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INVESTIGATION OF SOLVENT TYPE, CONCENTRATION, EXTRACTION TIME AND EXTRACTION TEMPERATURE ON TOTAL PHENOLIC YIELD OF SELECTED NIGERIA'S INDIGENOUS LEGUMES

¹James, S., ²Nwabueze, T.U., ²Onwuka, G.I., ²Ndife, J., ¹Yakubu, C.M., ³Bake, G.G. and ⁴Nwokocha, L.

¹Department of Food Science and Technology, Federal University of Technology, Minna, Nigeria

²Department of Food Science and Technology, College of Applied Food Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria

³Department of Water Resources, Aquaculture and Fisheries Technology, Federal University of Technology, Minna, Nigeria

⁴Department of Hospitality and Tourism Management, Delta State Polytechnic, Ogwashi-Ukwu, Delta State, Nigeria

Corresponding Author's E-mail: samaila.james@futminna.edu.ng

ABSTRACT

This study evaluated the effect of extraction variables in a single factor experiment to optimize the recovery of total phenolics from lesser legumes namely, African oil bean (*Pentaclethra mycrophylla* Benth.), African yam bean (*Sphenostylis stenocarpa*) seed, African breadfruit (*Treculia africana*) and groundnut (*Arachia hypogea*) indigenous to Nigeria. Total phenolic was extracted using standard method with different solvents (acetone, ethanol and methanol), at different concentrations (20 – 100%, v/v), different extraction time (30 – 50 minutes) and incubated at different extraction temperatures (50 – 60°C). The results revealed that extraction variables significantly ($p < 0.05$) affected the total phenolic yield of the samples evaluated. Acetone used at 40% concentration (v/v) incubated at 50°C for 30 minutes gave the highest total phenolic yield in African breadfruit (221.28 mg/100 g) and groundnut (225.56 mg/100 g). For African oil bean, 60% acetone (v/v) incubated at 50°C for 50 minutes gave the highest total phenolic (326.89 mg/100 g) while, 100% acetone (v/v) incubated at 50°C for 30 minutes gave the highest total phenolic (196.94 mg/100 g) in African yam bean. Therefore, for optimal recovery of total phenolic in lesser legumes, the need to adopt the stated variables.

Keywords: extraction variables; lesser legumes; optimization; total phenol



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INTRODUCTION

Legumes belong to the family Leguminosae. In the tropics, legumes are the second important food crops after cereals and are excellent sources of cheap plant proteins and minerals when compared with animal products (Apata and Ologhobo, 1997; Ubom, 2007; Annor *et al.*, 2014; Singh *et al.*, 2016). Indigenous legumes therefore are an important source of affordable alternative protein to people with poor financial resources in many developing countries most especially Africa and Asia where the grains constitute part of the daily staple food (Nwabueze *et al.*, 2007; James and Nwabueze, 2013).

African oil bean tree (*Pentaclethra macrophylla* Benth) is cited among the lesser known and under exploited legumes. The nutrient composition of the seed has been extensively studied (Mears and Mabry, 1981; Achinewhu, 1983; Enujiugha and Akanbi, 2002; Onwuliri *et al.*, 2004; Enujiugha and Akanbi, 2005). There is dearth of information on the bioactive constituents of the seed. African yam bean (*Sphenostylis stenocarpa*) seed is one of Africa's under-utilized plant species with potential to increase food supply. The plant produces small tuberous roots which contains more protein than other tubers such as sweet potatoes, Irish potatoes and cassava roots. The plant produces good yield of edible seeds and leaves which are utilized as edible vegetable. The nutrient composition of the seed has been studied extensively (Oboh *et al.* 1998; Amoetey *et al.*, 2000; Wokoma and Aziagba, 2001; Okeola and Machuka, 2001; Klu *et al.*, 2001; Oke *et al.*, 2013); however, there is scarce information on its phytochemical constituents. The African breadfruit is an annual crop found mainly in the high rain forest zone of southern part of Nigeria and other African countries (Ajiwe *et al.*, 1995, Nwabueze *et al.*, 2007; Nwabueze and Iwe, 2010). The seed serves as nutrient reserve most especially during scarce period when conventional sources foods are short in supply, usually before the rainy season sets in (Nwabueze *et al.*, 2007). Nwabueze *et al.* (2007); James and Nwabueze (2013) reported that the crop contains 15.76% protein, 11.45% fat, 3.00% ash, 60.59% carbohydrate, 1715.55 kcal/kg energy. The seed is reported to be rich in minerals, essential amino acids and vitamins. Groundnut is one of the most popular commercial crops in Nigeria and the country accounts for



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41% of the total groundnut production in West Africa (Echekwu and Emeka, 2005). Despite the industrial potentials of the nut in the production of different products, its uses in Nigeria remain largely with the rural women. United States Department of Agriculture (2011); Settaluri *et al.* (2012) reported that peanut per 100 g contains 1.55 g water, 21.51 g carbohydrates, 8.0 g fibre, 49.66 g lipids, 23.68 g proteins and 2448 kJ (585 kcal) total calories. Furthermore, the nut is rich in minerals such as calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, copper, manganese and selenium; vitamins for example thiamin, riboflavin, niacin, folate, vitamin E and pantothenic acid; different compositions of saturated and unsaturated fatty acids (Settaluri *et al.*, 2012).

Studies on conventional legumes have shown that they constitute a large reservoir of phytochemicals which are beneficial in preventing and managing the occurrence of degenerative diseases (Hu, 2003; Jacobs and Gallaher, 2004; Vadivel and Janardhanan, 2005; Enujiugha, 2010; Ade-Omowale *et al.*, 2015). There is immense research efforts on the possibilities of utilizing natural sources of bioactive compounds for the dietary management of certain chronic diseases due to the adverse effects associated with the use of synthetic chemicals. Hence, the need to assess the potentials of indigenous legumes in total phenolic yield to determine the best extraction variables which will provide the basis for their possible explorations.

MATERIALS AND METHODS

Materials

Indigenous and lesser legumes for this study were African oil bean (*Pentaclethra mycrophylla* Benth.) seed, African yam bean (*Sphenostylis stenocarpa*) seed, African breadfruit (*Treculia africana*) and groundnut (*Arachia hypogea*). The samples were procured in the month of December, 2017 from Umuahia Local Market and botanically identified by the Department of Crop Production, Federal University of Technology, Minna, Nigeria.

Reagents

Extraction solvents used in this study were acetone manufactured by Lobal Chemie Pvt. Ltd., India with CAS No. (64-17-5), ethanol manufactured by Guangdong Guanghua Sci-Tech. Co.



Ltd. India with CAS No. (67-64-1) and methanol manufactured by Lobal Chemie Pvt. Ltd., India with CAS No. (67-56-1). All the extraction solvents were procured from Finlab Abuja, Nigeria.

Extraction variables for total phenolic compounds

Extraction conditions for total phenolics recovery were based on solvent type, solvent concentration, extraction time and extraction temperature. One factor was varied at a time while keeping others constant. Fixed factors were particle size of 0.50 mm and solvent-to-solid ratio of 10:1 (Tan *et al.*, 2013).

Total phenolic content (TPC) was extracted with 60% (v/v) methanol, 60% (v/v) ethanol, 60% (v/v) acetone. The independent variables were constant solvent composition of 60% (v/v), extraction time (180 minutes) and extraction temperature ($28 \pm 2^\circ\text{C}$). The optimal extraction solvent was selected upon the highest value of TPC (mg GAE/100 g DW) (Tarzi *et al.*, 2012).

Total phenolic was extracted from the sample using solvent that gave the highest content. The best solvent was used at different concentrations ranging from 20% (v/v) to 100% (v/v) for the extraction while, holding the other two independent variables, which were the extraction time (180 min) and extraction temperature ($28 \pm 2^\circ\text{C}$) at a constant level. The optimal solvent concentration was selected upon the highest value of TPC (mg GAE/100 g DW) (Tarzi *et al.*, 2012; James *et al.*, 2020).

Based on the solvent type and solvent concentration selections giving optimal extraction of phenolics, extraction was repeated for different time periods of 30, 40 and 50 minutes at constant temperature ($28 \pm 2^\circ\text{C}$). The optimal extraction time was selected upon the highest value of TPC (mg GAE/100 g DW) (Tarzi *et al.*, 2012; James *et al.*, 2020).

Upon the evaluation of optimal solvent type, solvent concentration and extraction time, extraction was performed at different temperatures of 40, 50 and 60°C . The optimal extraction temperature was selected upon the highest value of TPC (mg GAE/100 g DW) (Tarzi *et al.*, 2012; James *et al.*, 2020).

Determination of total phenolics in the extract



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Folin-Ciocalteu method as described by Li *et al.* (2008) was used with some modification. An aliquot of 10 μL of the sample solution was mixed with 100 μL of commercial Folin-Ciocalteu reagent and 1580 μL of water. After 5 minutes incubation at room temperature ($27 \pm 2^\circ\text{C}$), 300 μL of saturated sodium carbonate was added. The colour generated was read after 2 h at room temperature at 760 nm using a UV-Vis spectrophotometer (UV-9200, UK). The correlation between the absorbance and gallic acid concentrations creates a calibration standard curve. The phenolic compounds concentration of the samples was expressed as gallic acid equivalents in mg/L, then the Total Phenolic Compounds yields (TPC) were calculated by transforming milligrams of Gallic Acid Equivalent (GAE) per litre (mg GAE/L) into grams of GAE per 100 g dry matter (g GAE/100g DM).

Experimental design

Two factorial design was adopted for this study.

$$Y_{ijk} = \beta_o + \beta_i X_1 + \beta_j X_2 + \beta_i \beta_j X_1 X_2 + \epsilon_{ijk}$$

Where, β_o = over all mean or intercept; X_1 = legume types; X_2 = extraction variables; ϵ_{ijk} = random error; β_i , β_j , $\beta_i \beta_j$ = model factor coefficients; Y_{ijk} = response.

Statistical analysis

The total phenolic content from each sample was determined in triplicates. The data generated were analysed using analysis of variance (Steel and Torrie, 1980). The difference between mean values was determined by the least significant different test. Significance was accepted at the 5% probability level. Processed data were expressed in a bar chart format as mean \pm standard error (SE).

RESULTS AND DISCUSSION

Effects of solvent types on total phenolic content

The effect of solvent types on total phenolic content of indigenous legumes is shown in Fig. 1. The results showed that acetone gave a significantly ($p < 0.05$) high yield of total phenolics in African oil bean (160.68 mg/100 g), African breadfruit (125.00 mg/100 g), groundnut (129.53 mg/100 g) and African yam bean seed (131.49 mg/100 g). Methanol gave the second highest



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yield of total phenolics (122.50 mg/100 g) in African oil bean while, ethanol gave the lowest yield (94.86 mg/100 g). In African breadfruit, ethanol gave the second highest yield (104.32 mg/100 g) while, methanol gave the lowest yield (97.57 mg/100 g). Acetone and methanol significantly ($p < 0.05$) gave high yield of total phenolics 129.53 mg/100 g and 125.07 mg/100 g, respectively, while, ethanol gave the lowest yield (104.32 mg/100 g). For African yam bean seed, ethanol gave the second highest yield of total phenolics (107.64 mg/100 g) while, methanol gave the lowest yield (95.27 mg/100 g). Goli *et al.* (2004) and Nur *et al.* (2014) in their study revealed that phytochemicals are either water soluble such as polyphenols or lipid soluble such as carotenoids. In this study all the legumes which contain appreciable amount of lipids African oil bean, African breadfruit, groundnut and African yam bean seed had significantly high yield of total phenolics in acetone extract. This agrees with the findings of Tan *et al.* (2013), Wang *et al.* (2008) and Tabart *et al.* (2007) who reported that acetone gave the highest yield of total phenolics and antioxidant property in plant matrix than ethanol. The total phenolics extracted using acetone from African oil bean (160.68 mg/100 g), from African breadfruit (125.00 mg/100 g), from groundnut (129.53 mg/100g) and from African yam bean seed (131.49 mg/100 g) were all low compared with 3120 – 6600 mg/100 g DM from wild legumes with methanol; 5500 mg/100 g DM from fava bean and 6760 mg/100 g DM from lentil extracted with methanol and acetone solvents and their mixtures (Amarowicz and Pegis, 2008; Villigiri and Biesalski, 2012; Salem *et al.*, 2014). To rationalize the disparity in the total phenolic yield of different food materials, Nobre *et al.* (2005), Liyana-Patthirana and Shahidi (2005) and Chew *et al.* (2014) reported that extraction solvent, solvent concentration, food particle size, extraction time and temperature, extraction pH, plant species and agronomic practice play significant roles in the recovery of total phenolic content in a sample matrix.

Therefore, based on the total highest phenolic yield, acetone was selected for African oil bean, African breadfruit, groundnut and African yam bean seed.

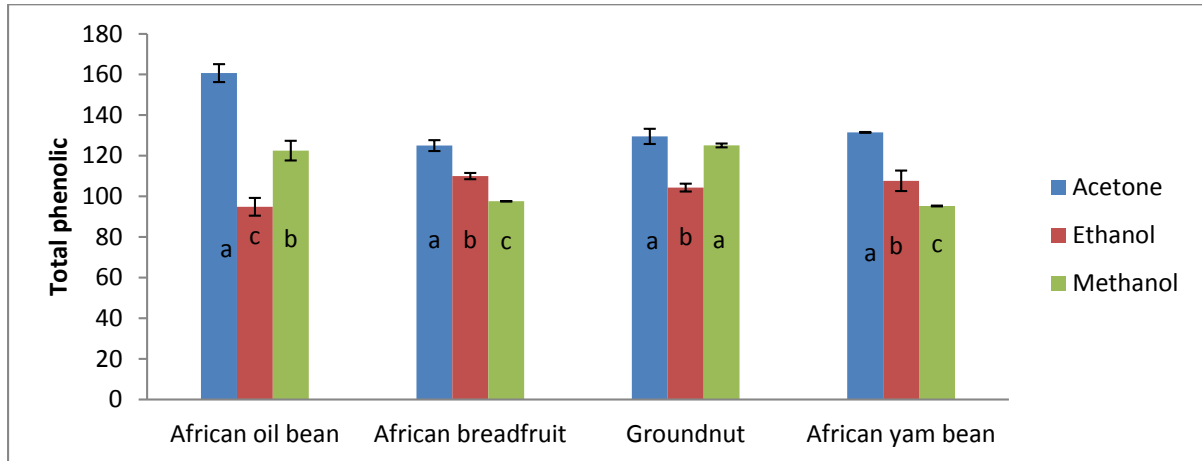


Figure 1: Effect of solvent type on total phenolic content

Key: Set of bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

Effects of acetone concentration on total phenolic content in African oil bean

The effects of acetone concentrations (20 - 100%, v/v) on African oil bean total phenolic content is shown in Fig. 2. The results show that solvent concentrations significantly ($p > 0.05$) affected the total phenolic yield. Extraction solvent used at 60% (v/v) had the highest yield of total phenolics (249.26 mg/100 g). This was followed by 80% (v/v) (249.26 mg/100 g), 40% (v/v) (184.53 mg/100 g) and 70% (v/v) (166.08 mg/100 g). However, acetone with the least concentration of water, 20% (v/v) had the lowest total phenolic yield (160.68 mg/100 g). It can be deduced that there was increase in the total phenolic yield with increase in acetone concentration.

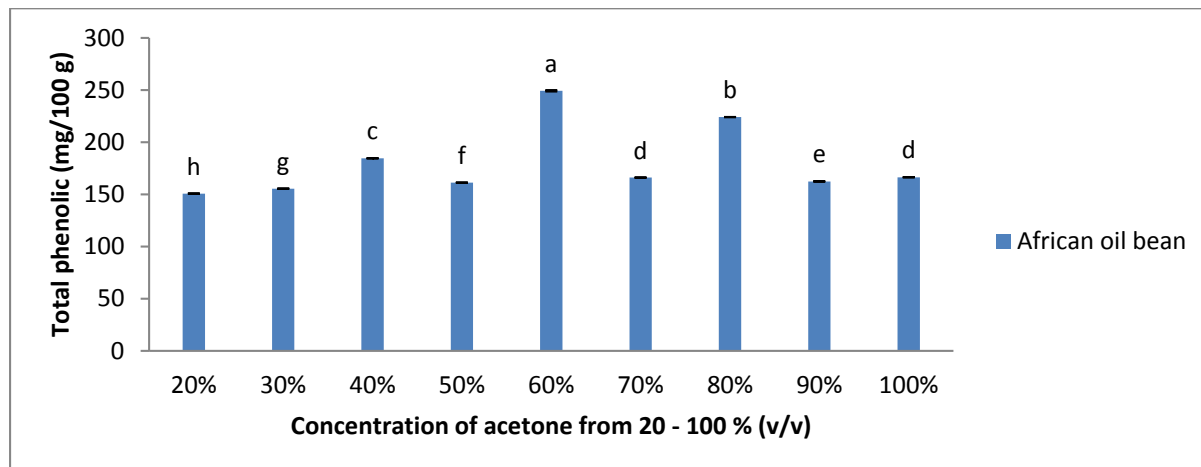


Figure 2: Effect of acetone concentrations on African oil bean total phenolic content
Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

The extraction of phenolic compounds from plant materials is directly related to the compatibility of the phenolic compounds with the extraction solvent, hence, when the compounds are well matched in polarity with the solvent, they are easily dissolved and extracted (Tan *et al.*, 2013; Chew *et al.*, 2014). In African oil bean, total phenolic content reached its peak at 60% acetone (v/v) (249.26 mg/100 g). This suggests that phenolic compounds in African oil bean are in moderate polar profile. Furthermore, increase in acetone concentration modulated the polarity of the solvent complex which matches with the polarity of the total phenolic profile which led to high total phenolic recovery at high acetone concentrations. The results of this findings agree with the study of Chirinos *et al.* (2007) and Tan *et al.* (2013) who reported a similar trend of total phenolic recovery in henna stem extracted with acetone at different concentrations. Therefore, a moderately polar solvent of 60% acetone (v/v) which gave the highest total phenolics was chosen for the evaluation of extraction times and extraction temperatures.

Effects of acetone concentrations on African breadfruit total phenolic content

Results in Fig. 3 revealed the effects of acetone concentrations (20 – 100%, v/v) on African breadfruit total phenolic content. The results show that acetone at 40% (v/v) gave the highest

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total phenolic (156.49 mg/100 g). This was followed by acetone at 80% concentration (v/v) (142.97 mg/100 g) while, 100% acetone gave the least total phenolics (132.23 mg/100 g). The result implies that moderate addition of water to acetone created a moderately polar medium that ensures high extraction of polyphenol and thus improving the overall extraction efficiency. However, extraction of African breadfruit total phenolics at low and high acetone concentrations gave low yield of total phenolics. It can be deduced that moderate addition of water to acetone increases the polarity of the solvent so that the ratio of more polar phenolic compounds in African breadfruit extract increases with moderate water ratio. This study agrees with the findings of Zhang *et al.* (2007), Chirinos *et al.* (2008) and Tan *et al.* (2013) who reported similar trend of total phenolic recovery in acetone in different plant matrix. Also, another possible reason of increase extraction efficiency in the presence of some water might be due to increase swelling power of the plant material by water which sufficiently exposes the contact surface area between the plant matrix and the extraction solvent (Tan *et al.*, 2013; Hemwimon *et al.*, 2017). A moderate polar solvent of 40% acetone (v/v) was chosen for the evaluation of extraction time and temperature.

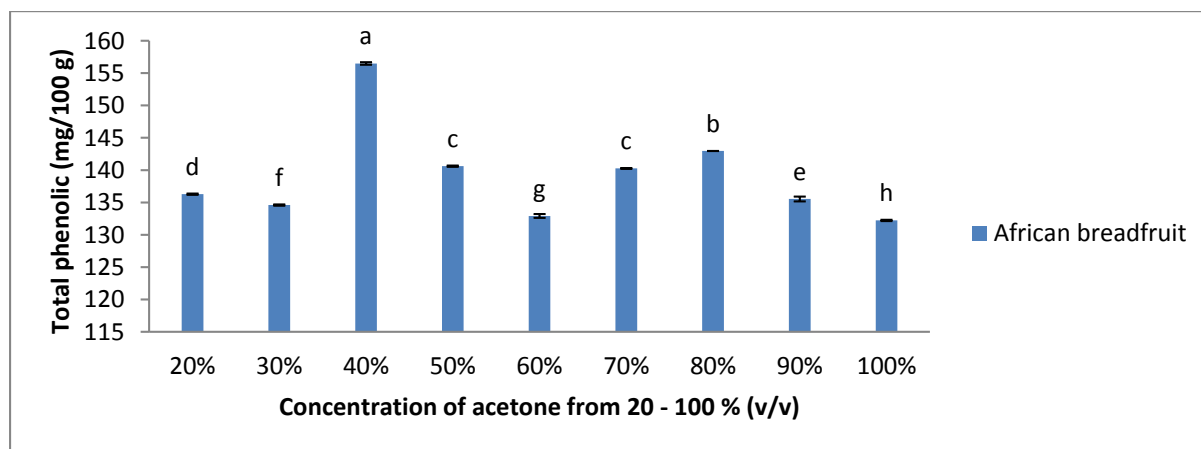


Figure 3: Effect of acetone concentrations on African breadfruit total phenolic content.
Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

Effects of acetone concentrations on African yam bean seed total phenolic content

Effect of acetone concentrations (20 – 100%, v/v) on African yam bean seed total phenolics is shown in Fig. 4. The result shows that acetone at 100% (v/v) gave the highest yield (190.34 mg/100 g) of total phenolics. It can be observed that acetone with moderate water concentrations 30% (v/v), 40% (v/v) and 50% (v/v) gave good yield of total phenolics 150.68 mg/100g, 150.68 mg/100 g and 176.28 mg/100 g, respectively. However, acetone concentrations of 20% (v/v) and 80% (v/v) gave the least yield of total phenolics 136.76 mg/100 g. This goes to suggest that African yam bean has diverse phenolic profile at different polarities. It can be deduced that African yam bean total phenolics can be maximally extracted in either more polar or less polar medium. However, the result is in contrast with the findings of Nur Syukriah *et al.* (2014) who reported low yield of total phenolics in 100% (v/v) acetone, though in *Quercus infectoria*. Furthermore, the results of this finding imply that, African yam bean seeds have similar polarities with the extraction solvent used at the adopted concentrations, unlike in *Quercus infectoria* which has polarity at 100% acetone (v/v). Since the highest yield of total phenolics from African yam bean was achieved in 100% (v/v) acetone, hence, it was chosen for time and temperature evaluations.

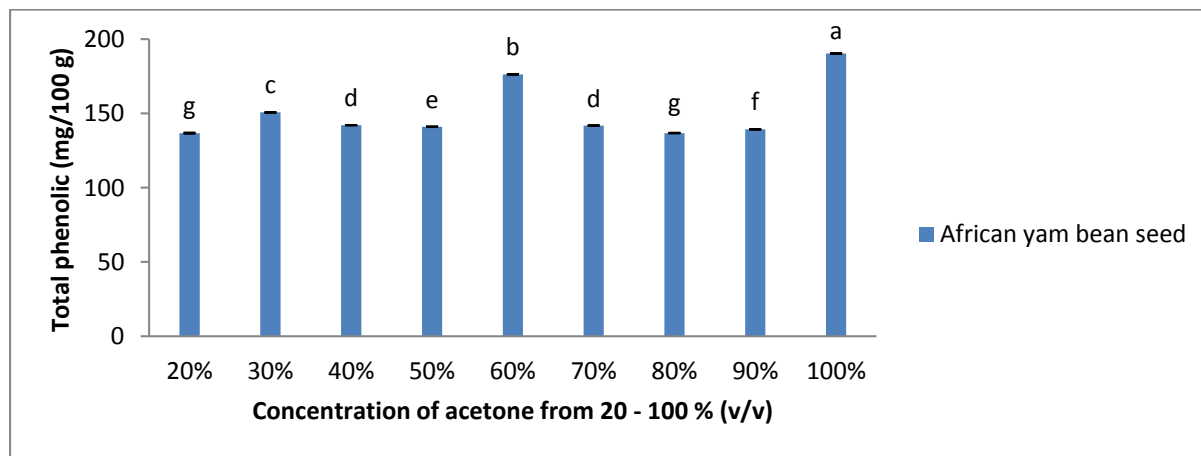


Figure 4: Effect of acetone concentrations on African yam bean seed total phenolic content. Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

Effects of acetone concentration on total phenolic content in groundnut

Fig. 5 shows the effect of acetone concentrations, 20 – 100%, (v/v) on groundnut total phenolics. The result shows that 40% acetone (v/v) gave significantly ($p < 0.05$) the highest yield (261.55 mg/100 g) of total phenolics. Extraction solvents with less acetone concentrations 20% and 30% (v/v) as well as those with less water concentrations 70% and 80% (v/v) gave good yield of total phenolics. However, 50% acetone (v/v) gave the lowest yield (142.91 mg/100 g) of total phenolics. The result suggests that groundnut total phenolics can be extracted at different polarities. The total phenolics ranged from 142.91 mg/100 g to 261.55 mg/100 g. The value obtained in this study compares favourably with 256.00 mg/100g total phenolics in grey peanut, however, the value is low when compared with 8453 mg/100 g, 1633 mg/100 g and 2211 mg/100g total phenolics extracted from the testa of pink, red and yellow peanut, respectively (Khaopha *et al.*, 2012). The recovery of phenolic compounds from legume grains depends mainly upon the type of solvent used, plant genotype, extraction time, extraction temperature among others (Agboola *et al.*, 2009; Vadivel *et al.*, 2012).

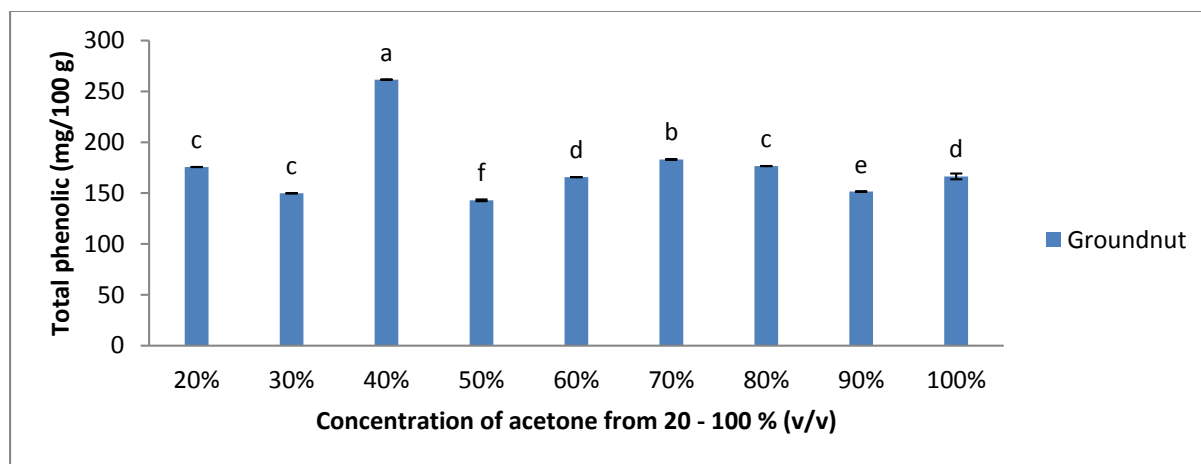


Figure 5: Effect of acetone concentrations on groundnut total phenolic content.

Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

The result of this study implies that, despite the good yield of total phenolics at both low and moderate polarities, high yield can be achieved at 40% acetone (v/v), hence, it was chosen for time and temperature evaluations.

Influence of extraction times on total phenolic content of the legumes

The effect of extraction time (min) on the yield of total phenolic content of legume samples is shown in Fig. 6. The results revealed that extraction time significantly ($p < 0.05$) affected the total phenolic yield of African oil bean, African breadfruit and groundnut, however, extraction time had no effect on the total phenolic yield of African yam bean seed. Extraction time is an important parameter that influences the total phenolic recovery. Its plays a role in minimising total cost and energy of the process (Chew *et al.*, 2011; Tan *et al.*, 2013). Longer extraction time exposes phenolic compounds to oxygen, light and unfavourable temperature which increase the chance of their being oxidized (Naczka and Shahidi, 2006; Chirinos *et al.*, 2007; Chew *et al.*, 2011).

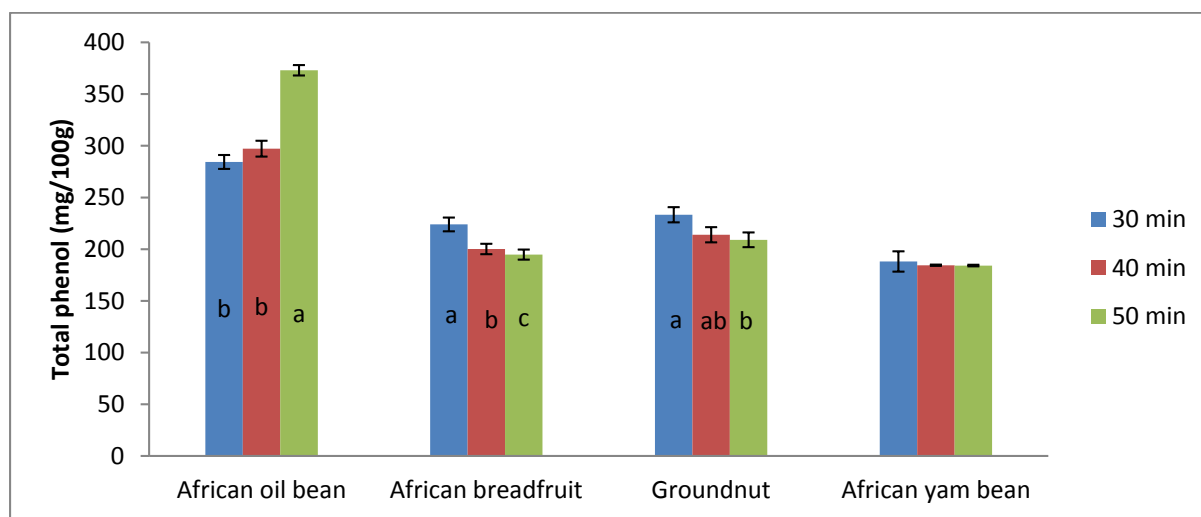


Figure 6: Effect of extraction time on total phenolic content

Key: Set of bar charts with different alphabets are significantly ($p < 0.05$) different from each other.



African oil bean total phenolic extracted with 60% (v/v) acetone (373.00 mg/100 g) at 50 minutes was significantly ($p < 0.05$) high than those extracted at 40 minutes and 60 minutes. In African breadfruit, the total phenolics extracted with 40% acetone (v/v) at 30 minutes (223.94 mg/100 g) was significantly ($p < 0.05$) higher than the ones extracted at 40 minutes and 50 minutes. Groundnut total phenolic extracted with 40% acetone (v/v) at 30 minutes (233.33 mg/100 g) was significantly ($p < 0.05$) higher than those extracted at 40 minutes and 50 minutes. The gradual increase in the total phenolics with corresponding increase in extraction time in African oil bean (284.33 - 373.00 mg/100 g) can be well explained by Fick's second law of diffusion which reveals that a final equilibrium will be attained between the solution concentration in the solid matrix and the solvent after a particular duration (Pinelo *et al.*, 2006; Silva *et al.*, 2007). Therefore, for temperature evaluation on total phenolic yield, extraction time of 50 minutes was chosen. However, the phenomenon of solvent equilibration did not apply to African breadfruit and groundnut whose high total phenolic contents were achieved at a shorter time (30 minutes). This can be attributed to possible oxidation of their total phenolics at extended time (40 minutes and 50 minutes) adopted in this study. This view agrees with the findings of Chew *et al.* (2011) and Tan *et al.* (2013) who in their studies reported low yield of total phenolics in plant matrix at extended time. Therefore, for temperature evaluation on African breadfruit and groundnut total phenolics, 30 minutes extraction time was chosen. Since extraction time did not significantly ($p > 0.05$) affect the total phenolic recovery of African yam bean seed, 30 minutes which gave the highest yield was chosen.

Effects of extraction temperatures on total phenolic content of the legumes

The effect of temperature variations on total phenolic yield of African oil bean, African breadfruit, groundnut and African yam bean seed is presented in Fig. 7. The results revealed that extraction temperature significantly ($p < 0.05$) affected the total phenolic yield of African breadfruit, groundnut and African yam bean seed. However, extraction temperature did not significantly ($p > 0.05$) affect the total phenol content of African oil bean.

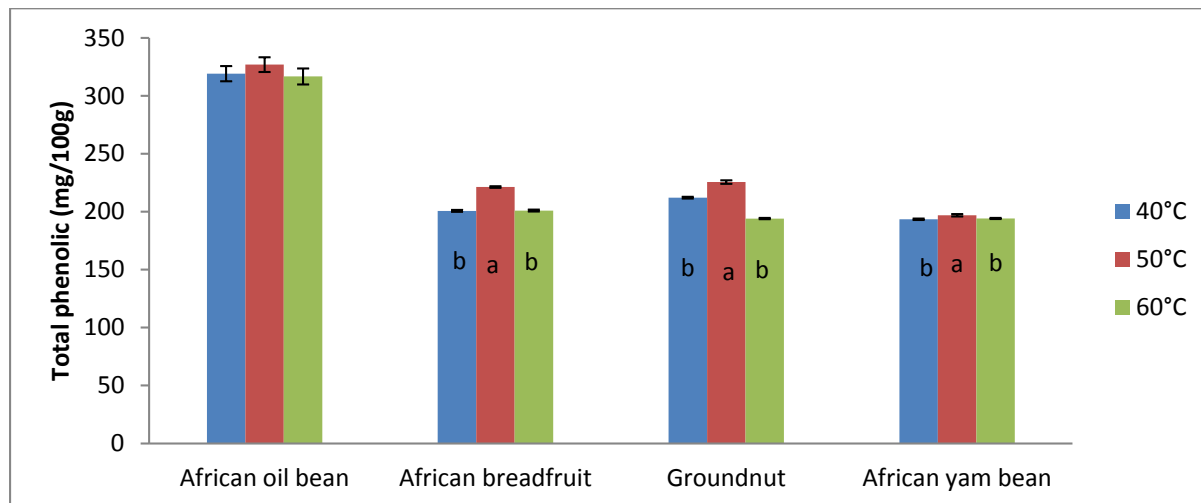


Figure 7: Effect of extraction temperature on total phenolic content.

Key: Set of bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

The results of this study revealed that there was a steady increase in the total phenolics with corresponding increase in temperature from 200.67 to 221.28 mg/100 g, 212.11 to 225.56 mg/100 g and 193.44 to 196.94 mg/100 g in African breadfruit, groundnut and African yam bean seed, respectively. The increase in total phenolics observed in this study from 40 to 50°C extraction temperature could be attributed to increase solubility and diffusion coefficient of the phenolic compounds, adequate mass transfer and penetration of solvent into the sample matrix, disruption of phenolic-macromolecule complex as well as softening of the plant matrix for easy penetration of mild heat and solvents (Shi *et al.*, 2005; Al-Farsi and Lee, 2008; Wang *et al.*, 2008; Tan *et al.*, 2013). Therefore, from the result of this study, it can be deduced that, for optimal yield of total phenolic from African breadfruit, groundnut and African yam bean extraction temperature of 50°C is recommended. However, at 60°C extraction temperature, there was significant decrease in the total phenolic content. This trend agrees with the findings of Liyana-Pathirana and Shahidi (2005) and Tan *et al.* (2013) who reported that elevating the extraction temperature to a certain level might results into concurrent disruption of the total phenolic mobilised at low temperature.



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Therefore, for maximum yield of total phenolics it is recommended that African oil bean should be extracted with 60% acetone (v/v) for 50 minutes at 50°C; African breadfruit extracted with 40% acetone (v/v) for 30 minutes at 50°C; groundnut extracted with 40% acetone for 30 minutes at 50°C and African yam bean seed extracted with 100% acetone (v/v) for 30 minutes at 50°C.

CONCLUSION

The present study has established that for maximum yield of total phenolic compound from the selected indigenous legumes namely African oil bean, African breadfruit, groundnut and African yam bean seed, their extraction should be carried out with 60% acetone (v/v) for 50 minutes at 50°C; with 40% acetone (v/v) for 30 minutes at 50°C; with 40% acetone for 30 minutes at 50°C and with 100% acetone (v/v) for 30 minutes at 50°C, respectively.

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