



PHYTOCHEMICAL STUDIES AND EFFECT OF ETHANOIC, METHANOIC AND AQUEOUS EXTRACTS OF *Tetrapleura tetraptera* FRUIT

*Adebayo, Masaudat A., ²Asimi Tajudeen, ¹Adegbola M.V., ¹Gbadamosi T. K., ¹Suleiman I.O., ²Rasheed O.M.

¹Department of Biochemical Sciences, School of Science and Technology, Federal Polytechnic Ede, Nigeria.

²Department of Chemical Sciences and Technology, School of Science and Technology, Federal Polytechnic Ede, Nigeria.

* E-mail of the corresponding author: adeola.adebayo@federalpolyede.edu.ng, smdeola@gmail.com

ABSTRACT-*This study focuses on the phytochemical analysis of Tetrapleura tetraptera fruits. Oxidative stress is considered as an imbalance between pro and antioxidant species, which results in molecular and cellular damage. Hence there is need to study the content of the Tetrapleuratetraptera and compared it with other biological herbs' contents to know if the plant worked in curing the oxidative stress in Albino rats. The medicinal value of this plant lies in the bioactive phytochemical constituents that produce certain physiological action on the human body. The aim of this research was to investigate the phytochemical contents of Tetrapleura tetraptera, also to evaluate the phytochemical constituents of ethanol, methanol and aqueous extracts, To investigate the free radicals activities such as ABTS and DDPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activities of Tetrapleura tetraptera were also determined. The study showed that the extract contained alkaloids, flavonoids, saponins, tannins, and phenols. In addition, the extract exhibited significant antioxidant and anti-inflammatory properties. . The results suggest that Tetrapleura tetraptera extract have potential therapeutic benefits against oxidative stress-induced organ damage.*

Keywords: phytochemical, *Tetrapleura tetraptera*, oxidative stress.

INTRODUCTION

1.1 TETRAPLEURA TETRAPTERA

Tetrapleuratetraptera is a flowering plant in the pea family native to Western Africa. It belongs to the order fabales and family fabaceae. It is a deciduous tree commonly known as Aidan tree (*Uyoh et al., 2013*). *Tetrapleuratetraptera* is a deciduous, single- stemmed, robust perennial tree with grey/brown, smooth/rough bark, of about 30 m high. The plant has yellow/pink to orange flowers with sessile, glabrous leaves, spreading branches and naturally distributed in the rainforest belt of West, Central and East Africa. The fruit of the plant is shiny, dark – brown, glabrous, slightly curved, with four longitudinal, wings – like ridges and small, brownish – black seeds. Two wings are woody, while the other two filled with soft, sugary pulp, oily and aromatic. It is green when tender and darker brown when fully ripe. The plant belongs to the family of Fabaceae (formerly Leguminoceae: Mimosaceae .(*GmelinR, et al, 1967*) and locally called different names by different ethnic groups in Nigeria. It is known as Aidan in English, Osakirisa or Oshosho in Igbo, Aridan in Yoruba, Dawo in Hausa, Abogolo in Igala, Osirisa in Ikwo dialect. (*Aladesanmi, et al, 2007*). There has been an appreciable increase in research on bioactivity of natural products. The biological aspects most researched are antimicrobial, molluscicidal, insecticidal, parasitic, toxicity tests and anti-tumour in decreasing order while advancing a look at African endemic and often neglected diseases such as malaria, leishmaniasis, schistosomiasis, pneumonia, lymphatic filariasis and ochacerciasis, African trypanosomiasis and chargas disease, leprosy, dergue and tuberculosis(*AdelajaBA, et al, 2012*). The use of modern drugs for the treatment of trematode infection has been like a burst of sunshine after a long and particularly dreary night. Where there had been, for half a century, a trickle of drugs of modest efficacy and awesome toxicity, there was the rapid discovery of several excellent medications. In which the outstanding among

them is *Tetrapleuratetraptera* (Aidan plant). Also, in West Africa, *Tetrapleuratetraptera* is mainly used as a spice, medicine and as dietary supplement rich in vitamins (Osei-Tutu et al., 2010). The extract of this plant is known for its anti-inflammatory properties and this advocates its inhibitory impacts against certain human pathogens.

The presence of bioactive substances (alkaloids, flavonoids, saponins, tannins, phenols, and glycosides) in the species is what gives it its therapeutic properties. (Okwu 2003). According to (Ojewole and Adewunmi 2004), *Tetrapleuratetraptera* fruit has anti-arthritis, anti-inflammatory, and anti-diabetic effects. Additionally, the use of *Tetrapleuratetraptera* in treating schistosomiasis, a chronic parasitic condition brought on by blood flukes, was mentioned by (Aladesanmi, 2007) and (Soladoye et al. (2014). (trematode worms). The critical dietary micronutrients contained in the dried fruit, such as iron and zinc, are what provide *T. tetraptera* its nutritious qualities (Akin-Idowu et al. 2011;) “(Uyoh et al. 2013).

Different solvents extracts of the fruit have been proved to have hypolipidaemic and hypokalaemic effects. The continuous availability of the fruit is threatened by extinction as a result of over exploitation. This then begs for conservation and breeding efforts on this plant. Moreover, (Uyoh et al 2013). reported highly significant differences in the nutrient composition of *Tetrapleuratetraptera* obtained from different localities in Cross River State, Nigeria. The

objective of this research was to determine proximate and phytochemical composition of *Tetrapleuratetraptera* fruit.

Fig 1.0: *Tetrapleuratetraptera* fruit, (Esther Kemigisha 2018)

Oxidative stress can occur when there is an imbalance of free radicals and antioxidants in the body. The body's cells produce free radicals during normal metabolic processes. However, cells also produce antioxidants that neutralize these free radicals.

In general, the body is able to maintain a balance between antioxidants and free radicals. Several factors contribute to oxidative stress and excess free radical production. The effects of oxidative stress vary and are not always harmful. For example, oxidative stress that results from physical activity may have beneficial, regulatory effects on the body. Exercise increases free radical formation, which can cause temporary oxidative stress in the muscles. the infection or repairs the damaged tissue.

However, oxidative stress can also trigger the inflammatory response, which, in turn, produces more free radicals



that can lead to further oxidative stress, creating a cycle. Chronic inflammation due to oxidative stress may lead to several conditions, including diabetes, cardiovascular disease, and arthritis.

3.0 MATERIALS AND METHODS

3.1 REAGENTS AND APPARATUS

3.1.1 APPARATUS

Apparatus that were used are test-tubes, test-tubes racks, analytical weighing balance, filter paper, clean white handkerchief, mechanical shaker, spatula, measuring cylinder, mortar and pestle, water bath, spectrophotometer, beakers, universal bottle, plain bottle, EDTA bottle, pH meter, Wistar Albino rats, stirrer, funnel, and dissecting kits.

The materials used were: Rat pellet,

3.1.2 REAGENT

Methanol, Ethanol, Granulated Tetrapleuratetraera, distilled water, DPPH, ABTS, Randox (ALP, ALT, AST, ALB) Sodium Phosphate Monobasic and Sodium Phosphate Dibasic, Ferric chloride, Hydrochloric Acid, Tetraoxosulphate(iv) Acid, Chloroform, Aqueous ferric chloride, Ammonium acetate, Distilled water, Sodium Hydroxide, Phosphate buffer (pH 7.4), Mayer's reagent(Potassium mercuric iodide), Glacial acetic acid.(ALP, ALT, AST, DPPH, ABTS, Creatinine) were purchased from Octopus.

METHODOLOGY

3.2 PREPARATION OF *TETRAPLEURA TETRAPTERA* EXTRACT

Identification and preparation of plant Materials Dry fruits of Tetrapleuratetraera plants were purchased from herb sellers at Oje Market Ede, Osun State Nigeria. The sample of the plant specimen was identified. The dry fruit of Tetrapleuratetraera plants was sun-dried for 4 weeks and subsequently dried (after which the lightly coloured part of the fruit turned to dark red) and ground into fine powder using an electric blender.

20g of the sample (Tetrapleuratetraera) was weighed from the powdered sample and dissolved in 200ml of distilled water and it was shaken with the aid of mechanical shaker 1hour at 1000rpm. Subsequently, appropriate concentrations of the extract were made by dilution with distilled water into 120-180mg/kg body weight and weight and administered to the rats.

3.2.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Phytochemical screening study for the active materials were carried out for extracts using the methods described with some modifications.

Test for Flavonoids

2ml of Tetrapleuratetraera extract was pipetted into test-tubes, 6 drops of sodium hydroxide(NaOH) solution was added into each test-tube and 3ml of tetraoxosulphate(iv) acid(H₂SO₄) was then added and orange yellow color was observed which is a positive test for Flavonoids.

Test for Alkaloids

2ml of Tetrapleuratetraera extracts was dissolved in 5 ml of 1% dilute hydrochloric acid and filtered. Filtrate was treated with Mayer's reagent (Potassium mercuric iodide). The formation of a yellow colored precipitate gave a positive result for alkaloids in the extracts.

Test for Terpenoids (Salkowski's test)

To 2ml of Tetrapleuratetraera extracts, 0.5 ml of chloroform was added followed by 1ml of concentrated sulphuric acid. The formation of reddish-brown precipitate gave an indication of the presence of terpenoids in the extracts.

Test for Tannins (Ferric chloride test)

A volume of 2ml of Tetrapleuratetraera extracts was mixed with an equal volume of distilled water in a test tubes and three drops of dilute ferric chloride was added. The formation of brownish blue or dark colour gave an indication of the presence of tannins in the extracts.

Test for Steroids (Liebermann-Burchard's test)

2ml of Tetrapleuratetraera extracts was mixed with 2 ml of chloroform. 2 ml of concentrated sulphuric acid was then added to the mixture in a test tube. The appearance of red colour in the lower chloroform layer gave a positive result for steroids in the extracts.

Test for Saponins (Foam test)

2ml of Tetrapleuratetraoptera extracts, 6 ml of distilled water was added and shaken vigorously in a graduated cylinder for 15 min lengthwise. The formation of bubbles or persistent foam for 10 min gave an indication of the presence of saponins in the extracts.

Test for Phenols (Ferric chloride test)

To 2ml of Tetrapleuratetraoptera extracts, 2 ml of 5% aqueous ferric chloride was added. The formation of bluish colour gave a positive result for phenols in the extracts.

3.7.4 DETERMINATION OF DPPH (2, 2-diphenyl-1-picrylhydrazyl) RADICAL SCAVENGING ACTIVITY

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang et al. (2001). The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517nm. Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1, 1 Diphenyl 2- Picryl Hydrazyl is a stable (in powder form) free radical with red color which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (HA) can be written as, $DPPH-H + (A) \rightarrow (DPPH) + (H-A)$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1mM of DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

WORKING PROCEDURE

Different volumes (2 - 20 μ l) of plant extracts were made up to 40 μ l with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the plant extracts was calculated using the following formula,

$$\%RSA = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

Where, RSA is the Radical Scavenging Activity; Abs control is the absorbance of DPPH radical + ethanol; Abs sample is the absorbance of DPPH radical + plant extract.

3.7.5 DETERMINATION OF ABTS (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

The ABTS radical scavenging activity was assessed using a method described by Yu et al. (2013) with modifications. The ABTS solution was prepared by mixing an equal volume of a 7 mmol/L ABTS stock solution with a 2.45 mmol/L potassium persulfate solution.

The mixture was then stored in the dark at room temperature for 12–16 h. The ABTS solution was diluted with 10 mmol/L phosphate-buffered saline (PBS, pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 50 μ L of the sample solution was added to 3 mL of the diluted ABTS solution.

The absorbance of the mixture was immediately measured at 734 nm after a 6-min incubation in the dark at room temperature. The control was prepared by replacing the ABTS solution with PBS; the blank was prepared in an identical manner, substituting distilled water for the sample.

The ABTS radical scavenging activity (%) was calculated as $[1 - (A_s - A_c)/A_0] \times 100$, where A_s , A_c , and A_0 are the absorbance values of the sample, control, and blank, respectively.

NOTE:

This is a great tool for calculating Mass Molarity.

ABTS 7mM = 192mg in 50ml

Potassium Persulfate 2.4mM = 32mg in 50ml

SOLUBILITY / SOLUTION STABILITY

ABTS dissolves at 50 mg/mL water to give a clear to very slightly hazy solution that is light yellow green to green in color. Reduced ABTS is colorless, whereas oxidized ABTS is dark green in solution. 5,6 ABTS solutions are sensitive to oxidation, particularly in the presence of heavy metal ions. Solutions should be prepared fresh each day, stored on ice until use. Under these conditions, the solution shows essentially no change in absorbance in ten hours. A solution at room temperature exposed to oxygen and light may turn dark green within an hour or two.

4.0 RESULTS ON PHYTOCHEMICAL STUDIES OF *Tetrapleuratetraptera*

Result for Flavonoids

Aqueous extract of *Tetrapleuratetraptera* with an initial color of amber color shows that there was no color changes before the addition of 6 drops of 2% of sodium hydroxide (NaOH) and 3ml of diluted sulphuric acid to 2ml of the extract (*Tetrapleuratetraptera*). And after the addition of 6 drops of 2% sodium hydroxide and 3ml of diluted sulphuric acid to 2ml of the extracts (*Tetrapleuratetraptera*), there was color change from amber color to orange yellow and lastly to colorless. Effervescence was also observed. This indicates the presence of flavonoids, while the result for methanoic and ethanoic extract of (*Tetrapleuratetraptera*) shows color changes from amber color to deep dark red which also indicates the presence of Flavonoids.

Result for Alkaloids

Aqueous extract of *Tetrapleuratetraptera* shows that there was color changes from brown color to very light yellow then to lemon when 10ml of 1% dilute hydrochloric acid (HCl) was reacted with 2ml of the extract and treated with 25ml of Mayer's reagent. Formation of bubbles was also observed. This indicated the presence of alkaloids. While the results for the methanoic and ethanoic extract show color change from very light yellow to lemon which also indicated the presence of alkaloids

Result for Terpenoids

Aqueous extract of *Tetrapleuratetraptera* with an initial color of brown color shows color changes from brown to white color which was immiscible and formed effervescence and then turned dark red when 2ml of the extract was reacted with 0.5ml of chloroform and 1ml of H₂SO₄. This indicated the presence terpenoids. While the methanoic and ethanoic extract show color change from yellow to amber color which later turns dark red which also indicated the presence of Terpenoids

Result for Saponins

Aqueous extract of *Tetrapleuratetraptera* with an initial color of brown color shows color changes from brown to lemon orange when 2ml of the extract was reacted with 5ml of distilled water and shaken lengthwise for 10mins . Effervescence was also observed. This indicated the presence of saponins, While the methanoic and ethanoic extract also show color change from amber to lemon yellow which also indicated the presence of saponins.

Result for Phenol

Aqueous extract of *Tetrapleuratetraptera* of initial color of brown color shows color changes from brown to yellowish brown when 2ml of the extract was reacted with diluted ferric chloride. This indicated the presence of phenol. The methanoic and ethanoic extract also show color change from amber to reddish-brown (turmeric) which also indicated the presence of phenol.

Result for Steroids

Aqueous extract of *Tetrapleuratetraptera* of 2ml with initial color of brown color shows color changes on reaction with 2ml of chloroform and 2ml of H₂SO₄ from brown color to violet red color at the lower layer of the solution, there was also explosion while adding H₂SO₄. This indicated the presence of steroids. Also the extract of methanol and ethanol show color change from amber to light yellow

Result for Tannins

Aqueous extract of *Tetrapleuratetraptera* of 2ml with initial color of brown shows color changes on reacting it with equal volume of distilled water and 3 drops of ferric chloride solution from brown color to dark colored insoluble precipitate. This indicates the presence of tannins. While the extract of methanol and ethanol also show color change from amber to dark kelp, Which also indicates the presence of tannins.

Result for DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH scavenging activities using Aqueous extract of *Tetrapleuratetraptera* using spectrophotometer the absorbance of 1.890nm was read. The result for DPPH scavenging activities using Methanoic extract of *Tetrapleuratetraptera* the absorbance of 1.836 was read. Also, the result for DPPH scavenging activities of *Tetrapleuratetraptera* using Ethanoic extract of *Tetrapleuratetraptera* the absorbance of 1.878nm was read.

Result for ABTS (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid)

ABTS scavenging activities using Methanoic extract of *Tetrapleuratetraptera* read an absorbance of 2.004nm, the Ethanoic extract also read an absorbance of 2.083nm. Also, the Aqueous extract read absorbance of 2.077nm.

4.1 RESULTS FOR THE PHYTOCHEMICAL SCREENING

TESTS	AQUEOUS		ETHANOIC		METHANOIC	
	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
FLAVONOIDS	+++	NIL	+++	NIL	+++	NIL
ALKALOIDS	+++	NIL	+++	NIL	+++	NIL
TERPENOIDS	+++	NIL	+++	NIL	+++	NIL
TANNINS	+++	NIL	+++	NIL	+++	NIL
STEROIDS	+++	NIL	+++	NIL	+++	NNIL
SAPONINS	+++	NIL	+++	NIL	+++	NIL
PHENOL	+++	NIL	+++	NIL	+++	NIL

DDPH	1.890nm	1.034nm	1.836
ABTS	2.077nm	2.083nm	2.083nm

Table 4.1: Results for the phytochemical Studies of *Tetrapleuratetraptera*

Using a UV-VIS spectrophotometer with Absorbance (Abs) at 517nm.

Keynote:Positive(+)

Negative(-)

Nil(Indifference)

4.3 DISCUSSION

The results of qualitative phytochemical studies of Aqueous, Ethanoic &Methanoic extracts of *Tetrapleuratetraptera*fruits indicated the presence of flavonoids, alkaloids, terpenoids, saponins, phenol, steroids and tannins (Table 4.1). Likewise, the presence of flavonoids, alkaloids, terpenoids, saponins, phenol, steroids and tannins has been detected in the *Tetrapleuratetraptera*fruits. In contrast, something and something were not detected in both methanol and ethanol extracts of the *Tetrapleuratetraptera*fruits.

The result of this study revealed that the administration of the Aqueous extract of *Tetrapleuratetraptera*fruits has medicinal and antioxidant properties in the wistar rats. This present study further confirmed the previous report on the hematinic potentials of *Tetrapleuratetraptera*fruits. The extraction contains flavonoids, alkaloids, and phenolic compounds which are powerful antioxidant. The medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances includes tannins, alkaloids, carbohydrates, triterpenoids, steroids and flavonoids. The medicinal value of *Tetrapleuratetraptera*fruits lies in the bioactive phytochemical constituents that produce certain physiological action on the human body.

Flavonoids (or **bioflavonoids**; from the Latin word *flavus*, meaning yellow, their color in nature) are a class of polyphenolic secondary metabolites found in plants, and thus commonly consumed in the diets of humans, and such as its presence in the extract of *Tetrapleuratetraptera* fruits aid in the prevention of oxidative stress. *Delage B (November 2015)*.

Alkaloids have a wide range of pharmacological activities including antimalarial (e.g. quinine), antiasthma (e.g. ephedrine), anticancer (e.g. homoharringtonine), cholinomimetic (e.g. galantamine), vasodilatory (e.g. vincamine), antiarrhythmic (e.g. quinidine), analgesic (e.g. morphine), antibacterial (e.g. chelerythrine), and antihyperglycemic activities (e.g. piperine). *Roberts, M. F. et al (1998)*.

Many have found use in traditional or modern medicine, or as starting points for drug discovery. Other alkaloids possess psychotropic (e.g. psilocin) and stimulant activities (e.g. cocaine, caffeine, nicotine, theobromine), and have been used in entheogenic rituals or as recreational drugs. Alkaloids can be toxic too (e.g. atropine, tubocurarine). *Kittakoop P, et al (2014)*.

Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke a bitter taste. *Rhoades and David F (1979)*

Tannins (or **tannoids**) are a class of astringent, polyphenolic biomolecules that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids.

The tannin compounds are widely distributed in many species of plants, where they play a role in protection from predation (acting as pesticides) and might help in regulating plant growth. *Richard W. et al (2006)*.

The astringency from the tannins is what causes the dry and puckery feeling in the mouth following the consumption of unripened fruit, red wine or tea. *McGee and Harold (2004)*. Likewise, the destruction or modification of tannins with time plays an important role when determining harvesting times.

Tannins have molecular weights ranging from 500 to over 3,000 (gallic acid esters) and up to 20,000 Daltons (proanthocyanidins). *Bate-Smith and Swain (1962)*.

The result of this research also indicates that the extract of Tetrapleuratetraptera fruits may function as blood booster in anemic condition and this could possibly be as a result of its direct effect on the hematopoietic systems.

Antioxidants are compounds that inhibit oxidation (usually occurring as autoxidation), a chemical reaction that can produce free radicals. Autoxidation leads to degradation of organic compounds, including living matter. Antioxidants are frequently added to industrial products, such as polymers, fuels, and lubricants, to extend their useable lifetimes, *Klemchuk, Peter P. (2000)*.

Food are also treated with antioxidants to forestall spoilage, in particular the rancidification of oils and fats. In cells, antioxidants such as glutathione, mycothiol or bacillithiol, and enzyme systems like superoxide dismutase, can prevent damage from oxidative stress.

The only dietary antioxidants are vitamins A, C, and E, but the term *antioxidant* has also been applied to numerous other dietary compounds that only have antioxidant properties in vitro, with little evidence for antioxidant properties in vivo. Dietary supplements marketed as antioxidants have not been shown to maintain health or prevent disease in humans. *Derek A.J et al., (2021)*.

5.1 CONCLUSION

This study has unveiled that the extract of *Tetrapleuratetraptera* fruits, which has been reported to be commonly used in the traditional systems of medicine, contains an appreciable amount of phytochemicals and also possesses antioxidants. The phytochemical screening tests revealed the presence or absence of many phytochemicals including phenols, flavonoids, alkaloids, tannins, terpenoids, steroids and saponins in the three extracts (aqueous, ethanolic and methanolic) of the *Tetrapleuratetraptera* fruits. It was also discovered from this study that the fruits had

significant antioxidant activity and is therefore recommended for antioxidant role than the whole fruit or seeds. The presence of many active phytochemical compounds and antioxidants in the fruit of *Tetrapleuratetraptera* advocate its antioxidant role and ethno-pharmacological uses in traditional medicine.

RECOMMENDATION

The present investigation focuses on the phytochemical component of *Tetrapleuteratetraptera* with the effect of *tetrapleuteratetraptera* as a protective agent of oxidative stress. Also further research should be made on *tetrapleuteratetraptera* to find out if its contain peptide sequences that are toxic to person, as it was found in wheat, barley and rye and is therefore a safe food for celiac and anaemic patients with increasing interest related to the unique properties of *Tetrapleuteratetraptera*, its value as a food in helping improving human health and to prevent diseases. It has some biological functions such as antioxidant, anti-inflammatory activities and extra effort should be made to introduce *Tetrapleuteratetraptera* as a therapeutic agent to be utilized for treatment of oxidative stress in nephropathy.

Ademiluyi AO, Oboh G, Agbebi OJ, Boligon AA and Athayde ML. (2014). Sorghum

[*Sorghum bicolor* (L.) Moench] leaf sheath dye protects against cisplatin-induced hepatotoxicity and oxidative stress in rats. *J Med Food*.

Adetuyi AO, Akpambang VOE, Oyetayo VO and Aetuyi FO. (2007). The nutritive value And antimicrobial property of Sorghum Bicolor L. stem (POPORO) flour used as food colour and its infusion drink. *Am J Food Technol*, **2(2)**, 79- 86.

Akogou FUG, den Besten HMW, Kayodé APP, Fogliano V and Linnemann AR. (2018). Antimicrobial evaluation of red, phytoalexin-rich sorghum food biocolorant.

Avwioro OG, Owolagba GK, Anibor E, Bankole JK, Oduola T, Adeosun OG, Aloamaka CP (2009). Biochemical observations in Wistar rats fed with the histological dye extracted from Sorghum bicolor. *Int. J. Med. Med. Sci.*, **1(10)**: 464-466.

Avwioro OG, Aloamaka CP, Olabampe OB, Oduola T (2006). Collagen and muscle stain obtained from Sorghum bicolor. *Scand J. Clin. Lab. Invest.* **66**: 161-168

Awika, J.M.; Rooney, L.W.; Waniska, R.D. (2004) Properties of 3-deoxyanthocyanins from sorghum. *J. Agric. Food Chem*, **52**, 4388–4394.

Ayala-Soto FE, Serna-Saldívar SO, García-Lara S and Pérez-Carrillo E. (2014). Hydroxycinnamic acids, sugar composition and antioxidant capacity of arabinoxylans extracted from different maize fiber sources. *Food Hydrocoll*, **35**, 471-475.

Benson KF, Beaman JL, Ou B, Okubena A, Okubena O and Jensen GS. (2013). West African Sorghum bicolor leaf sheaths have anti-inflammatory and immune modulating properties in vitro. *J Med Food*, **16 (3)**, 230–238.

Borokini FB. (2017). Identification and quantification of polyphenols in Sorghum bicolor (L) Moench leaves extract using reverse-phase HPLC-DAD. *Clin Exp Pharmacol*, **7, 2**.

Bukantis R In: Boca Raton, FL. Chevellier A (1980). Energy inputs in sorghum production, (1996). *The Encyclopedia of Medical Plant*. London. Dorling Kindersley Ltd. **p. 103-108**.

Cox, S.; Noronha, L.; Herald, T.; Bean, S.; Lee, S.-H.; Perumal, R.; Wang, W.; Smolensky, D. (2015) Evaluation of ethanol-based extraction conditions of sorghum bran bioactive compounds with downstream anti-proliferative properties in human cancer cell. **5, e01589**.

Dalziel JM (1948). . Duke JA, Wain KK (1981). ‘The useful plants of West Africa’.

Published by the Crown Agents for the colonies, p. 546

- Dia, V.P.; Wang, Z.; West, M.; Singh, V.; West, L.; DeMejia, E.G. (2015) Processing method and corn Cultivar affected anthocyanin concentration from dried distiller's grains with solubles. *J. Agric. Food Chem.* **63**, 3205–3218.
- Dia, V.P.; Pangloli, P.; Jones, L.; McClure, A.; Patel, A. **2016**, Phytochemical concentrations and biological activities of Sorghum bicolor alcoholic extracts. *Food Funct.* **7**, 3410–3420.
- Duodu KG and Awika JM. (2019). Chapter 8: Phytochemical-related health-promoting attributes of sorghum and millets. In sorghum and millets, Second Edition, **225-258**.
- Dykes L, Seitz L, Rooney WL and Rooney LW. (2009). Flavonoid composition of red Sorghum genotypes. *Food Chem*, **116**, 313–317.
- Eisenberg DN, Kessler RC, Foster C, Norlock EE, Calkins DR, Delbano TL (1993). Unconventional medicine in the United States: Prevalence costs and pattern or use. *New England Med. J.*, **328:246-252**.
- Gilchrist, A.K.; Smolensky, D.; Ngwaga, T.; Chauhan, D.; Cox, S.; Perumal, R.; Noronha, L.E.; Shames, S.R. **2020**, High-polyphenol extracts from Sorghum bicolor attenuate replication of Legionella pneumophila within RAW 264.7 macrophages. *FEMS Microbiol. Lett.* 367, 053.
- Gomes, A.; Fernandes, E.; Lima, J.L. **2005** Fluorescence probes used for detection of reactive oxygen species. *J. Biochem. Biophys. Methods*, **65**, 45–80.
- Hagerman, A.E.; Riedl, K.M.; Jones, G.A.; Sovik, K.N.; Ritchard, N.T.; Hartzfeld, P.W.; Riechel, T.L. 1998 High molecularweight plant polyphenolics (tannins) as biological antioxidants. *J. Agric. Food Chem*, **46**, 1887–1892.
- Hargrove, J.L.; Greenspan, P.; Hartle, D.K.; Dowd, C. 2011 Inhibition of aromatase and α -amylase by flavonoids and proanthocyanidins from Sorghum bicolor bran extracts. *J. Med. Food*, **14**, 799–807.