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Comparative antioxidant studies of methanol pericarp, mesocarp, seed and whole-fruit extracts and fractions of *Citrullus lanatus*

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ABSTRACT

Aim: This study evaluated the antioxidant potentials of methanol seed, pericarp, mesocarp, and whole-fruit extracts and fractions of *Citrillus lanatus*. **Methods:** Various extracts and fractions (dichloromethane, ethyl acetate, n-butanol and aqueous) of *Citrillus lanatus* whole-fruit were investigated for 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activities, total phenolic content (TPC) and ferric reducing antioxidant power (FRAP). **Results:** In the DPPH assay, the seed extract showed the highest activity (48%) followed by the whole-fruit extract (27%), pericarp (26%) and the mesocarp (22%) at 100 ug/ml. The whole-fruit fractions also showed improved activity with DCM fraction (64%), ethyl acetate (62%), aqueous fraction (46%) and n-butanol (24%) at 100 ug/ml but the activity observed with ascorbic acid, was much higher (84%). The seed extract had the highest phenolic content (14.30 mg) gallic acid equivalents per gram followed by the pericarp (9.58 mg/g), whole-fruit (6.94 mg/g) and mesocarp (2.78 mg/g). The DCM fraction exhibited the highest TPC (78.19 mg/g) followed by ethyl acetate (61.11 mg/g), aqueous (26.66 mg/g) and n-butanol (10.69 mg/g). FRAP assay showed strongest activity with the whole-fruit extract (0.640nm) followed by seed, mesocarp and pericarp extracts. The DCM fraction showed the highest antioxidant potential (0.735nm) followed by ethyl acetate, aqueous and n-butanol fractions but not comparable to ascorbic acid. **Conclusion**: The results revealed that *C. lanatus* seeds, pericarp, mesocarp and whole-fruit extracts and fractions contain varying amounts of flavonoids, tannins and phenolic compounds which exhibit potent antioxidant and free radical scavenging activities.

Keywords: Antioxidant, *Citrillus lanatus*, DPPH, FRAP

INTRODUCTION

Normal metabolic processes usually produce a variety of highly reactive entities known as free radicals or reactive oxygen species (ROS) [1]. Free radicals have dual functions whereby in one instance they can play a beneficial role while in another, they can have adverse effects or both [2]. The functions of cells in a normal biological system depend on reduction and oxidation of pro-oxidants and antioxidants – redox balance [2, 3]. It is the role of antioxidants to maintain a delicate balance between the production and neutralization of ROS. Whenever this balance is interfered with, the cells begin to suffer the consequences of oxidative stress [4, 5]. The consequences of oxidative stress include a series of chronic pathological conditions such as liver disorders, hypertension, rheumatoid arthritis, atherosclerosis, stroke, neurological disorders, renal disorders, adult respiratory distress syndrome, diabetes mellitus, cataracts, obesity, autism, Alzheimer's disease, auto-immune deficiency diseases, degenerative disorders related to ageing, Parkinson's disease, Huntington's diseases, vasculitis, glomerulonephritis, lupus erythematous, gastric ulcers and preeclampsia [6-8]. These conditions are associated with free radical-mediated damage to lipids, proteins [9]. The human body does produce antioxidant enzymes to neutralize free radicals but exogenous antioxidants are needed to help the body to protect itself [10]. Therefore, antioxidants can directly scavenge ROS or indirectly act to up-regulate antioxidant defenses or inhibit ROS production [11]. There are various mechanisms by which antioxidants act such as inhibition of free radical oxidation reactions, interruption of propagation of the autoxidation chain reaction, inhibition of pro-oxidative enzymes; inhibition of formation of free lipid radicals, reduction of hydro peroxides converting them into stable compounds, chelation of metals and converting metal pro-oxidants into stable products and through synergism with other antioxidants, and quenching of single oxygen species [12]. Hence an antioxidant is known as a molecule that acts as radical scavenger and protects the body from oxidative damage [13]. Because of the importance of antioxidants, it is recommended in dietary guidelines that humans should increase the consumption of plant-based foods

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that are rich in carotenoids. Carotenoids are a bright-coloured micro-component in fruits and vegetables. *Citrullus lanatus* (Watermelon) is known to contain high levels of carotenoids such as lycopene, beta-cryptoxanthin, beta-carotene, and vitamin E and it is proven to scavenge free radicals [14, 15, 16]. It has also been demonstrated that *Citrullus lanatus* contains chemicals which can cause reduction of lipid peroxidation [17]. *Citrullus lanatus* is reported to be rich in flavonoids, alkaloids, saponins, glycoside, tannins and phenols and has nutritive values good for human health. The plant itself grows well in Africa, India, USA, China, Russia, Romania and Bulgaria [18].

The aim of this study was to evaluate and compare the antioxidant potentials of the different parts of the *Citrullus lanatus* fruit such as pericarp, mesocarp, seeds and whole-fruit, all of which are edible.

MATERIALS AND METHODS

Collection and Identification of Plant

The plant was collected from Uyo, Akwa Ibom State, Nigeria and identified and authenticated by Prof. Mrs. Margaret Bassey, a Taxonomist in the Department of Botany and Ecological Studies, University of Uyo. A voucher specimen was deposited at the Faculty of Pharmacy Herbarium, University of Uyo.

Preparation of Extract

The fruit was washed and allowed to drain and then cut into its different parts. The pericarp was cut into small cubic sizes and reduced to paste using mortar and pestle. The seed was reduced to paste using mortar and pestle while the mesocarp was chopped into small cubic sizes but not ground because of its texture. The whole fruit was mashed together and reduced to paste. These samples were separately macerated in 99.5 % methanol (Sigma, USA) for 72 hours and filtered using Whatman Filter paper No. 7. The filtrate was evaporated to dryness using water bath regulated at 45 ºC to obtain pericarp, mesocarp, seed, and whole-fruit extracts respectively.

Partitioning of Extract

The extract of *C. lanatus* whole fruit (200 g) was dissolved in 300 ml of distilled water and partitioned successively with dichloromethane, ethyl acetate and n-butanol using separating funnel (Pyrex, England) to obtain dichloromethane (DCM), ethyl acetate (Ethyla), n-butanol and aqueous (Aq) fractions. These fractions were concentrated *in vacuo* at 40 ºC to dryness. The extracts and the fractions were stored at -4 ºC until needed.

Phytochemical Screening and Analysis

Phytochemical screening of the crude extracts of *C. lanatus* seeds, pericarp, mesocarp and the whole fruit was carried out using the standard procedures and tests [19].

Antioxidant Studies

Determination of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Activity

To evaluate the free radical scavenging activity of the *C.lanatus* whole-fruit, seed, pericarp and mesocarp extracts and fractions of the whole fruit, 1, 1- diphenyl-2-picryl hydrazyl (DPPH) was used. DPPH is a relatively stable nitrogen centered free radical that readily accepts an electron or hydrogen radical to become a stable diamagnetic molecule. DPPH radicals react with suitable reducing agents as a result of which the electrons become paired off forming the corresponding hydrazine. The capacity of DPPH radical to cause reduction is determined by the decrease in its absorbance at 517nm, induced by antioxidants [20, 21]. In summary, 0.1 mM solution of DPPH in methanol was prepared. This solution (1 ml) was added to 3 ml. of different extracts and fractions in methanol at different concentration (20, 40, 60, 80 and 100 µg/ml). The mixture was shaken vigorously and allowed to stand at room temp for 30 min. then absorbance was measured at 517 nm using spectrophotometer. The reference standard drug used was ascorbic acid and the experiment was done in triplicate and the results expressed as mean values ± standard deviation. Lower absorbance of the reaction mixture indicated higher free radical activity. The percentage DPPH scavenging effect was calculated by using the following equation:

DPPH scavenging effect (%) or Percent inhibition = A0 - A 1 / A0 × 100.

Where A0 was the Absorbance of control reaction and A1 was the Absorbance in presence of test or standard sample [22, 23, 24].

Reducing Power Assay

In the reducing power assay, various concentrations (20, 40, 60, 80, 100 μg/ml) of extracts, fractions and ascorbic acid (2.5mL) were mixed with 2.5mL of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1 % Potassium ferricyanide. This mixture was incubated at 50 ºC for 20min, 2.5ml of 10% TCA was then added to the blend and centrifuged at 650 rpm for 10 min. The upper layer of the solution (5ml) was assorted with deionized water (5ml) and Ferric Chloride (0.1%), and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicates increased reducing power. The experiment was conducted in triplicates and results expressed as mean values $\pm$ standard deviation [25, 26].

Assay of Total Phenolic Content

The total phenolic content of the extracts and fractions of the whole fruit was determined spectrophotometrically with Folin–Ciocalteu reagent. Approximately, 0.5mL (1mg/ml) of crude extract of the seeds, pericarp, mesocarp and whole fruit and the fractions of the whole fruit was mixed with 2.5mL of 10 % Folin–Ciocalteu reagent and 2mL of sodium carbonate (Na_2CO_3) in water (7 %). The resulting mixture was then be vortexed for 15 seconds and incubated at 40 ºC for 30minutes for colour development. The absorbance of the samples was measured at 765nm wavelength. Follin-ciocalteu reagent (2.5mL) was also added to different concentrations (20-100 µg/ml) for the calibration curve of gallic acid. The total phenolic content was calculated from the calibration curve, and the results expressed as mg of gallic acid equivalent per gram dry weight. The experiment was performed in triplicates [27].

Statistical Analysis

Data were collected and analyzed using SPSS version 22.0 and results expressed as Mean ± standard deviation (SD). The statistical significance was considered to be at $p \leq 0.05$.

RESULTS

Phytochemical Screening

The preliminary qualitative phytochemical analysis revealed that the fruit extracts contained saponins, tannins, cardiac glycosides, anthraquinones, flavonoids and alkaloids in varying degrees as shown in Table 1.

Table 1: Phytochemical Constituents of *Citrullus lanatus* Extracts

Test	Extracts			
	Mesocarp	Pericarp	Seeds	Whole fruit
Saponins	+ + +	+ + +	+ + +	+ + +
Tannins	+ + +	+ + +	+	+ +
Flavonoids	-	+	+ + +	+
Cardiac glycosides	+ + +	+ + +	+ + +	+ + +
Anthraquinones	-	-	+	-
Alkaloids	+	+ +	+ +	+ +

Key: + + + Highly positive + Slightly positive
+ + Moderately positive - Negative

Antioxidant Assay

DPPH Radical Scavenging Activity

DPPH is a well- known radical and a scavenger for other radicals. Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. The seeds, pericarp, mesocarp, whole-fruit extracts and fractions of *C. lanatus* exhibited varied levels of antioxidant activity in DPPH model that was concentration dependent, though not as active as the standard drug, ascorbic acid. The highest activity (48%) was exhibited by the seed extracts, followed by the whole-fruit extract which was (27%), pericarp (26%) and the mesocarp (22%) at 100μg/ml. The antioxidant activity of the whole-fruit fractions also showed improved activity in the DPPH model. The highest activity was demonstrated by DCM fraction (64%), followed by ethylacetate (62%), aqueous fraction (46%) and n-butanol (24%) at 100μg/ml. Although the activities of DCM and ethylacetate were significant, they were not comparable to the standard drug, ascorbic acid (ASA) which was (84%) as shown in Figure 1.

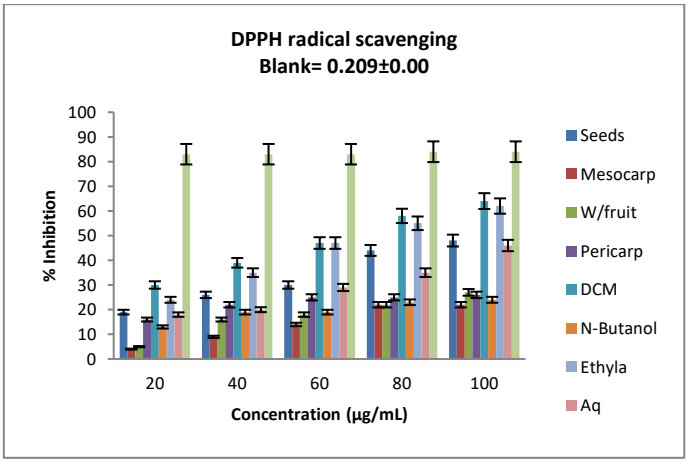

Figure 1: DPPH radical scavenging Activity of methanol pericarp, mesocarp, seed and whole-fruit extracts and fractions of *Citrullus lanatus*

Reducing Power Assay (FRAP)

In FRAP assay, the whole-fruit, seeds mesocarp and pericarp extracts also demonstrated significant antioxidant activity though not comparable to that of the standard drug, ascorbic acid. The best antioxidant activity was demonstrated by the whole-fruit extract (0.650nm) followed by the seed extract (0.588nm), mesocarp extract (0.566nm) and the pericarp extract (0.537nm). The antioxidant activity of the whole-fruit fractions equally demonstrated good antioxidant potential in this FRAP assay. Dichloromethane (DCM) exhibited the highest (0.735nm) antioxidant potential, followed by ethylacetate fraction (0.623nm), aqueous fraction (0.50nm) and n-butanol (0.44 nm) at 100μg/ml. Though the activities of DCM fraction and ethylacetate were significant, they were not as comparable to that of the standard drug, ascorbic acid (1.217nm) at 100μg/ml. These results are shown in Figure 2.

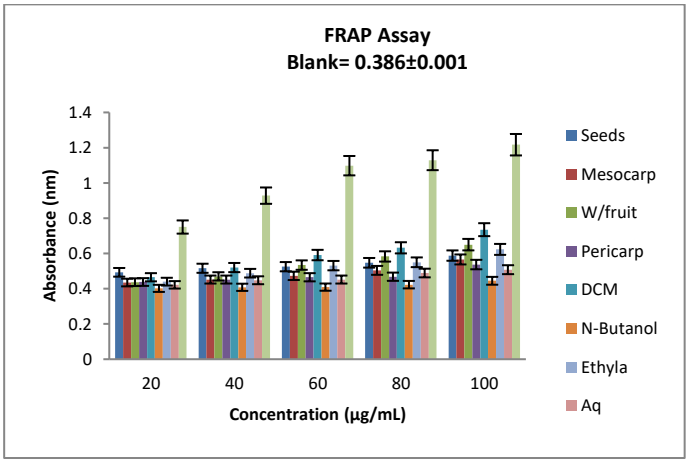

Figure 2: FRAP Assay of methanol pericarp, mesocarp, seed and whole-fruit extracts and fractions of Citrullus lanatus

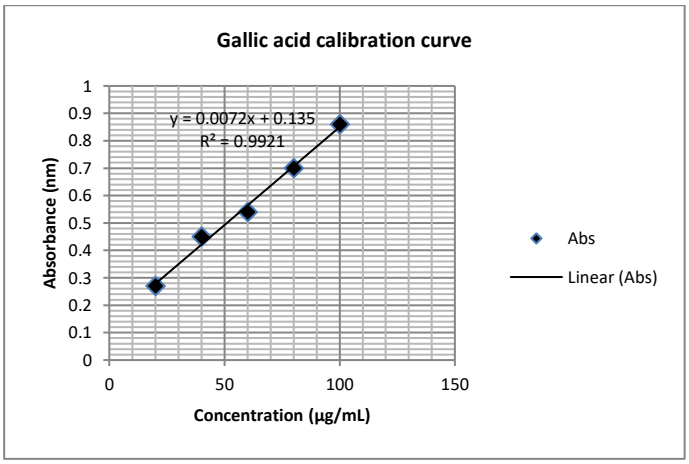

Figure 3: Gallic acid Calibration curve for the estimation of the total phenolic content of methanol pericarp, mesocarp, seed and whole-fruit extracts and fractions of *Citrullus lanatus*

Total Phenolic Content

The quantitative determination of total phenolic content of the crude extracts as well as fractions of the whole fruit was done using garlic acid calibration curve. The seed shows the highest phenolic content (14.305mg/g) followed by the pericarp (9.583mg/g), whole-fruit (6.944mg/g) and mesocarp (2.777mg/g). The DCM fraction exhibited

the highest total phenolic content (78.194mg/g) followed by ethyl acetate fraction (61.111mg/g), aqueous fraction (26.666 mg/g) and n-Butanol (10.694 mg/g) as shown in Figure 3. This is a quantitative assay used to ascertain which part of the plant or fraction has a higher phenolic content in it.

DISCUSSION

Fruits and vegetables have been known to contain phytochemicals which can play a vital role in preventing diseases associated with oxidative stress. Oxidative stress releases free radicals which are unstable highly reactive and energized molecules having unpaired electrons such as oxygen derived free radicals like super oxide (O_2^-), hydroxyl (OH^-), hydroperoxyl (HOO^-), peroxyl (ROO^-) and alkoxyl (RO^-) radicals. There are other reactive oxygen species that the body produces such as nitric oxide (NO^-) and the peroxynitrite anion ($ONOO^-$) [28]. Oxidative stress releases oxygen radicals in the body resulting in pathological disorders such as cataracts, cancers, cardiovascular disease, rheumatism, ageing and autoimmune diseases. It has been reported that there is a significant association between fruits and vegetable intake and reduced rate of mortalities from heart diseases, common cancers, ageing and other degenerative diseases. There is also reduced risk of cancers of the mouth and pharynx, oesophagus, lung, stomach and colon as well as pancreas, bladder and breast cancer in those who consume fruits and vegetables [29, 30]. Fruits and vegetables contain polyphenols which are a group of several low- and high-molecular weight compounds possessing antioxidant properties capable of preventing lipid oxidation. Polyphenols make up the majority of antioxidant activity when compared with ascorbic acid in fruits [31, 32]. Natural antioxidants are mainly phenolics which can be found in all parts of plants such as fruits, leaves, nuts, roots, barks and seeds [33]. The fruit of *C. lanatus* is rich in Lycopene, β-carotene, vitamin C. Some carotenoids such as β-carotene and lycopene show antioxidant activities. Vitamin C, vitamin E and β-carotene are collectively called antioxidant vitamins [34, 35].

The phytochemical screening of *C. lanatus* shows the presence of flavonoids and tannins. The DPPH scavenging capacity of the extract is seed > whole fruit > pericarp > mesocarp. This accounted for by the seeds having the highest flavonoid content than all the other extracts. Also, the scavenging capacity of dichloromethane fraction > Ethyl acetate > Aqueous > n-butanol, but the standard drug Ascorbic acid recorded the highest scavenging capacity over extracts and fractions. This result is similar to an earlier report that *Citrullus lanatus* seed methanolic extract showed a strong antioxidant activity by scavenging DPPH and inhibiting lipid peroxidation. The extract was also found to contain noticeable amount of total phenols and flavonoids, which could play a major role in controlling oxidation generated by free radicals and concluded that the methanolic seed extract of *Citrullus lanatus* can be used as an easily accessible source of natural antioxidant [36]. It is reported that medicinal plant tissues are commonly rich in phenolic compounds such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological activities which includes antioxidant effects [37]. Flavonoids cause reduction of free radicals through upregulation, quenching, or protection of antioxidant defenses and chelation of intermediates of free radical compounds [38]. Hence the antioxidant activities of *C. lanatus* may be ascribed to its flavonoid, tannins and phenolic contents. The seed has the highest phenolic content followed by the pericarp, whole-fruit and mesocarp respectively. The total phenolic content of dichloromethane fraction > ethyl acetate > aqueous > n-butanol. The phenolic content of any plants has a direct relationship to their antioxidant properties because phenolic compounds can act as reducing agents, hydrogen donors, and have the capacity to scavenge free radicals [39]. This is clearly seen in the seed extract having the highest DPPH radical scavenging capacity. The presence of tannins also adds to the antioxidant effects of the extract as tannins are 15–30 times more effective in quenching peroxyradicals than simple phenolics [40].

The antioxidant activity in the FRAP assay was significant with whole fruit extract > seed > mesocarp > pericarp. The antioxidant activity of the whole-fruit fractions equally demonstrated good antioxidant potentials with dichloromethane fraction > ethylacetate > aqueous > n-butanol. However, their effects were not comparable to that of the standard drug, ascorbic acid.

CONCLUSION

This study has shown that *C. lanatus* seed, pericarp, mesocarp and whole-fruit extracts and fractions contain varying amounts of flavonoids, tannins and phenolic compounds, especially the seeds, which exhibit antioxidant and free radical scavenging activities. These suggest that preparing a fruit juice with whole fruit along with the seeds has a better antioxidant and health benefit than that of the mesocarp alone and also demonstrated that purification improved activity as the fraction of the whole-fruit extract exhibited higher antioxidant activity. These results corroborate with the ethnomedicinal use of *C. lanatus* fruit to reduce oxidation and prevent its accompanying pathological disorders.

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