Fjrs.futa.edu.ng
FUTA Journal of Research in Sciences
ISSN: 2315 – 8239 (Print); E-ISSN: 2489 - 0413

FUTA Journal of Research in Sciences, Vol. 15(2), October, 2019: 292-299

COMPARATIVE STUDIES ON THE EFFECT OF FREE AND BOUND PHENOLIC EXTRACTS FROM SELECTED EGGPLANT (*SOLANUM SPP*) FRUITS ON CARBOHYDRATE HYDROLYSIS ENZYMES

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ABSTRACT
Tropical Eggplant (*Solanum spp*) fruits are known to have numerous therapeutic properties, however little information is known on the distribution of phenolic constituents of these selected eggplant *Solanum melongena depressum* (green), *Solanum gilo* (white) and *Solanum melogena* (purple) fruits commonly found in Nigeria. Most literature only report free phenolic content while bound phenolic content is not included. Therefore this study aims to determine the anti-diabetic and anti-hypertensive effect of free and bound phenolics from these eggplant fruits i*n-vitro*. The dried pulverised fruits were subjected to solvent extraction, base digestion, and solid-phase extraction process using 80% acetone and ethyl acetate for free and the bound phenolics. The extracts were used to determine total phenol, total flavonoid, ferric reducing antioxidant capacity (FRAP), as well as their inhibitory effect on carbohydrate hydrolysis enzymes (α-glucosidase and α-amylase) and angiotensin-I-converting enzyme (ACE) linked to hypertension. Results show that the free phenolic significantly ($P<0.05$) had higher total phenol, total flavonoid than bound phenolic, in the same vein free phenolics inhibited α-amylase, α-glucosidase, and ACE activities more than the bound phenolics. Free phenolic extract from *Solanum depressum* had the highest inhibitory effect on α-amylase (EC_{50}= 79.48µg/mL) while its bound phenolic extract had the highest inhibitory effect on α-glucosidase (EC_{50}= 170.24µg/mL) as well as on ACE (EC_{50}= 98.68µg/ml) activities. This study revealed that these eggplants are rich sources of free and bound phenolics which could serve as a good bioactivity index for dietary diabetes and hypertension prevention.
Keywords: eggplant, free phenolic, bound phenolic, α-amylase; α-glucosidase

INTRODUCTION
Diabetes Mellitus (DM) can be classified into two, the insulin dependent diabetes mellitus (IDDM) or type1 and non-insulin dependent diabetes mellitus (NIDDM) or type 2 (Robiah *et al.*, 2017). NIDDM is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes mellitus (Singh *et al.*, 2017). Inhibition of pancreatic α-amylase and α-glucosidase is part of the mechanism adopted by many commercially available drugs for the management of NIDDM (Oridupa and Saba, 2017). This inhibition of intestinal α-glucosidase aggressively delays the digestion of starch and disaccharides to absorbable monosaccharide, which suppresses a rise on postprandial hyperglycaemia (Adisakwattana, 2017). This is the mechanism most anti-diabetes drugs, as well as functional foods simulate. Hypertension which one of the risk factors of NIDDM which increases the risk of long term cardiovascular complication of type-2 diabetes such as stroke, chronic renal failure, heart diseases, peripheral vascular diseases and death (Kadivec *et al.*, 2015; Nwanna *et al.*, 2016)

Vegetables and fruits are an important part of a healthy diet and the principal source of natural antioxidants such as vitamin C, α-tocopherol, and

phenolic compounds (Ames *et al*. 1993). These antioxidants have been linked to the protection against diseases provided by fruits and vegetables (Harasym and Oledzki 2014). Eggplant, commonly called garden egg in West Africa is an edible fruits vegetable crop belonging to the family Solanaceae. The family is one of the largest and most important families of vegetable which are tropical in origin (Nwanna *et al*., 2016). Eggplant not only provides antioxidants but also helps in the development of blood vessels, required to prevent tumour growth and metastasis, and also inhibits inflammation that could lead to atherosclerosis (Kadivec *et al*., 2015). The phenolic contents and antioxidant activities of tropical eggplant fruits are underestimated, to a large extent, the bound fractions are not considered in some way. Furthermore, the extraction procedure used for the isolation of the phenolic compounds from plant materials could have a significant impact of the outcome of any investigation aimed at evaluating the properties of the phenolic compounds in plant materials, because it would dictate the nature and quantity of the phenolic compounds obtained in the extracts (Kajdzanoska *et al*., 2011). This study aims to extract the free and the bound phenolic from *Solanum melongena depressum*, *Solanum gilo* and *Solanum melogena* fruits predominant in South West Nigeria, and also assess the therapeutic effect of these extracts on carbohydrate hydrolysing enzymes (α-glucosidase and α-amylase) and hypertension (angiotensin-I-converting enzyme) in *in-vitro*.

MATERIALS AND METHODS

The selected species of the eggplants *Solanum gilo* (white), *Solanum melongena* (purple), and *Solanum melongena depressum* (green) were purchased from Akure main market. The identification and authentication of the samples were carried out at the Crop, Soil, and Pest management (CSP) Department of the Federal University of Technology, Akure. The free soluble and the bound phenolic were extracted using the method of Chu *et al* (2002).

All chemicals and reagents used were of analytical grade, and were sourced from Sigma Co. (St Louis MO) while water used was glass distilled.

Determination of Total Phenol Content

The total phenol content was determined according to the method of Singleton *et al* (1999). Appropriate dilutions of the free and bound phenolic extracts were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45^{o}C and the absorbance was measured at 765 nm using Jenway 6305 spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of Total Flavonoid Content

The total flavonoid content of the free and bound phenolic extracts was determined using a slightly modified method reported by Meda *et al* (2005). Briefly, 0.5 ml of appropriately diluted sample extract were mixed with 0.5 ml methanol, 50 µl 10% $AlCl_3$, 50 µl 1 M potassium acetate and 1.4 ml water and allowed for incubation at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid content was calculated as quercetin equivalent.

Determination of Reducing Property (FRAP)

The reducing property of the free and bound phenolic extracts was determined by assessing the ability of the extracts to reduce $FeCl_3$ solution as described by Oyaizu (1986). 2.5 ml aliquot was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min and then 2.5 ml of 10% trichloroacetic acid was added. This mixture was centrifuged at 2000 x g for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm and ferric reducing power was subsequently calculated as ascorbic acid equivalent

Angiotensin I-Converting Enzyme (ACE) Inhibition

The angiotensin-I converting enzyme assay was done using a slightly modified method of Cushman and Cheung (1971). The free and bound phenolic eggplant extracts (0 – 50 µl) and 50 µl ACE (EC 3.4.15.1) solution was incubated at 37 ^{o}C for 15 min. The enzymatic reaction was initiated by adding 150 µl of 8.33 mM of the substrate Bz–Gly–His–Leu in 125 mM Tris–HCl buffer (pH 8.3) to the mixture. After incubation for 30 min at 37^{o}C, the reaction was arrested by adding 250 µl of 1 M HCl. The Gly–His bond was then cleaved and the Bz–Gly produced by the reaction was extracted with 1.5 ml ethyl acetate. Thereafter the mixture was centrifuged to separate the ethyl acetate layer; then 1 ml of the ethyl acetate layer was transferred to a clean test tube and evaporated. The residue was dissolved in distilled water and its absorbance was measured at 228 nm using UV spectrophotometer (Jenway). The ACE inhibitory activities were later expressed as percentage inhibition.

α- amylase Inhibition assay

The free and bound phenolic eggplant extracts (500 µl) and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25°C for 10 min. Then, 500 µl of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to each tube. The reaction mixtures was incubated at 25°C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid colour reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water, and absorbance measured at 540 nm using the spectrophotometer Jenway 6305. (Worthington, 1993). The percentage (%) enzyme inhibitory activity of the aqueous extracts was calculated

α - glucosidase Inhibition assay

Appropriate dilution of the frre and bound phenolic eggplant extracts (0 – 200 µl) and 100 µl of α-glucosidase (EC 3.2.1.20) solution in 0.1 M phosphate buffer (pH 6.9) was incubated at 25 ^{o}C for 10 min. Then, 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added. The mixtures were incubated at 25 ^{o}C for 5 min, before taking the absorbance at 405 nm in the spectrophotometer. The α-glucosidase inhibitory activity was expressed as percentage inhibition (Apostolidis *et al*., 2007)

Data analysis

The results of replicate readings were pooled and expressed as means of standard deviation ±SD. (Zar, 1984). A significant difference was taken to be ($P<0.05$). The IC_{50} was calculated using non-linear regression analysis.

RESULTS

Table 1: Phenolic content of three species of African eggplant

Sample	Free phenolic (mgGAE/g)	Bound phenolic (mgGAE/g)
Green	1.11±0.01^{a}	0.68±0.04^{a}
Purple	1.54±0.01^{b}	0.65±0.02^{a}
White	1.37±0.17^{a}	0.49±0.01^{b}

Data represent mean of triplicate determinations. Values with the same superscript letter along the same column are not significantly different ($p< 0.05$).mgGAE: Milligram Gallic acid equivalent.

Table 2: Total Flavonoid content of the eggplant.

Sample	Free phenolic (mgQE/g)	Bound phenolic (mgQE/g)
Green	0.59±0.18[a]	0.26±0.03[a]
Purple	0.33±0.05[a]	0.23±0.07[a]
White	0.55±0.13[a]	0.10±0.03[b]

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$). (mg.QE/g) Milligram Quercetin equivalent.

Table 3: Ferric reducing antioxidant capacity of the eggplant

Sample	Free phenolic (mg/g)	Bound phenolic (mg/g)
Green	9.40±0.10[b]	7.70±0.10[a]
Purple	10.3±0.20[b]	8.20±0.20[b]
White	12.3±0.25[a]	6.50±0.27[a]

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$).

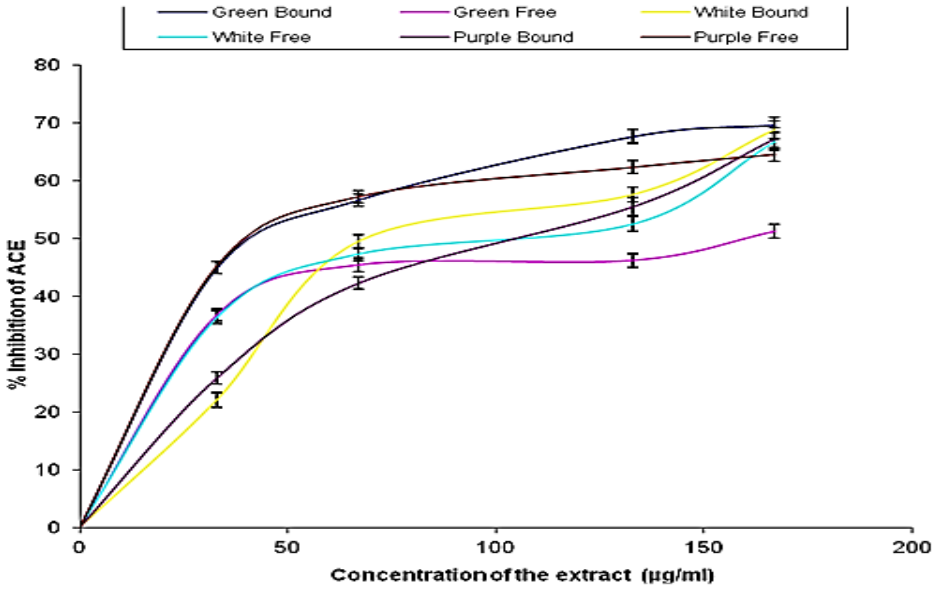

Figure 1. Effect of extract inhibition on angiotensin-1-converting enzyme in concentration-dependent manner. Values represent means of replicate readings.

Table 4: IC_{50} of the free and bound phenolics on α-amylase enzyme (µg/ml) activity.

Sample	Free phenolics	Bound phenolics
Green	79.48±4.27[a]	128.63±4.14[a]
White	81.43±4.86[a]	127.87±4.65[a]
Purple	86.52±4.71[a]	157.67±7.61[b]

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$).

Table 5: IC_{50} of the free and bound phenolic on α-glucosidase enzyme (µg/ml) activity

Sample	Free phenolics	Bound phenolics
Green	105.55±5.55[a]	170.24±6.50[a]
White	106.52±5.16[a]	458.70±11.50[c]
Purple	104.84±4.79[a]	373.69±10.05[b]

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$).

Table 6: IC_{50} of the free and bound phenolic on ACE (µg/ml) activity.

Sample	Free phenolics	Bound phenolics
Green	134.84±7.70[a]	98.68±4.35[a]
White	111.95±4.47[a]	110.28±5.20[b]
Purple	105.93±4.40[a]	114.63±5.10[b]

Data represent means of triplicate determinations. Values with the same letter along the same column are not significantly different ($P < 0.05$).

DISCUSSION

The phenolic contents and antioxidant activities of the eggplant fruits could be underestimated if the bound phenolic compounds are not considered. In this study, the extraction efficiencies of various solvents were investigated in terms of the total content of the free and bound phenolic compounds, as well as the phenolic profiles and antioxidant activities of the extracts. It is well known that phenolic compounds exist in both free and bound forms in plant cells, and that the free phenolic compounds are solvent extractable. In contrast, the bound phenolic compounds, which are covalently bound to the plant matrix, cannot be extracted into water or aqueous/organic solvents mixtures (Perez-Jimenez and Torres, 2011). Although the total phenolic content and antioxidant activities of some tropical eggplant fruits have been reported previously, a study by Fetegbe *et al*., (2012) revealed that total phenolic content measures the total amount of phenolic, which include the flavonoids. Findings from this study are consistent with the one reported by Fetegbe *et al*., (2012) which reveal that extracts from *Solanum* species are rich in phenolic and flavonoids compounds with strong antioxidant properties (Chu *et al*., 2002, Nwanna *et al*., 2016). Free phenols are more readily absorbed and thus, exert beneficial bioactivity in early digestion, it is also possible that different plant foods with different amounts of bound phytochemicals can be digested, absorbed at different sites of the gastrointestinal tract and play their unique health benefits as evidence from the results of this study (Table 1 and 2). These eggplants free phenolic was significantly ($P<0.05$) higher than bound phenolic however the green (*Solanum melongena depressum*) and the purple (*Solanum melogena*) species had a twofold of the bound phenolic when compared with the white (*Solanum gilo*) specie which could be attributed to the bound form content. Reducing power is a novel anti-oxidation defence mechanism; the mechanisms available to affect this property are by electron transfer and hydrogen atom transfer (Rong, 2010). The ability of the free and bound extracts of the eggplants to reduced Iron (III) to Iron (II) is due to their constituents but the free phenolics have higher reducing power ability than the bound phenolics as revealed in (Table 3) this observed activity could be as a results of delay in the bioavailability of bound phenolic than the soluble free phenolics. Whitaker and Stommel (2003) showed that eggplants contain unusual pigments and large amounts of other

phytochemicals, particularly phenolic compounds, which are thought to confer much strong antioxidant activity as reported from this study. The inhibition of α-amylase, α-glucosidase by both the free and bound phenolic extracts of eggplants could have contributed to their use in the management of diabetes by inhibiting starch hydrolysing enzymes reducing the amount glucose absorbed into the blood thereby ameliorating the diabetes incidence. The Free and bound phenolic extracts inhibited α-amylase, α-glucosidase and Angioten 1 converting enzymes (Table 4-6) also the IC_{50} values depict the inhibition concentration, indicates their potential benefit for type-2 diabetes and hypertension (Kwon *et al*., 2008). In *in-vitro* experiments the lower the IC values the better the inhibitory potential. This study reveals no significant difference ($p>0.05$) in the inhibitory potential of the eggplant species except for *Solanum melongena depressum* which seems to have the highest inhibition of these enzymes. Pharmaceutically, stronger inhibition of α-amylase when compared to α-glucosidase is of great importance in addressing some of the side effects associated with drugs such as metformin, acarbose and voglibose presently used for the management of diabetes (Nwanna *et al*., 2013). The free phenolic was able to have better inhibition of these enzymes which however agree with the results of their total phenolics and flavonoids. Both the free and bound phenolic extracts from the eggplant species inhibited angiotensin -1- converting enzymes (ACE) activity in a concentration-dependent manner from Figure 1, although the bound phenolics had a better inhibition from the IC_{50} Table 6. This activity could not be far-fetched from the synergistic ability of the bound phenolics content. Also, Kahlon *et al* (2007) found than eggplant showed amongst other plant fruits the lowest bile acid-binding capability which helps in solubilizing fats because increased of fat in blood vesicles could lead to atherosclerosis as one of the risk factor of hypertension. Thus the findings from this study showed that the bound *Solanum melongena depressum* and *Solanum melongena* were found to have the highest inhibitory effect on the enzymes linked to hypertension (ACE) than the their free phenolic content which means that these eggplants could serve as a better agent in the management and treatment of hypertension. The use of acetone yielded the highest total contents of free phenolics, flavonoid compounds from the extract which exhibited the highest antioxidant activity and anti-diabetic property. The use of an acid hydrolysis method resulted in lower extraction efficiency and antioxidant activity for the bound phenolic compounds as seen from the different species of the eggplant used except that the anti- hypertensive ability was better with bound form extract, the results from this study is the reverse to that obtained on litchi pulp extracts as reported by Su *et al*.,(2014), which could be due to the uniqueness and characteristic of the eggplant fruits which clearly shows that it is rich in soluble polyphenols and flavonoids.

CONCLUSION

This study reveals the in-vitro therapeutic property of the free and bound phenolic extracts of selected eggplant fruits could exhibit anti-diabetic and antihypertensive properties. *Solanum melongena depressum* and *Solanum melongena* were found to have a better output based on their free and bound phenolic content.

REFERENCES

Adisakwattana, S., (2017). Cinnamic acid and its derivatives. Nutrients, 9 (2), p.163.

Ames B.N., Shigenaga M.K and Hagen T.M. (1993): Oxidants antioxidants and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America, 90: 7915–7922.

Apostolidis E, Kwon YI, Shetty K (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and hypertension. Inno. Food Sci. Emerging Technol. 8:46-54.

Chu Y, Sun J, Wu X, Liu RH. (2002). Antioxidant and antiproliferative activity of common vegetables. Journal of Agriculture and Food Chemistry 50:6910–6.

Cushman, DW. Cheung,H.S. (1971). Spectrophotometric assay and properties of the angiotensin I-converting enzyme of rabbit lung, Biochem. Pharmacol. 20 1637–1648.

Fetegbe MA, Rocha JBT, Ibukun EO, Kade IJ (2012). A comparative study on ripe and unripe eggplant (S.melongena) as dietary antioxidant sources. J Med Plant Res. 6(18):90.

Harasym J and Oledzki R. (2014): Effect of fruit and vegetable antioxidants on total antioxidant capacity of blood plasma. Nutrition, 30: 511–517.

Kadivec, M., Kopjar, M., Žnidarčič, D. and Požrl, T., (2015). Potential of eggplant peel as by-product. Acta alimentaria, 44(1), pp.126-131.

Kajdzanoska M, Petreska J and Stefova M. (2011). Comparison of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. J Agric Food Chem 59: 5272–5278.

Kahlon TS, Chapman MH, Smith GE (2007). In vitro binding of bile acids by okra, beets, asparagus, eggplant, turnips, green beans, carrots, and cauliflower. Food Chemistry. 103(2): 676–680.

Kwon, Y.I., Apostolidis, E. and Shetty, K., (2008). In vitro studies of eggplant (*S melongena*) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension. Bioresource Technology, 99(8), pp.2981-2988.

Meda A, Lamien CE, Romito M, Millogo J, Nacoulma O.G (2005) Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91:571–577.

Nwanna, E.E., Ibukun, E.O. and Oboh, G. (2013). Inhibitory effects of methanolic extracts of two eggplant species from South-western Nigeria on starch hydrolysing enzymes linked to type-2 diabetes. African Journal of Pharmacy and Pharmacology. 7(23), pp. 1575-1584

Nwanna, E.E., Ibukun, E.O. and Oboh, G. (2016). Effect of some Tropical Eggplant Fruits (*Solanum Spp*) Supplemented Diet On Diabetic Neuropathy In Experimental male Wistar rats In-vivo. Functional Foods in Health and Disease, 6(10), pp.661-676.

Oridupa, O.A. and Saba, A.B. (2017). Diabetes mellitus in Nigeria and the on-going search for a cure from medicinal plants: a review. Afr. J. of Diabe Med. 25(2).

Oyaizu M (1986). Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine. Japanese J. of Nutr. 44:307–315.

Perez-Jimenez J, and Torres J.L.(2011). Analysis of nonextractable phenolic compounds in foods: the current state of the art. J Agric Food Chem .59:12713–12724.

Robiah, S., Jenie, R.P., Dahrul, M., Nurdin, *et al* (2017). Infra Red Light Emitting Diode in 1200 nm Range has Moderate Performance in Detecting Glucose in Human Blood Glucose Model. Earth and Environmental Science 58:1

Rong T. (2010). Chemistry and biochemistry of dietary polyphenol. Nutrients.2:1231 - 1246.

Singh, S., Nigam, N., Gupta, S. and Kumar, S. (2017). Adverse drug reactions monitoring of newer oral hypoglycemic drugs in a tertiary care hospital of North India: a prospective study. In. J.of Basic & Clinical Pharma. 6(10), 2434 -2440.

Singleton VL, Orthofer R, Lamuela-Raventós RM. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by folin-ciocalteu reagent. Enzym299, 152-178.

Su D, Zhang R, Hou F, Zhang M, Guo J, Huang F,Deng Y and Wei Z. (2014). Comparison of the free and bound phenolic profiles and cellular antioxidant activities of litchi pulp extracts from different solventsBMC Complementary and Alternative Medicine 14:9.

Whitaker, B.D, Stommel, J.R (2003). Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.). J. Agric and Food chem.51 (11): 3448–3454.

Worthington V (1993). Alpha amylase. In: Worthington Enzyme Manual. Worthington Biochemical Corp. Freehold, NJ, pp. 36-41.

Zar JH. (1984). Biostatistical analysis. USA: Prentice-Hall.