~~Comparative Phytochemical Screening and~~ Antioxidant Activity of the Fruit and Stem bark of *Tetrapleura tetraptera*

ABSTRACT

~~*Tetrapleura tetraptera* belongs to the family *Mimosaceae*~~. The present investigation was carried out to evaluate the antioxidant and phytochemical activities of ethanol extracts of *Tetraptera* stem bark and fruit. DPPH radical scavenging assay and Fe^{+3} reducing assay were carried out to determine the antioxidant activities of the extracts. The extracts exhibited marked antioxidant activity by scavenging DPPH free radical in a concentration dependent manner. In Fe^{+3} reducing assay, increase in the absorbance revealed the reducing power of the extracts. For the stem bark, the value ranged from 0.393 to 1.641 mg Ascorbic acid equivalent/ml of extract and fruit (0.342 to 1.325 mg Ascorbic acid equivalent/ml of extract. The DPPH for the stem bark ranged from 28.74% to 85.26% while that of fruit ranged from 10.56% to 66.01%. ~~The Preliminary phytochemical analysis of ethanol extract of the stem bark showed the presence of tannins, saponins, flavonoids, glycosides, and anthraquinones while for the fruit extract glycosides and anthraquinones were absent~~.

Key words: *Tetrapleura tetraptera,*% DPPH radical scavenging assay, % Fe^{+3}reducing assay and phytochemical

1.0 INTRODUCTION

Obtaining adequate nutrients from various foods plays a vital role in maintaining normal function of human body. With recent advances in medical and nutrition sciences, natural products and health-promoting foods have received extensive attention from both health professionals and the common population. New concepts have appeared with this trend, such as nutraceuticals, nutritional therapy, phytonutrients and phytotherapy [1,2, 3]. These functional or medicinal foods are maintaining well-being, enhancing health and modulating immune

function to prevent specific diseases. They also hold great promise in clinical therapy due to their potential radiotherapy and significant advantages in reducing the health care cost[4] .The history of plants being used for medicinal purpose is probably as old as the history of mankind. Extraction and characterization of several active phyto-compounds from these green factories have given birth to some high activity profile drugs. The potential natural anticancer drugs like vincristine, vinblastine and taxol can be the best examples[5]. Free radicals are found to be a product of normal phytonutrients or phytomedicines play positive roles in metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc [6;7] . Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin[8]. Antioxidants that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important [9]. Synthetic antioxidants are widely used but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly[8]. The objective of the present investigation was to evaluate the phytochemical and antioxidant potential of different concentrations of the stem bark and fruit of *T.tetraptera.*

2.0 MATERIALS AND METHODS

2.1 Collection of Plant Material: Fresh stem bark and fruit of *Tetrapleura tetraptera* were bought in a local market in Ondo, Nigeria. The plant parts were washed thoroughly 2-3 times with running water and once with distilled water and then air-dried on sterile blotter under shade

2.2 Solvent Extraction: Thoroughly washed plant parts were dried in shade for five days and then powdered with the help of blender. The powdered plant parts were extracted successively with ethanol solvent in Soxhlet extractor for 48 h. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

2.3 Phytochemical Analysis: The ethanol extracts were subjected to preliminary phytochemical screening to screen the presence of various secondary metabolites[10;11].

Reducing property: The reducing property was determined by assessing the ability of extracts to reduce $FeCl_3$ solution as described by Pulido, et al [12]. Briefly, extracts (0-250 µL of stock) were mixed with 250 µL 200mM Sodium phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferrocyanide, the mixture was incubated at 50^{o}C for 20mins, thereafter 250 µL of 10% trichloroacetic acid was added, and subsequently centrifuged at 650rpm for 10mins, 1000 µL of the supernatant was mixed with equal volume of water and 100 µL of 0.1g/100mL ferric chloride, the absorbance was later measured at 700nm. A higher absorbance indicates a higher reducing power.1,1-diphenyl-2 picrylhydrazyl free radical scavenging ability: The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical was evaluated as described by Halliwell, et al.[9]. Briefly, appropriate dilution of the extracts (1mL) was mixed with 1mL of 0.4mM methanol solution containing DPPH free radicals, the mixture was left in the dark for 30mins and the absorbance was measured at 516nm. The DPPH free radical scavenging ability was subsequently calculated.

Scavenging activity (%) = A – B / A x 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

3.0 RESULTS

~~Table 1: Phytochemical analysis~~

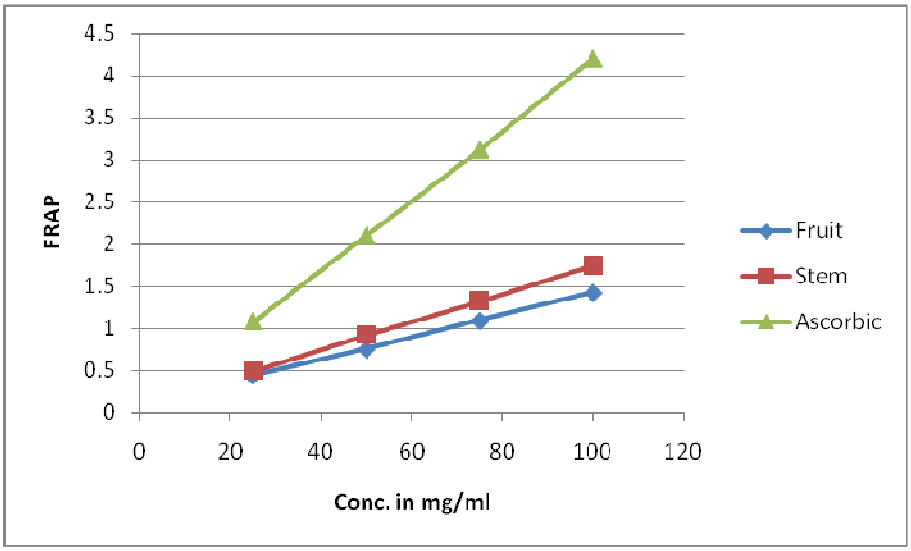
Sample	Alkaloids	Flavonoids	Saponins	Tannins	Glycosides	Anthraquinones
Stem bark	-	+	+	+	+	+
Fruit	+	+	+	+	-	-

Key: +ve = present; -ve = absent.

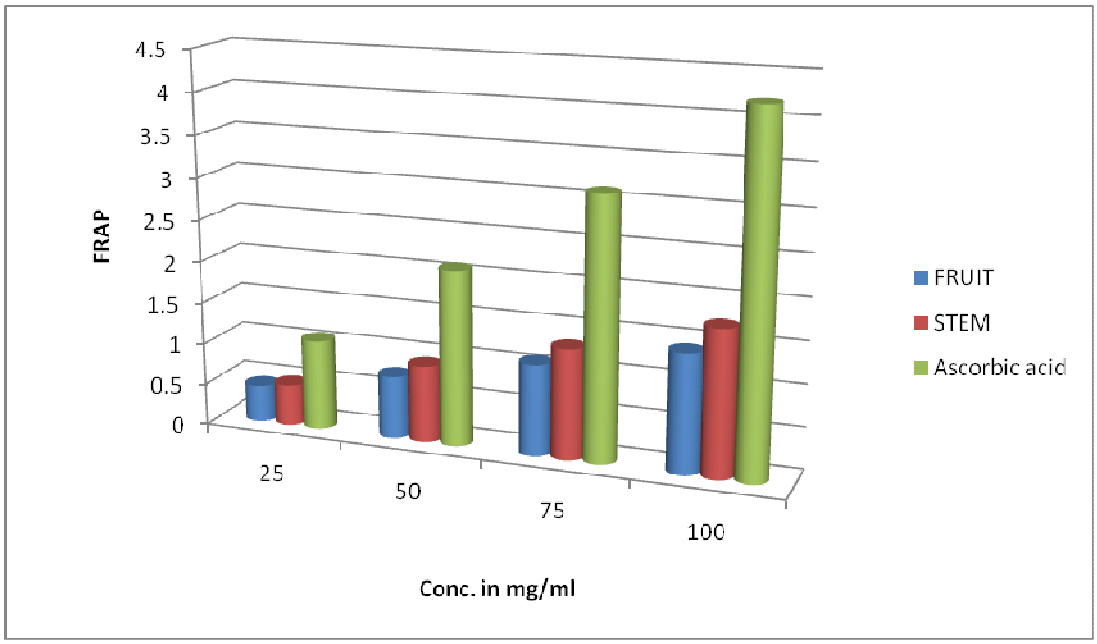
~~Preliminary phytochemical analysis of ethanol extract revealed the presence of tannins, saponins, glycosides, flavonoids and anthraquinones in the stem bark while only alkaloids was absent. The fruit extract contains all except glycosides and anthraquinones. (Table 1)~~

Antioxidant activities

Figure 1: Ferric Reducing Antioxidant Property

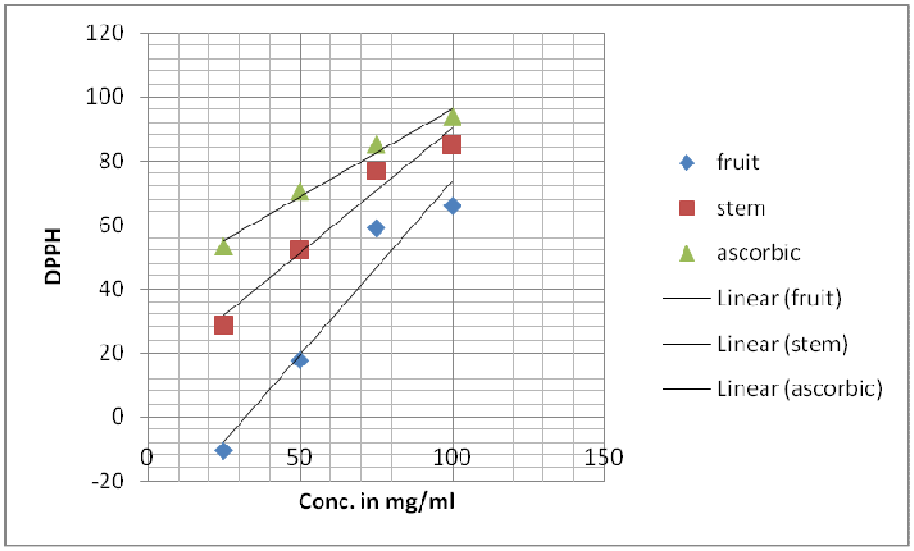


84 Figure 2: Ferric reducing antioxidant property



85

86 Figure 3: DPPH free radical scavenging ability (%)

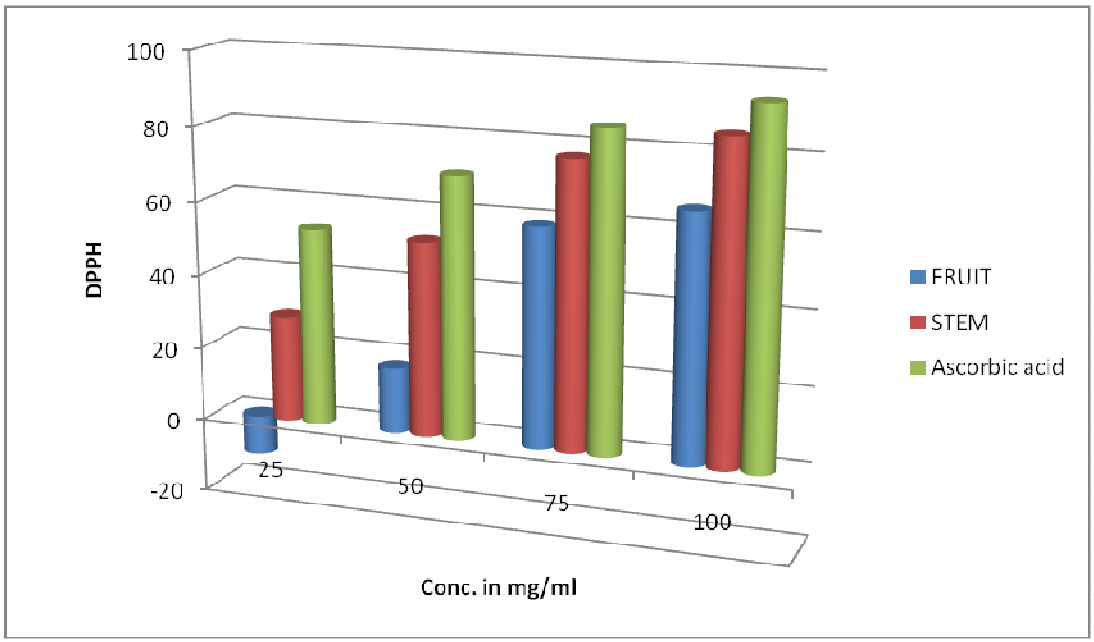


87

88

89

90 Figure 4: DPPH free radical scavenging ability (%)



4.0 DISCUSSION

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural plant origin and natural products play an important role in drug development programs in the pharmaceutical industry [13]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. [14]. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness [15]. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological activities. [16]

Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in

order to attain stability. In recent years much attention has been devoted to natural antioxidant and their association with health benefits [17]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [18;19]. There are several methods available to assess antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid and sensitive method for the antioxidant screening of plant extracts. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.[20] The antioxidant activities have been reported to be the concomitant development of reducing power.[21].

The result of antioxidant activity of different concentrations of ethanol extracts and standard (Ascorbic acid) is shown in Figures 3&4. The extracts exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. The extracts have shown concentration dependent radical scavenging activity, conversely, at 25 mg/ml, the extract of the fruit of *Tetrapleura tetraptera* is pro-oxidant. The scavenging activity of the standard was higher than those of ethanol extracts. Though the DPPH radical scavenging abilities of the extract were less than those of Ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavenger, acting possibly as primary antioxidant. Fe^{+3} reducing power assay was carried out for the measurements of reductive ability of different concentrations of ethanol extracts and standard, ascorbic acid. An increase in the absorbance revealed the reducing power of extracts. In this study, the reducing power of ethanol extracts was found to increase with the dose (Figures 1&2). The reducing capacity of a compound may serve as significant indicator of its potential antioxidant activity.[22].

CONCLUSION

The efficacy of the fruit and stem bark extracts of *T.tetraptera*, may be attributed to the phytochemicals present in the solvent extract. Further studies on isolation of active constituents and their biological activities are to be carried out.

REFERENCES

1 .Bagchi, D.(2006). Nutraceuticals and functional foods regulations in the United States and around the world. *Toxicol*., 221: 1-3.

2.Berger, M.M. and A. Shenkin(2006). Vitamins and trace elements: Practical aspects of supplementation. Nutrition, 22: 952-55.

3.Bland, J.S., (1996). Phytonutrition, phytotherapy and phytopharmacology. *Altern. Ther. Health Med*., 2: 73-76.

4. Ramaa, C.S., A.R. Shirode, A.S. Mundada and V.J. Kadam, (2006). Nutraceuticals- an emerging era in the treatment and prevention of cardiovascular diseases. *Curr Pharm Biotechnol.,* 7: 15-23.

5. Huie, C.W.(2002). A review of modern sample techniques for the extraction and analysis of medicinal plants. *Anal. Bio . Chem.*,373: 23-30.

6.Gutteridge, J.M.(1993). Free radicals in disease processes: a compilation of cause and consequence. *Free Radical Research,* 19: 141-158.

7. Knight, J.A.(1995). Diseases related to oxygen-derived free radicals. Annals of Clinical and Laboratory Sciences, 25(2): 111-121.

8. Rechner, A.R., G. Kuhnle, P. Bremmer, G.P. Hubbard, K.P. Moore and C.A. Rice-Evans, (2002). *Free Radical Biology and Medicine,* 33: 220-235..

9.Halliwell, B., J.M. Gutteridge and C.E. Cross, (1992).Free radicals, antioxidant and human disease: where are we now? 119: 598-620. *J. Laboratory and Clinical Medicine,*

10. Manjunatha, B.K., H.S.R. Patil, S.M. Vidya, T.R.P. Kekuda, S. Mukunda and R. Divakar, (2006). Studies on the antibacterial activity of *Mucunaaeruginosa Monosperma* DC.

156 *Indian Drugs*. 43: 150-152.

157 11.Parekh, J. and S.V. Chanda,(2007). *In vitro* Antimicrobial Activity and Phytochemical

158 Analysis of Some Indian Medicinal Plants. *Turk J. Biol.*, 31: 53-58.

159 12. Pulido, R., Bravo, L., Sauro-Calixo, F. (2000). Antioxidant activity of dietary

160 poluphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric.*

161 *Food chem.*, 48: 3396-3402. DOI PMid: 10956123

162 13. Nair, R., T. Kalariya and S. Chanda, (2005). Antibacterial Activity of Some Selected

163 Indian Medicinal *Flora.Turk. J. Biol.*, 29: 41-47

164 14.Edeoga, H.O., D.W. Okwu and B.O. Mbaebie, (2005)Phytochemical constituents of some

165 Nigerian medicinal plants. *African J. Biotechnol.*, 4(7): 685-688.

166 15. Adwan, G., B. Abu-Shanab, K. Adwan and F. Abu- Shanab, (2006). Antibacterial Effects

167 of Nutraceutical Plants Growing in Palestine on *Pseudomonas Turk. J. Biol.,* 30: 239-242.

168 16.Gupta, M., U.K. Mazumder, R.S. Kumar, T. Sivakumar and M.L.M. Vamsi(2004).

169 Antitumor and Antioxidant status of *Caesalpiniabonducella*against Ehrlich ascites

170 Carcinoma in swiss albino mice. *J. Pharmacological Sci.*, 94: 177-184.

171 17.Ali, S.S., N. Kasoju, A. Luthra, A. Singh,H. Sharanabasava, A. Sahu and U. Bora, (2008).

172 Indian medicinal herbs as sources of Antioxidants. Food Research International, 41: 1-15.

173 18.Cook, N. and S. Samman, (1996). Flavonoids- Chemistry, metabolism, cardioprotective

174 effects and dietary sources. *The J. Nutritional Biochem.* 7(2): 66-76.

175 19. Kumpulainen, J.T. and J.T. Salonen, (1999). Natural Antioxidants and Anticarcinogens in

176 Nutrition, Health and Disease, The Royal Society of Chemistry,UK, pp: 178-187.

177 20. Koleva, I.I., T.A. Vanbreek, J.P.H. Linssen, A.D.E. Groot and L.N. Evstatieva, (2002).

178 Screening of plant extracts for antioxidant activity: A comparative study on the three testing

179 methods. *Phytochem. Anal.*, 13: 8-17.

180 21.Yang, J.H., H.C. Lin and J.L. Mau, (2002). Antioxidant properties of several commercial

181 mushrooms. Food Chemistry, 77: 229-235.

182 22. Meir, S., J. Kanner, B. Akiri and S.P. Hadas, (1995).Determination and involvement of

183 Aqueous reducing compounds in Oxidative Defence systems of various senescing Leaves.

184 *J. Agric. Food Chem.*,43: 1813-1817.