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

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ABSTRACT

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

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

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 e*t 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

41% of the total groundnut production in West Africa (Echekwu and Emeka, 2005). D industrial potentials of the nut in the production of different products, its uses in Niger largely with the rural women. United States Department of Agriculture (2011); Settal (2012) reported that peanut per 100 g contains 1.55 g water, 21.51 g carbohydrates, 8. 49.66 g lipids, 23.68 g proteins and 2448 kJ (585 kcal) total calories. Furthermore, t rich in minerals such as calcium, iron, magnesium, phosphorus, potassium, sodium, zinc manganese and selenium; vitamins for example thiamin, riboflavin, niacin, folate, vitan pantothenic acid; different compositions of saturated and unsaturated fatty acids (Settal 2012).

Studies on conventional legumes have shown that they constitute a large rese phytochemicals which are beneficial in preventing and managing the occurrence of deg diseases (Hu, 2003; Jacobs and Gallaher, 2004; Vadivel and Janardhanan, 2005; E 2010; Ade-Omowale *et al.*, 2015). There is immense research efforts on the possib utilizing natural sources of bioactive compounds for the dietary management of certair diseases due to the adverse effects associated with the use of synthetic chemicals. H need to assess the potentials of indigenous legumes in total phenolic yield to determine 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 mycrophyll* seed, African yam bean (*Sphenostylis stenocarpa*) seed, African breadfruit (*Treculia afric* groundnut (*Arachia hypogea*). The samples were procured in the month of December, 2 Umuahia Local Market and botanically identified by the Department of Crop Production, University of Technology, Minna, Nigeria.

Reagents

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

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 ± 2°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 ± 2°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 ± 2°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

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

Experimental design

Two factorial design was adopted for this study.

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

Where, β_o = over all mean or intercept; X_1 = legume types; X_2 = extraction variable 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 ge were analysed using analysis of variance (Steel and Torrie. 1980). The difference betwee values was determined by the least significant different test. Significance was accepted at probability level. Processed data were expressed in a bar chart format as mean ± standa (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 The results showed that acetone gave a significantly ($p < 0.05$) high yield of total phen African oil bean (160.68 mg/100 g), African breadfruit (125.00 mg/100 g), groundnut mg/100 g) and African yam bean seed (131.49 mg/100 g). Methanol gave the second

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), Luyana-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.

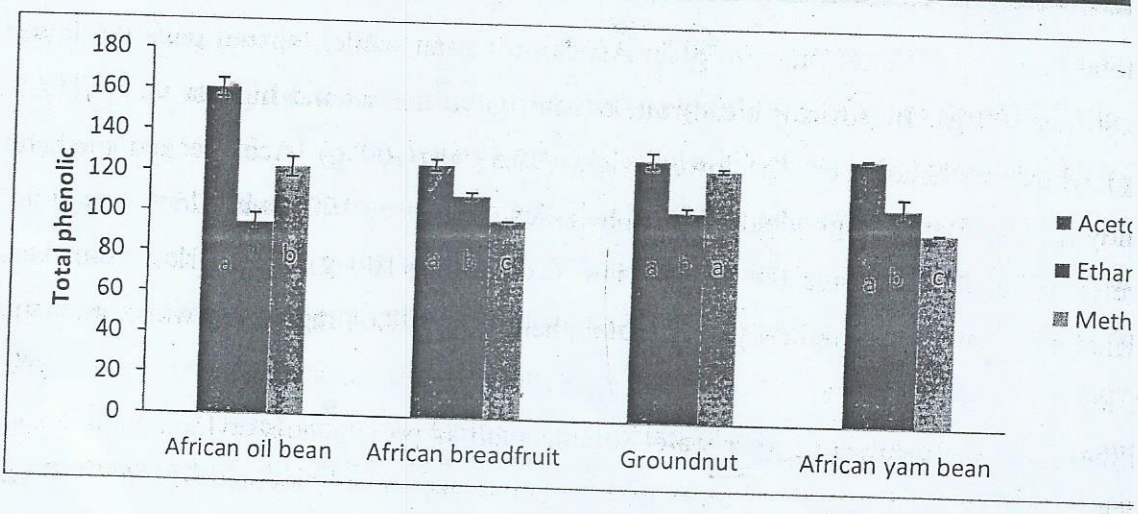

Figure 1: Effect of solvent type on total phenolic content
Key: Set of bar charts with different alphabets are significantly ($p < 0.05$) different fr other.

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

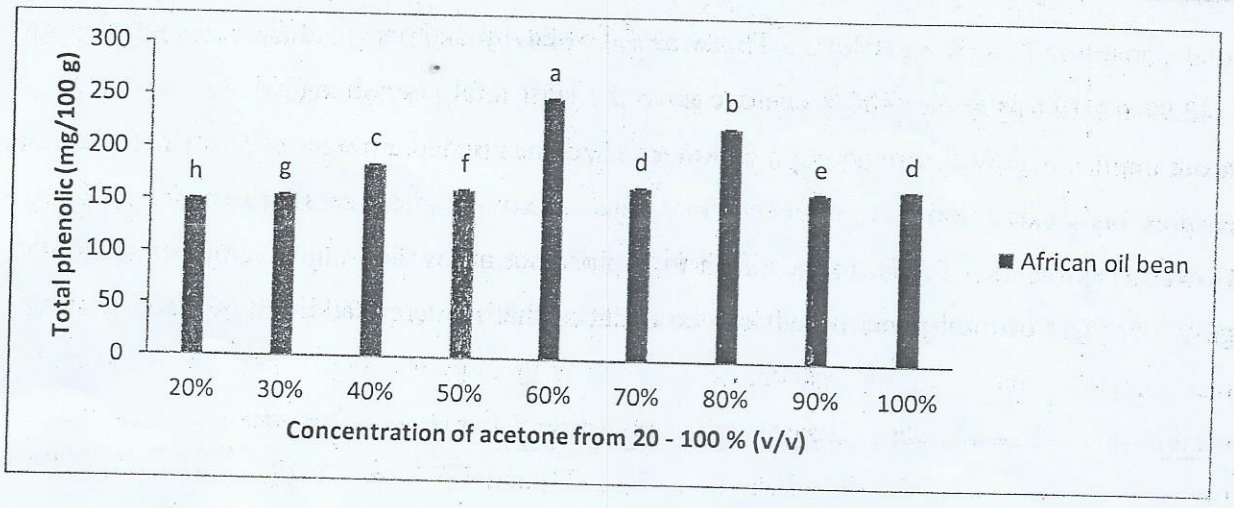

Figure 2: Effect of acetone concentrations on African oil bean total phenolic content
Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

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

total phenolic (156.49 mg/100 g). This was followed by acetone at 80% concentratio (142.97 mg/100 g) while, 100% acetone gave the least total phenolics (132.23 mg/100 result implies that moderate addition of water to acetone created a moderately polar medi ensures high extraction of polyphenol and thus improving the overall extraction effi However, extraction of African breadfruit total phenolics at low and high acetone concen gave low yield of total phenolics. It can be deduced that moderate addition of water to increases the polarity of the solvent so that the ratio of more polar phenolic compo African breadfruit extract increases with moderate water ratio. This study agrees w findings of Zhang *et al.* (2007), Chirinos *et al.* (2008) and Tan *et al.* (2013) who reported trend of total phenolic recovery in acetone in different plant matrix. Also, another p reason of increase extraction efficiency in the presence of some water might be due to i swelling power of the plant material by water which sufficiently exposes the contact surfa between the plant matrix and the extraction solvent (Tan *et al.*, 2013; Hemwimon *et al.*, 2(moderate polar solvent of 40% acetone (v/v) was chosen for the evaluation of extractio and temperature.

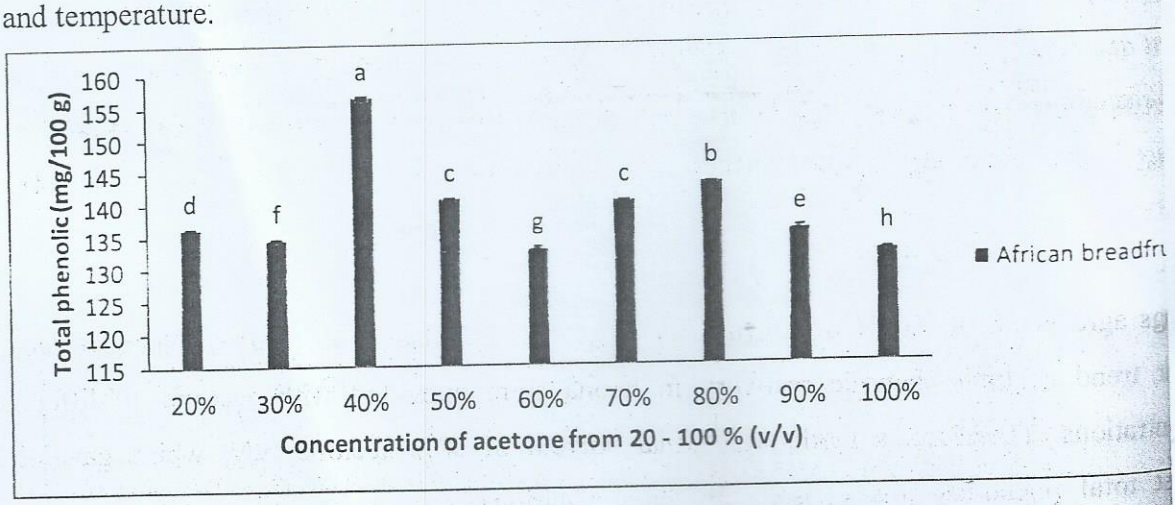

Figure 3: Effect of acetone concentrations on African breadfruit total phenolic content.
Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each oth

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 suggests 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.

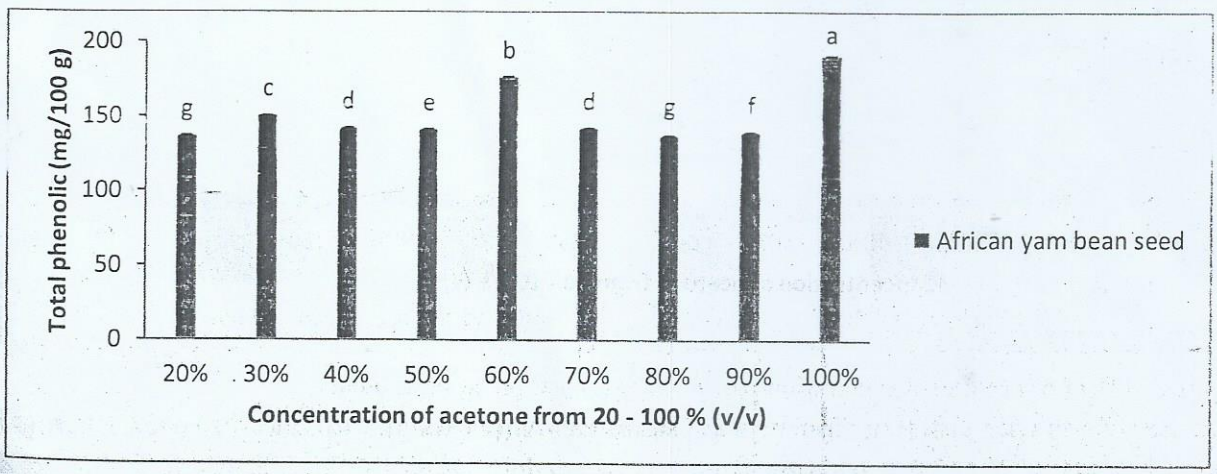

Figure 4: Effect of acetone concentrations on African yam bean seed total phenolic content.
Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

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total phenolics extracted from the testa of pink, red and yellow peanut, resp
(Khaopha *et al.*, 2012). The recovery of phenolic compounds from legume grains
mainly upon the type of solvent used, plant genotype, extraction time, extraction tem
among others (Agboola *et al.*, 2009; Vadivel *et al.*, 2012).

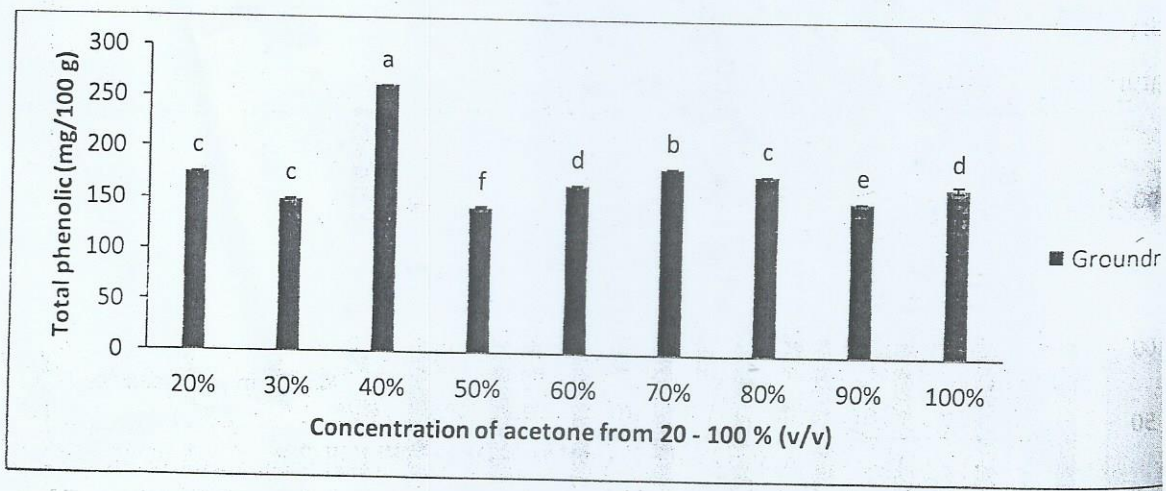

Figure 5: Effect of acetone concentrations on groundnut total phenolic content.
Key: Bar charts with different alphabets are significantly ($p < 0.05$) different from each oth

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 (Naczk and Shahidi, 2006; Chirinos *et al.*, 2007; Chew *et al.*, 2011).

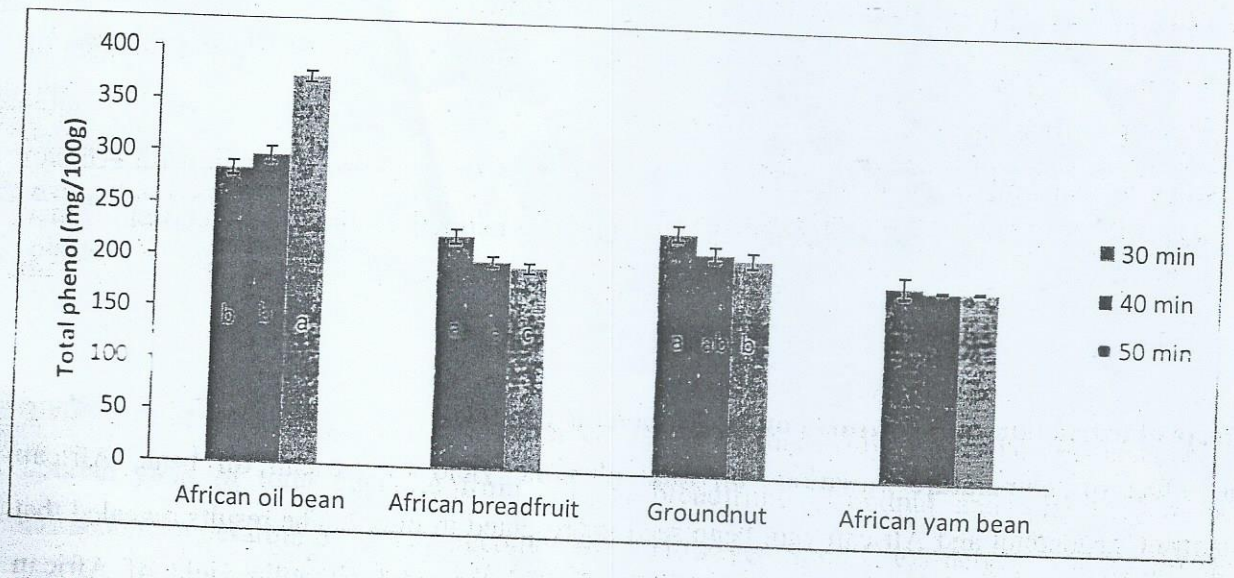

Figure 6: Effect of extraction time on total phenolic content
Key: Set of bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

African oil bean total phenolic extracted with 60% (v/v) acetone (373.00 mg/100 g minutes was significantly ($p < 0.05$) high than those extracted at 40 minutes and 60 min African breadfruit, the total phenolics extracted with 40% acetone (v/v) at 30 minutes mg/100 g) was significantly ($p < 0.05$) higher than the ones extracted at 40 minutes minutes. Groundnut total phenolic extracted with 40% acetone (v/v) at 30 minutes mg/100 g) was significantly ($p < 0.05$) higher than those extracted at 40 minutes and 50 n The gradual increase in the total phenolics with corresponding increase in extraction African oil bean (284.33 - 373.00 mg/100 g) can be well explained by Fick's second diffusion which reveals that a final equilibrium will be attained between the s concentration in the solid matrix and the solvent after a particular duration (Pinelo *et al.* Silva *et al.*, 2007). Therefore, for temperature evaluation on total phenolic yield, extracti of 50 minutes was chosen. However, the phenomenon of solvent equilibration did not a African breadfruit and groundnut whose high total phenolic contents were achieved at a time (30 minutes). This can be attributed to possible oxidation of their total phenolics at ex time (40 minutes and 50 minutes) adopted in this study. This view agrees with the findi Chew *et al.* (2011) and Tan *et al.* (2013) who in their studies reported low yield o phenolics in plant matrix at extended time. Therefore, for temperature evaluation on A breadfruit and groundnut total phenolics, 30 minutes extraction time was chosen. extraction time did no significantly ($p > 0.05$) affect the total phenolic recovery of Africa 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, A breadfruit, groundnut and African yam bean seed is presented in Fig. 7. The results reveale extraction temperature significantly ($p < 0.05$) affected the total phenolic yield of A breadfruit, groundnut and African yam bean seed. However, extraction temperature d significantly ($p > 0.05$) affect the total phenol content of African oil bean.

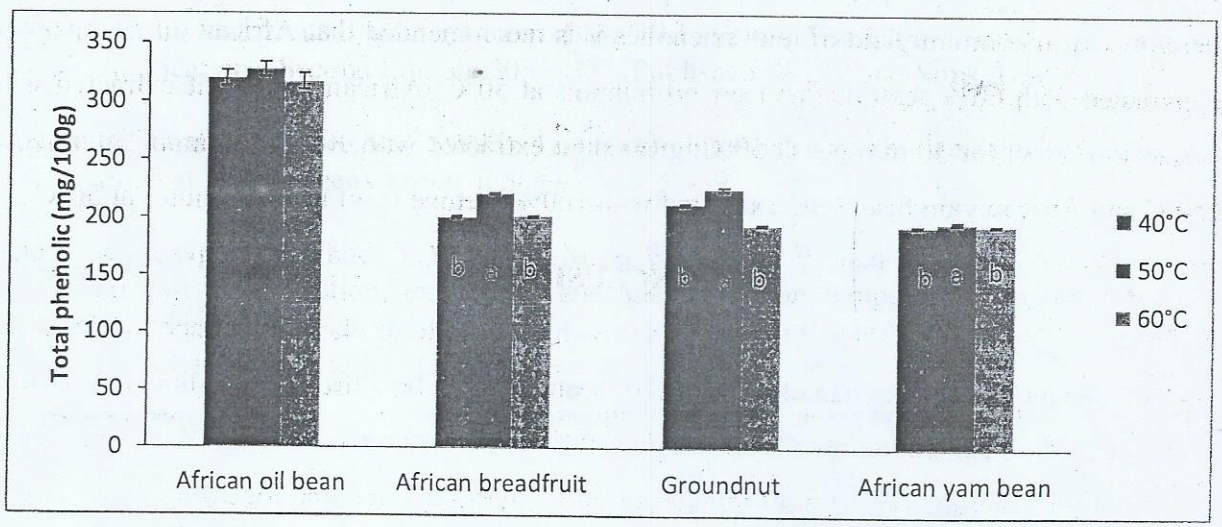

Figure 7: Effect of extraction temperature on total phenolic content.
Key: Set of bar charts with different alphabets are significantly ($p < 0.05$) different from each other.

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.

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

CONCLUSION

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

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