3. Akanji, M. A., Salau, A. K. & Yakubu, M. T. (2013). 'Safety evaluation of aqueous extract of *Crateva adansonii* leaves on selected tissues of rats'. *Fountain Journal of Natural and Applied Sciences*, 217-28.

4. Abu, A.H. & Uchendu, C.N. (2011). '*In vivo* trypanocidal activity of hydroethanolic extract of *Hymenocardia acida* stem bark in rats'. *Veterinary World*, *4*, 113–116.

5. Akinmoladun, C., Ibukun E.O., Obuotor, E. M. & E. O. Farombi (2007). Phytochemical constituent and antitoxidant activity of extract from leaves *Ocimum gratissimum, Science Research Essay*, 2:163-166.

6. Kushi, L.H., Byers, T., Doyle, C., Bandera, E.V. & McCullough, M. (2006). American Cancer Society Guidelines on Nutrition and Physical Activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. *Journal of Clinical Pathology:* 56: 254-281.

7. Wachtel-Galor, S., Siu, P.M. & Benzie, I.F. (2014). 'Antioxidants, Vegetarian Diets and Aging. In R. V. Preedy (Ed.), Aging: Oxidative Stress and Dietary Antioxidants. 81-91.

8. Badarinath, A. V., Mallikarjuna, K. R., Madhu, S. C., Ramkanth, S., Rajan, T. V. & Gnanaprakash, K. (2010). A review on in-vitro antioxidant methods: comparisions, correlations and considerations. *International Journal of PharmaTech Research*, 2(2), 1276-85.

9. San, S., Chakraborty, R., Verma, A., De, B., Devanna, N., & Dey, K. B. (2014). Evaluation of antioxidants activities of *Saraca indica* leaves extracts using *in vitro* and ex *vivo* models. *Journal of Scientific and Industrial Research*, 73: 157-62.

10. Shad, M. A., Nawaz, H., Rehman, T., & Ikram, N. (2013). 'Determination of some biochemical, phytochemicals and antioxidant properties of different parts of *Cichorium intybus L*: A comparative study. *The Journal of Animal and Plant Sciences*, 23(4):1060-6.

11. Sies H. & Stahl W. (1995). "*Withania somnifera*, vitamin E and C, [beta]-carotene and other carotenoids as antioxidants. *American Journal of Clinical Nutrition*, 62, 1315S – 1321S.

12. Mandal, S., Yadav, S., Yadav, S. & Nema, R. K. (2009). 'Antioxidants: a review'. *Journal of Chemical and Pharmaceutical Research*. 1(1): 102-104.

13. Maestri, D.M., Nepote, V., Lamarque, A.L. & Zygadlo, J.A. (2006). 'Natural products as antioxidants. In Imperato, first Edition. *Phytochemistry: Advances in Research,* 105-135.

14. Liu, H., Qiu, N., Ding, H.H., Yao, R.Q (2008). Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Research Int.*, 41: 363-370.

15. Halliwell, B., Gutteridge, J.M.C. (2007). 'Free Radicals in Biology and Medicine'. Fourth Edition, Oxford University Press, Oxford, UK; Pp45-152.

16. Gul M. Z., Ahmad .F, Kondapi, A. K., Qureshi I.A. and Ghazi I.A (2013). Antioxidant and Antiproliferative activities of Abrus precatorius leaf extracts- An *in vitro* study, *BMC Complement Alternative Medicine,* 13:53.

17. Agboke, A., Attama, A. and Momoh, A. (2011). 'Evaluation of the antimicrobial properties of crude extract of *Cryptolepis sanguinolenta* and *Crateva adansonii* leaves and their interactions'. *Journal of Applied Pharmaceutical Science*, 1(10):85-89.

18. Hoet, S., Opperdoes, F. Brun, R. Adjakidje, V. & Quetin-Leclercq. J. (2004). *Journal of Ethnopharmacology*. 2(3); 201-208

19. Muanza, D. N., Dangala, N.L. & Olingo M. (1995). 'Antibacterial and antifungal activities of nine medicinal plants from Zaire'. *International Journal of Pharmacognosy,* 32(4): 337.

20. Burkill, H.M (1985). 'Families A–D. In *The Useful Plants of West Tropical Africa*; Royal Botanic Garden, Kew Publishing: London, UK, 1: 960.

21. Igoli, N.P., Gray, A.I., Clements, C.J., Igoli, J.O., Nzekwe, U. & Singla, R.K. (2012).'Scientific Investigation of Antitrypanosomal Activity of *Crateva adansonii* DC Leaves Extracts'. *Indo Global Journal of Pharmaceutical Science*. *2*: 226–229.

22. Mann, A. & Ogbadoyi, E. O. (2012). Evaluation of Medicinal Plants from Nupeland for Their in vivo Antitrypanosomal Activity. *American Journal of Biochemistry*, 2(1): 1-6.

23. Mpiana, P.T., Tshibanga, D.S., Shetonde, O.M. & Ngobula, K.N. (2007). **'**Medicinal use of hymenocardia acida**'.** *Phytomedicine,* 14, 192-195.

24. Harborne, J. B. (1973). Phytochemical methods: *A guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London; Pp49-188.

25. Sofowora, A. (1993): Medicinal plants and Traditional medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria: pp 289.

26. Trease, G.E. & Evans, W.C. (1989). 'Pharmacognosy'. 13th (edition). ELDS/Bailliere Tindall, London: pp345-6, 535-6, 772-3.

27. Oloyede, O.J., Odugbenro, P.O., Abioye, A.O. & Raji, N.L. (2005). "Some physical properties of African star apple (*Chrysophyllum alibidum*) seeds". *Journal of Food Engineering,* 67 (4): 435–440.

28. Harborne, J. B. (1973). Phytochemical methods: *A guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London; 279.

29. Edeoga, H.O., Okwu D.E. & Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *Afrcan Journal of Biotechnology*, 4, 685-688.

30. Makkar, H.P.S., Bluemmel, M., Borowy, N.K., Becker, K. 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. *Journal of Scientific Food and Agriculture*, 61: 161 -165.

31. Chang C, Yang M, Wen H, Chern J (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food Drug Analysis*, 10: 178-182.

32. Wheeler, E. L. and Ferrel, R. E. (1971) A Method for Phytic Acid Determination in Wheat and Wheat Fractions. *Cereal Chemistry*, 48, 312-320.

33. Molyneux, P. (2004). The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity.*Songklanakarin Journal of Science and Technology* 26; 211–219.

34. Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.

Analytical Biochemistry ,95(2):351-8.

35. Smith, H. L., & Rajan, T. V. (2001). Inhibitors of the Lipoxygenase Pathway Block Development of Brugia malayi L3 In vitro. *The Journal of Parasitology,* 87(2) : 242-249.

36. Dillard, C.J., German, J.B. (2000). Phytochemicals: Nutraceuticals and human health. *Journal of Science & Food Agriculture*, 80:1744–56.

37. Khopde, S.M.; Priyadarsini, K.I.; Mohan, H.; Gawandi, V.B.; Satav, J.G.; Yakhmi, J.V.; Banavaliker, M.M.; Biyani, M.K.; MittaL, J.P.(2001).Characterizing the antioxidant activity of amla (*Phyllanthus emblica*) extract. *Current Science*, 81, 2; 85-190.