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Evaluation of total polyphenols and antioxidant activity of African grape (*Lannea microcarpa*) Fruits

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Abstract
The aim of the research work is to evaluate the polyphenols and antioxidant activity of African grape fruits. The methanolic extract was evaluated for its phenolic contents using Folin-Ciocalteu reagent, antioxidant activity using 1, 1- diphenvi1-2 picrvlhidrazyl (DPPH) radical scavenging activity, and flavonoid using sodium nitrite solution. The total phenolic content was 380+2,11 mg/100 g gallic acid equivalent (GAE), antioxidant activity of 562.25±2.91%, and flavonoids contents of 20.63±1.80mg/100g quercetin equivalent (QE .), The results indicated the importance of *Lannea microcarpa* fruits as potential source of polyphenols which if property utilized could supplement the existing sources of antioxidants for the formulation of drugs and other therapeutic uses.

Keywords: *Lannea microcarpa*, polyphenols, antioxidant, fruit pulp, flavonoids

Introduction

The global overpopulation needs parallel increase in food and nutrition sources. Food security becomes vulnerable when it is only dependent on a few numbers of traditional crop plants and domestic animals. Food and nutrition security need to be addressed in the context of biodiversity, an important asset to domesticate new crops or improve the quality of traditional crop plants (Hegazy *et al*., 2013) [10]. Nutritionally, not only the quantity and energy contribution of foods are important to combat malnutrition but also their quality, including macro-and micronutrient content, and antioxidant activities. The gap between wild edible fruits and cultivated ones is wide and needs to be bridged by shedding more light on potential wild food biodiversity (FAO, 2010) [6]. Wild food plants represent a minor contribution to family meals, they are potentially important nutrient and cultural resources for local people around the world (Hegazy *et al*., 2013) [10]. They often contain higher amount of nutrients and bioactive compounds than many cultivated species, especially those which have been under cultivation for many generations (Hegazy *et al*., 2013) [10]. They have great potential as high-value nutraceuticals, and source of bioactive compounds for dietary supplements. Their fruits are edible and therefore important food items in traditional diets of local people, making an important contribution to the health of local communities. The edible fruits have been employed, for a long, in traditional and popular medicine (Delang, 2006) [4].

Fruits and vegetables are recommended as a source of dietary fiber, they are important part of a healthy diet, and variety is as important as quantity and no single fruit or vegetable provides all of the nutrients needed to be healthy. A diet rich in vegetables and fruits can lower blood pressure, reduce risk of heart disease and stroke, prevent some types of cancer, lower risk of eye defects, digestive problems and oxidative stress and also help the body to develop the capacity to fight against these by boosting immunity. This is based on the fact that they are home for many antioxidants such as ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids (provitamin A) and several phenolic compounds (flavones, isoflavones, flavanones, anthocyanins and catechins) (Shahidi and Naczk, 2004) [23].

African grape (*Lannea microcarpa*) (Fig 1) commonly known as "*Faaru*'' in Hausa speaking language belongs to the family *Anacardiacea*. It is found in the savanna and the drier forest re-growth zone of West Africa. In Nigeria it is commonly found in Northern states of Sokoto, Kebbi, Zamfara, Kaduna, Katsina, Kano, and Jigawa. The unripe fruits are green in color while ripe ones are purplish black. The fruits are often sold in both city street markets and along roadsides in West Africa. In Nigeria, the tree is cultivated commercially on a small scale and the trees can be seen in and around villages. The fruit can be eaten fresh or dried like raisins for longer-term storage. The fruit makes an excellent jam, can be made into wine, and the pulp fermented into a potent alcoholic drink.

Fig 1: Riped fruits of *Lannea microcarpa*

Lannea microcarpa is one of the most important species in Africa south of Sahara. The species belongs to the family of Anacardiaceae. It is a Soudano-Sahelean species also present in the Guineo-Congolese region. This species has a great socioeconomic importance in Burkina Faso (Abdoulaye *et al*., 2014) [1]. It is a source of food for both human (fruits) and livestock (forage), and used in local handicrafts (woodcarving, dyeing, and tanning). The plant is a real panacea: the parts (leaves, bark and fruits) are used in the composition of diverse formula in traditional pharmacopoeia (Abdoulaye *et al*., 2014) [1]. *Lannea microcarpa* ripe fruit which is dark red is assumed to be an interesting food Colorant. This color is due to the presence of flavonoids and anthocyanins pigments. Patricia *et al*. (2014) [19] reported that the fruits are eaten raw or dried, and a number of fermented or soft drinks are produced from its juice, however, important concentrations of phenolic compounds with strong antioxidant capacities have been found in the fruit (1005.75 mg/100 g of fruit). Partial reports on its seed and seed oil protein and lipid compositions are also available (Glew *et al*., 1997).

Wild plant fruits contain many natural antioxidants compounds such as carotenoids, vitamins, phenols, tannins, flavonoids, and many secondary metabolites; which have been identified as a free radical or active oxygen scavengers (Oliveira *et al*., 2009) [17]. Therefore, wild plant food sources need to be investigated not only from the nutritional value point of view, but also as a potential therapeutic agent against a wide range of human diseases (Oliveira *et al*., 2009) [17].

Materials and Methods

Sampling and Sample treatment

Fresh fruits of Arican grape (*Lannea microcarpa*) fruits were collected in September, 2021 from Chimola district of Gwadabawa Local government, Sokoto State, Nigeria. Five (5) trees were randomly selected and only ripped fruits were collected from different branches of the trees as described by Hassan and Umar (2004) [9]. The sample was collected in black polythene bags and transported to the laboratory. Prior to analyses, the sample was authenticated at the Herbarium section, Botany Unit, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. A representative sample was taken using alternate shovel method (Alan, 1996) [2]. The sample was thoroughly washed with distilled water and then air dried.

Preparation of the Extract

After drying, the fruits pulp was removed and crushed into powder with the help of pestle and mortar. Fifty grams (50g) of the powdered pulp were then soaked into 500cm^3 methanol and allowed to stand for four days at 4^{0}C. The extract was centrifuged at 1000rpm for 5minutes, filtered and then concentrated to dryness using rotary evaporator. The percentage extract was calculated using equation 1.

$$\% \text{ Extract} = \frac{\text{Weight of extract}}{\text{Sample weight}} \times 100 \quad \text{.....(1)}$$

The residue obtained was kept at 4^{0}C until when required (Motlhanka *et al*., 2012) [15].

Determination Total Polyphenols

The amount of total polyphenols in the sample was determined using modified Folin-Ciocalteu colorimetric method (Singleton *et al*., 1999) [22]. Stock solution of sample extract (25 µl) was dissolved in methanol and further dilution were performed to obtain readings within the standard curve made with gallic acid (R=0.997). The extract was oxidized by the Folin-Ciocalteu reagent (120 µl) and the neutralization was made with Na_2CO_3 (340 µl after 5 minutes. The absorbance was measured at 750 nm after 90 minute in the dark at room temperature. The result was expressed as milligram of gallic acid per 100 grams.

Determination of Flavonoids

The method reported by Kim *et al*. (2003) [13] was adopted. The methanolic extract (1.5mL) was added to 10mL volumetric flask filled with 5mL distilled water and 5% $NaNO_2$ followed by thorough mixing. To the content, 1.5mL of 2% methanolic $AlCl_3$ solution was added followed by 2mL of 1M NaOH solution and the volume made up to the mark with distilled water, the mixture was shaked vigorously and then incubated for 10minutes after which the absorbance measured at 367nm. The flavonoids content was calculated using a standard curve prepared from quercetin, and express as mg quercetin/100g of the extract.

Determination of Total Antioxidant Capacity

The total antioxidant capacity of the extract was determined by adopting the method reported by Pan *et al*. (2007) [18]. One mL of the extract was combined with 3mL reagent solution (0.6 M H_2SO_4, 28mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was capped incubated in a thermal block at 95 °C for 150 min after cooling at room temperature; the absorbance was measured at 695 nm against blank. Readings were taken every 30 min. The absorbance at 734 nm was measured to represent the total antioxidant activity and then calculated using equation 3.

$$\text{Total Antioxidant activity}(\%) = \left(1 - \frac{A_{Sample}}{A_{Control}}\right) \times 100 \text{.....(3)}$$

Where A_{sample} and $A_{control}$ represent the absorbance of the sample and control respectively.

Determination of DPPH Scavenging Activity

The reduction capacity of the DPPH radical was determined by the decrease of absorbance induced by antioxidants according to Hanane *et al*. (2017) [8]. The reaction system consisted of 0.1 mL of the extract and the standard diluted to different concentrations and 2.9 mL of a 0.025 g/L DPPH in methanol. The mixture was shaken vigorously and left standing at room temperature in the dark for 30 minutes. The absorbance was measured at 515 nm against a blank. The ability to scavenge the DPPH radical was calculated using the formula in equation 4.

$$\% \text{ Scavenging effect} = \frac{(ADPPH - A_{Sample})}{ADPPH} \times 100 \text{.....(4)}$$

Where A_{sample} is the absorbance of the solution when the sample extract was added while ADPPH represents the absorbance of the DPPH.

Statistical Analysis

The results obtained were statistically analyzed using one-way analysis of variance (ANOVA) with SPSS version 10.0 statistical packages and were reported as mean± standard error of mean.

Results

The result of the percentage yield, total polyphenols, flavonoids, antioxidant activity and DPPH scavenging activity of the fruits extract were expressed on dry weight basis (DW) and are presented in Table 1.

Table 1: Total polyphenols and Antioxidant activity of *Lannea microcarpa* fruits

Parameter	Concentration
Yield	6.74±1.65%
Total polyphenols	380±2.1 mgGAE/100 g
Total flavonoids	20.63±1.80 mgQE/100 g
Antioxidant activity	562.25±2.91%
DPPH Scavenging activity	65.77±7.80%

The values are mean± Standard error of mean; GAE = Garlic Acid Equivalent; QE = Quercetin Equivalent

Discussion

The Percentage yield

The percentage yield of the extract was 6.74±1.65g/100g of the fruit pulp which is an indication that the fruits contain some important nutritional and or medicinal phyto-compounds.

Total Polyphenols

The total polyphenols content of *Lannea microcarpa* fruits pulp was 380±2.11 mgGAE/100 g DW. The value recorded is lower than 424.84±20 mgGAE/100G DW for Straw berry, 398.25±0.1 mgGAE/100 gDW for African star apple fruits, and higher compared to 247.25±11 mgGAE/100 gDW for Black berry fruits (Ewa *et al*., 2009) [5]. The value obtained is an indication that the fruits if properly utilized could be a good source of polyphenols. Polyphenols are aromatics secondary plant metabolites that are widely spread throughout the plant kingdom and are associated with color, sensory qualities, nutritional and antioxidant properties. In food, polyphenols may contribute to the bitterness, astringency, color, flavor, odor and oxidative stability. Epidemiological studies and associated meta-analyses strongly suggested that long term consumption of diets rich in plant polyphenols offered some protection against development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases (Kanti and Syed, 2009) [12].

Total Flavonoids

The flavonoids content of the fruits is 20.63±1.80 mgQE/100 Gdw. The value is remarkably lower compared to 84.33±8 mgQE/100 Gdw for Straw berry and 29.07±1.12 mgQE/100 Gdw for Black berry (Andre *et al*., 2011) [3] also lower than that of *Adansonia digitata* (42.73 mgQE/100 Gdw) reported by Lmien – Meda *et al*. (2008) [14]. The result obtained indicates that *Lannea microcarpa* fruits are importance sources of flavonoids which are responsible for the attractive colors of flowers, fruits, leaves and also possess biological activities such as anti –inflammatory, anti-carcinogenic and anti-ant atherosclerotic activities (Olajire and Azeez, 2011) [16]. They are important in the plant for normal growth development and defense against infection and injury.

DPPH Scavenging and Antioxidant activities

2,2 – diphenyl -1 – picrylhydrazyl (DPPH) is a stable free radical that has been used as a tool to estimate the free radical scavenging activity of antioxidants. The *Lannea microcarpa* fruits analyzed showed DPPH scavenging activity of 65.77±7.80%. The value is higher compared to 46.64±1.65% reported for Blue berry fruits (Andre *et al*., 2011) [3]. DPPH is one of the compounds that possessed a proton free radical with a characteristic absorption which decreases significantly on exposure to proton radical scavengers. The DPPH scavenging by antioxidants is due to their hydrogen-donating ability. Antioxidants react with DPPH reducing a number of DPPH molecules equal to the number of available hydroxyl group (Hanane *et al*., 2017) [8].

The antioxidant activity of the fruit extract (562.25±2.91%) is probably due to its phenolic contents. It is a well – known fact that phenolic compounds are constituents of many plants, and have attracted great deal of public and scientific interest because of their health promoting effects as antioxidants. The phenolic compounds exhibit considerable free radical scavenging activities through their reactivity as hydrogen or electron donating agents, and as metal ion chelating properties (Rice-Evans *et al*., 1996) [20].

Conclusion

The study provides information on the phenolic composition, flavonoids content, and antioxidant activity. The results obtained indicated that the fruits if properly utilized can be a potential source of dietary polyphenols, and flavonoids which are important antioxidants and therefore their consumption should be stimulated based on the fact that the beneficial effects of polyphenols have been ascribed to their strong antioxidant activity and their ability to scavenge oxygen radicals and other reactive species. These features make *Lannea microcarpa* fruit a potentially interesting material for

the development of functional foods or possible therapy for the prevention of some diseases.

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