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Comparison of total phenolic contents and antioxidant activities of ripe and unripe fruits from extracts of *Vitellaria paradoxa*

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Abstract
In this study, total phenolic contents and antioxidant activities of ripe and unripe fruits from extracts (70% (v/v) acetone, 70% (v/v) ethanol and hot water (70 ºC) of *Vitellaria paradoxa* (Shea fruit) were investigated. The total phenolic contents and antioxidant activities of these extracts were compared. Additionally, the extraction potentials of 70% (v/v) acetone, 70% (v/v) ethanol and hot water (70 ºC) were also compared. The extracts were analyzed for their total phenolic contents using the Prussian blue assay and the antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. This study showed that the Shea fruit (*Vitellaria paradoxa*) is a good source of natural antioxidants and that unripe fruits of *Vitellaria paradoxa* contain higher phenolic content and antioxidant activity than the ripe fruits.

Keywords: *Vitellaria paradoxa*, Antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl, Total phenolic content

Introduction
Oxidation, a natural process that occurs in food and in living organisms, results in the production of free radicals. These free radicals are unstable compounds which affect molecular structure, chemical reactions in the body and have been known to have some negative physiological effects on humans (Akbarirad *et al*., 2016) [3]. Antioxidants are able to terminate the oxidation reactions by producing free hydrogen ions to be consumed in the reaction or to scavenge the reactive oxygen (Scalbert and Williamson, 2000) [20]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as chemical preservatives to prevent food deterioration despite their threat to humans' health (Mohammed *et al*., 2013) [17]. The adverse toxic and health risks of synthetic antioxidants have increased and this has attracted the attention of many researchers into the field of natural antioxidants (Mohammed *et al*., 2013) [17]. There is higher demand for natural antioxidants because they have the potential to provide protection against diseases that are free radical induced such as cancer and other cardiovascular diseases (Liu *et al*., 2011) [13]. Fruits and vegetables have been recognized to reduce the risk of chronic diseases on regular consumption due to their richness in antioxidants. Citrus fruits have been particularly identified as natural source of antioxidants because of the presence of vitamin C (Yevgenia *et al*., 2013) [23]. Polyphenols constitute the main active ingredients in fruits that have been proven as effective for preventing certain chronic diseases such as cancer, diabetes and coronary heart diseases because of the free radical-scavenging activities of the polyphenols (Aline *et al*., 2008) [4].
Vitellaria paradoxa (*Butryspermum paradoxum*) commonly known as shea butter tree is a well-known plant with several applications in herbal medicine (Ajijolakewu and Awarun, 2015) [2]. The plant is very common in the northern than southern parts of Ghana especially they are in abundance in Navrongo in the Upper East region of Ghana (Adelibigbe *et al*., 2017) [1]. It is a very common tree which occurs largely in off-reserves in some parts of Africa including northern Ghana (Maistrello *et al*., 2011; Jasaw *et al*., 2015) [14, 12]. The plant is a non-timber forest product (NTFP) which means that it is a conserved biodiversity for enhancement of local livelihood and environmental sustainability (Jasaw *et al*., 2015) [12].

Its kernels are used in production of shea butter which has gained popularity as a result of its application in food and cosmetic industries (Maistrello *et al*., 2011) [14]. The People employ the byproducts they obtain after extraction of shea-butter to prevent their houses from termites (Maistrello *et al*., 2011) [14]. The constituents of shea include saponins, alkaloids and polyphenols like tannins (Maranz *et al*., 2003; Ajijolakewu and Awarun, 2015) [15, 2]. Total phenolic and antioxidant capacity changes with aging of fruits (Patthamakanokporn *et al*., 2008) [18].

The objective of the current research was to compare the total phenolic contents and antioxidant activities of ripe and unripe fruits of *Vitellaria paradoxa* using different solvents such as hot water, aqueous ethanol and aqueous acetone.

Materials and methods

Collection and preparation of sample

The samples (unripe and ripe *Vitellaria paradoxa* fruits) were collected in June 2018. The fruits were plucked directly from shea trees at Gognia, near Navrongo in the Kasena Nankana Municipality, Upper East Region of Ghana. The fruits were washed thoroughly with running tap water and dried in an open air for an hour. They were then collected and the fruit pericarps were removed with a stainless steel kitchen knife. The epicarps were air dried for about three weeks and blended with electrical blender (Binatone) into powder. Blending was done for an average of 30 seconds with intermittent breaks after every 10 seconds to allow the heated blades of the blender to cool down. The powdered sample was stored in airtight polythene bags and kept in a cool dry place for further use.

Extraction

The extraction was carried out using 70% (v/v) ethanol, 70% (v/v) acetone and hot water (70 ^{o}C) as solvents by the method of maceration described by Sukhdev *et al*., (2008) [21].

The solid–to-solvent ratio was in the range of 1:10-1:50. Thus, 1.0 g of the sample was measured with an electronic balance into separate tubes. For each of the tubes, 10, 20, 30, 40 and 50 mL of solvent were added and corked to prevent evaporation. The tubes containing the aqueous ethanol and aqueous acetone were kept at 40 ^{o}C and that of the hot water at 70 ^{o}C in water bath and allowed to stand for an hour with agitation, by shaking in 10 minutes interval. The extracts were filtered using Whatmann's filter paper into separate tubes and labeled. The extracts were then kept in a refrigerator at -4.0^{o}C until further analysis.

Determination of Total Phenolic Contents

The Prussian blue assay was used for the determination of the total phenolic contents of the extracts. Gallic acid served as standard and a standard calibration curve (Fig. 1) was prepared using different concentrations of Gallic acid (100-20 mg/L). A 1.0 mL of each of the extracts was measured into separate test tubes and 1.0 mL potassium ferricyanate (0.526 g/100 mL distilled water) was added. In addition, 1.0 mL of ferric acid (0.526 g/100 mL distilled water) was then added and allowed to stand for 15 minutes and 2.5 mL of distilled water was added followed by 2.5 mL of stabilizer and allowed to stand for an hour. Absorbance was measured at 700 nm using Jerway 7315 spectrophotometer. The Total Phenolic Contents (TPC) of the extracts were determined by extrapolating the concentration from the calibration curve ($y = 0.0204x + 0.0658, R^2 = 0.9986$) and the total phenolic content (TPC) was then expressed as Gallic Acid Equivalent (GAE) in milligrams per litre (mgL^{-1}) of sample extract.

Determination of Antioxidant activity

The method as described by Govindarajan *et al*., (2013) [10] for DPPH radical scavenging activity was used to determine the antioxidant activity of the extracts using ascorbic acid as positive control (Govindarajan *et al*., 2013) [10]. Extracts were obtained by dissolving 1.0 g of the dry plant sample in the varying concentrations of the different solvents used. The DPPH reagent (control) was prepared by dissolving 0.02 g of DPPH in 100 mL methanol. A 1.0 mL of each of the extract was measured into different test tubes and 3.0 mL of DPPH reagent added. The mixtures were allowed to stand for about 1 hour under room temperature. A colour change from purple to yellow was observed and the absorbance measured at 517nm using Jerway 7315 spectrophotometer. The percentage radical scavenging activity (%RSA) was then calculated using the formula;

$$\% RSA = 1 - \left(\frac{Abs}{Abc}\right) \times 100 \ldots\ldots\ldots\ldots\ldots (1)$$

where Abs is the absorbance of the extracts and Abc is the absorbance of the control (ascorbic acid).

Statistical analysis

The data of the study was presented using frequency tables and Microsoft Excel was used in the data analysis.

Results and Discussion

Presentation of results

Total phenolic content

The results of the total phenolic contents of the various concentrations of the acetone, ethanol and water extracts were presented in Tables 1, 2 and 3.

For the unripe fruit, the highest phenolic content was found in the 70% (v/v) acetone extract (7.93 ± 0.60 mgL^{-1}GAE) followed by the 70% (V/V) ethanol extract (5.91 ± 1.34 mgL^{-1}GAE) which also provided a better yield than the hot water (70 ^{o}C) extract (5.48 ± 0.08 mgL^{-1}GAE). The same trend was found in the ripe fruit in which the highest phenolic content was found in the acetone extract (6.88 ± 0.04 mg GAEL^{-1}) followed by the ethanol extract (4.44 ± 0.12 mgL^{-1}GAE) and then followed by the hot water extract (2.88 ± 0.04 mgL^{-1}GAE).

DPPH radical scavenging activity of ripe and unripe fruits extracts from of *V. Paradoxa*.

The results for the percentage (%) DPPH radical scavenging activity of aqueous acetone, aqueous ethanol and hot water (70 ^{o}C) extracts for ripe and unripe fruit of *V. paradoxa* are shown in Table 4. The antioxidant activity of the unripe fruit was found to be highest in the aqueous acetone extract (79.56% RSA) followed by the aqueous ethanol extract (73.15% RSA) and then by the hot water extract (70.12% RSA). Additionally, the antioxidant activities of the ripe fruit were also found to increase in the order of 79.53% RSA, 70.32% RSA and 68.72% RSA for aqueous acetone, aqueous ethanol and hot water extracts respectively.

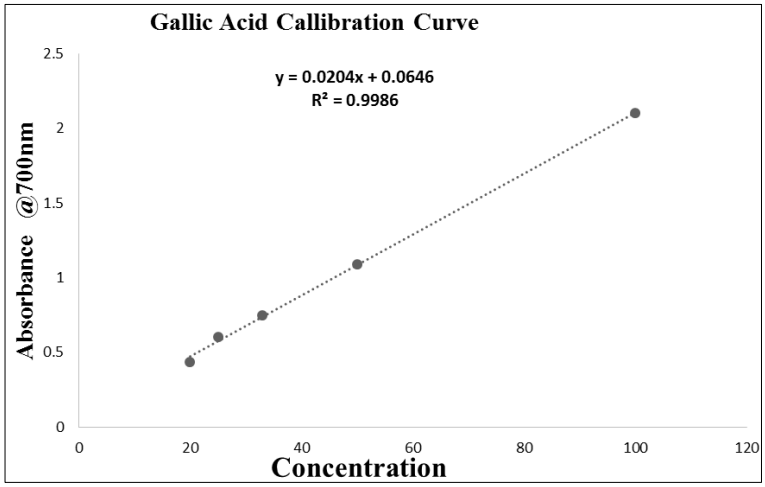

Fig 1: Gallic acid calibration curve

Table 1: Total phenolic contents of 70% (v/v) acetone extracts from ripe and unripe fruits of *V. paradoxa*

Solvent Concentration (mg/L)	TPC of 70% (v/v) acetone extract in mg GAE/L ± SD	
	Unripe	Ripe
10	1.80 ± 0.14	1.48 ± 0.02
20	3.93 ± 0.25	3.04 ± 0.04
30	6.10 ± 0.21	4.60 ± 0.03
40	7.56 ± 0.09	5.68 ± 0.02
50	7.93 ± 0.60	6.88 ± 0.04

Table 2: Total phenolic contents of 70% (v/v) ethanol extracts from ripe and unripe fruits of *V. paradoxa*

Solvent Concentration (mg/L)	TPC of 70% (v/v) ethanol extract in mg GAE/L ± SD	
	Unripe	Ripe
10	1.59 ± 2.39	1.49 ± 0.05
20	3.46 ± 0.18	3.09 ± 0.05
30	4.76 ± 0.64	3.56 ± 0.09
40	5.80 ± 0.86	3.84 ± 0.07
50	5.91 ± 1.34	4.44 ± 0.12

Table 3: Total phenolic content of water (70 ºC) extracts for ripe and unripe fruit of *V. paradoxa*

Solvent Concentration (mg/L)	TPC of water extract (70 ºC) in mg GAE/L ± SD	
	Unripe	Ripe
10	0.28 ± 0.22	0.89 ± 0.08
20	1.60 ± 0.51	2.07 ± 0.06
30	3.24 ± 0.82	2.41 ± 0.01
40	4.82 ± 0.05	2.74 ± 0.03
50	5.48 ± 0.08	2.88 ± 0.04

Table 4: DPPH radical scavenging activity of ascorbic acid standard; aqueous acetone, aqueous ethanol and hot water (70ºC) extracts for ripe and unripe fruit of *V. paradoxa*are

Concentration of solvent	%RSA of 70% (v/v) acetone extract ± SD		%RSA of 70% (v/v) ethanol extract ± SD		%RSA of water (70ºC) extract ± SD		%RSA Ascorbic acid standard
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe	
10	66.09 ± 0.06	64.16 ± 0.08	57.63 ± 0.06	56.46 ± 0.16	54.45 ± 0.14	52.19 ± 0.16	78.45 ± 0.09
20	67.04 ± 1.02	66.32 ± 0.05	62.54 ± 1.02	61.42 ± 0.12	59.62 ± 0.05	58.45 ± 0.08	86.72 ± 0.02
30	69.51 ± 0.08	68.43 ± 0.10	67.51 ± 0.06	66.38 ± 0.06	64.82 ± 0.06	64.70 ± 0.12	94.99 ± 0.01
40	74.64 ± 0.95	71.45 ± 0.12	70.11 ± 1.08	68.35 ± 0.04	66.75 ± 0.12	65.36 ± 0.06	96.81 ± 0.06
50	79.56 ± 0.08	79.53 ± 0.06	73.15 ± 0.08	70.32 ± 0.01	70.12 ± 0.21	68.72 ± 0.04	98.64 ± 0.08

Discussion

The highest phenolic contents was found in the aqueous acetone extract (1.80 ± 0.14 to 7.93 ± 0.60 mg GAE/g) followed by the aqueous ethanol extract (1.59± 2.39 to 5.91 ± 1.34 mg GAE/g) which also provided a better yield than the hot water (70ºC) extract (0.28± 0.22 to 5.48 ± 0.08 mg GAE/g). The values obtain for the phenolic content showed that the fruits of *Vitellaria paradoxa* contained significant amount of phenolic compounds. The total phenolic content which was found to be higher in the unripe fruit than the ripe fruit may be attributed to reduction in tannins and some other phenolic compounds during ripening of fruits. The higher phenolic content in the 70% aqueous acetone and 70% aqueous ethanol extracts than the hot water (70ºC) extract may be attributed to the presence of both polar and non-polar phenolic compounds. This means that the aqueous organic solvents were able to extract both types of compounds whiles the hot water extracted only the polar compounds leaving out

some water insoluble compounds. The results showed that the total phenolic content was dependent on the concentration of the extract and hence the different sample to solvent ratios recorded different phenolic content. In the result of Huda-Faujan *et al.* (2015) [11], the water extracts of some selected Malaysian vegetables had higher phenolic content than their ethanol extracts. This may be as a result of inability of the pure ethanol used by Huda-Faujan *et al.* to extract the polar phenolic compounds. In general, aqueous solvents have shown higher yield of phenolic compounds than any other solvents (Garcia-Salas *et al.,* 2010) [8].

The percentage radical scavenging activity of the control (Ascorbic acid) was recorded to be 78.5%, 86.7% 95.0%, 96.8% and 98.6% for the 1:10, 1:20, 1:30, 1:40 and 1:50 concentrations respectively. Using the ascorbic acid as the reference, the highest percentage radical scavenging activity of the three different extracts for the unripe fruits were found to be 79.56%, 73.15% and 70.12% for acetone, ethanol and hot water respectively. For the ripe fruit, the highest percentage radical scavenging activity of the three different extracts were found to be 79.53%, 70.32% and 68.72% for acetone, ethanol and hot water respectively. The results showed that all the extracts can be considered as having very good antioxidant activities. It can be clearly seen that the DPPH radical scavenging activity of the extracts was dependent on the concentration of the extracts and increased with the volume of solvent used. The results showed a positive correlation between the total phenolic content and antioxidant (DPPH radical scavenging) activity of both ripe and unripe fruits of *V. paradoxa.*

Conclusions

The study showed that 70% (v/v) acetone, 70% (v/v) ethanol and hot water (70 ^{o}C) extracts all gave significant levels of phenolic contents and DPPH radical scavenging activity. The extracts of the unripe and ripe fruits of *Vitellaria paradoxa* are very good sources of phenolic compounds. It was found that aqueous acetone gave a better yield of phenolic compounds than aqueous ethanol which also gave a better yield than hot water in the extraction of both the unripe and ripe *V. paradoxa* fruits. The unripe fruit comparatively showed a higher level of total phenolic contents and antioxidant activity than the ripe fruits.

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References

1. Adelibigbe EJ, Olamide OF, Ogunkule OC, Oladele AF. Eco-distribution of *Vitellaria paradoxa* in Kwara state, Nigeria. Notulae Scientia Biologicae 2017;9(4):503-507.
2. Ajijolakewu KA, Awarun FJ. Comparative antibacterial efficacy of *Vitellaria paradoxa* (Shea Butter Tree) extracts against some clinical bacterial isolates. Notulae Scientia Biologicae 2015;7(3):264-268.
3. Akbarirad H, Gohari AA, Kazemeini SM, Mousavi KA. An overview on some of important sources of natural antioxidants. International food Research Journal 2016;23(3):928-933.
4. Aline LM, Charles EL, Moussa MY, Roland NT, Martin K, Boukare Z *et al*. Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. Molecules 2008;13:581-594.
5. Castellarin SD, Gambetta GA, Wada H, Shackel KA, Mathews MA. Fruit ripening in *Vitis vinifera:* spatiotemporal relationships among turgor, sugar accumulation and anthocyanin biosynthesis. Journal of experimental botany 2011;62(12):4345-4354.
6. Eric AD. Biochemistry; Encyclopedia of physical science and technology. Edn 3, MacMillan Printing Press, New York 2014, 335-342.
7. Falana MB, Bankole MO, Ojo DA, Omemu AM. Comparative phytochemical investigation of the various parts of *Vitellaria paradoxa.* Journal of natural sciences research 2016;6(11):74-80.
8. Garcia-Salas P, Morales-Soto A, Segura-Carretero A, Fernandez-Gutierrez A. Phenolic-compound-extraction systems for fruit and vegetable samples. Molecules 2010;15(12):8813-26.
9. Giri GR, Padam A, Meena R. Isolation of Gallic acid and estimation of total phenolic content in some medicinal plants and their antioxidant activity. Nepal journal of science and technology 2013;14(1):95-102.
10. Govindarajan R, Rastoji S, Vijayakumar M, Shirwaikar A, Rawat AK, Mehrotra S *et al*. Studies on the antioxidant activities of Desmodiumgangeticum. Biological and Pharmaceutical Bulletin 2013;26:1424-1427.
11. Huda-Faujan N, Abdul RZ, Rehan MM, Ahmad FB. Comparative analysis of phenolic content and antioxidative activity of eight Malaysian traditional vegetables. Malaysian Journal of analytical sciences 2015;19(3):611-624.
12. Jasaw SG, Saito O, Takeuchi K. Shea (*Vitellaria paradoxa)* butter production and resource use by urban and rural processors in northern Ghana. Sustainability 2015;7:3592-3614.
13. Liu MNJ, Wang C, Wang Z, Zhang C, Lu S, Liu J. The antioxidant and free radical scavenging activities of extract and fractions from corn silk (*Zea mays* L.) and related flavone glycosides. Journal of food Chemistry 2011;126(1):261-269.
14. Maistrello L, Martini L, Macias-pavon I, Bortolini S, Marchettini N. Evaluation of polyphenols-rich compounds as treatment to prevent attacks by subterranean and drywood termites: preliminary results. Journal of entomology and Acarological Research 2011;43(2):261-267.
15. Maranz S, Wiesman Z, Garti N. Phenolic constituents of shea (*Vitellaria paradoxa*) kernels. Journal of Agriculture: Food Chemistry 2003;51(21):6268-6270.
16. Mehas KM, Rodgers SL. Food science, the biochemistry of food and nutrition. Edn 5, Amazon, Washinton 1997, 337-339.
17. Mohammed S, Hamzat IT, Bashir MA, Saidu H, Yusuf H, Jibrin NM *et al*. An Overview of Natural Plant Antioxidants: Analysis and Evaluation. Advances in Biochemistry 2013;1(4):64-72.
18. Patthamakanokporn O, Puwastien P, Nitithamyong A, Sirichakwal PP. Changes of antioxidant activity and total phenolic compounds during storage of selected fruits. Journal of food composition and analysis 2008;21(3):241-248.
19. Randhir R, Lin YT, Shetty K. Stimulation of phenolics, antioxidant and antimicrobial activities in dark germinated mung bean sprouts in response to peptide and

phytochemical elicitors. Process Biochemistry 2004;39:637-646.
20. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. Journal of nutrition 2000;130:2073-2085.
21. Sukhdev SH, Suman PSK, Gennaro L, Dev DR. Extraction technologies for medicinal and aromatic plants. International Centre for Science and High Technology. Edn 1, UNIDO, Austria 2008, 69-71.
22. Williams MH. Nutrition for health, fitness and sports. Edn 7, 2005, 278-279.
23. Yevgenia S, David I, Yael K, Zvy D, Yaron Y. Natural antioxidants: Function and sources. Food and nutrition sciences 2013;4:643-649.