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Effect of solvent types on phenolics content and antioxidant activities of *Acacia polyacantha* gum

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Abstract

Acacia polyacantha gum (APG) is a dried exudate which obtained from the stems and branches of *Acacia polyacantha* trees. APG is rich in soluble dietary fibers as well as organic compounds. In this study quantitation of the levels of total phenolics content (TPC) and antioxidant activities were conducted using ABTS and CUPRAC assays for APG extraction using pure solvents (methanol, ethanol, acetone) and their aqueous mixtures at 50% and 100%. The antioxidant levels were evaluated by 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{+}$) radical cation deculturization and cupric iron reducing capacity in the presence of neocuproine (CUPRAC) for the evaluation of reducing power, and (TPC) was evaluated by the Folin-Ciocalteu method. The solvent Methanol (50%) gave the best extraction ratio for APG presented by highest (TPC 60.78 mg GAE/100g of DW, CUPRAC 34.65 mg TE/100g DW, and, ABTS about 37.65 mg TE/100g DW respectively), followed by ethanol 50% extract. On the other hand, pure methanol showed the lowest TPC 5.33 mg GAE/100g of DW, ABTS 10.9 mg TE/100g DW, and CUPRAC 7.80 mg TE/100g DW, values respectively. Therefore, the variation in the antioxidant capacity of extracts was possibly due to the difference of polarity, immiscibility and the nature of the APG compounds extracted using various solvents. The higher content of antioxidant activity in APG shall be useful to human health if it is properly utilized.

Introduction

Include the objectives of the research work at the last paragraph clearly natural antioxidants, particularly in fruits and vegetables have gained increasing interest among consumers and the scientific community because epidemiological studies have indicated that frequent consumption of natural antioxidants is associated with a lower risk of cardiovascular disease and cancer (Renaud *et al*., 1998; Temple, 2000; Frankel, 2014) . Gum Arabic (GA) (*Acacia senegal*, L. Willd) is an important food ingredient and feed commodity in Sudan. This product is crucial for Sudanese economy, with a total production of 110.000 thousand tons in 2006/7 harvested season with an increase of 20% of the total production in the last 10 years (Ibrahim, 2015). Sudan ranks first among the world's largest producers of GA and exports for marketing annually (Abdel Nour, 1999; Ibrahim, 2015).

GA is a water-soluble (Tiss *et al.*, 2001; Glogauer *et al.*, 2011) polysaccharide based on branched chains of (1-3) linked β-Degalactopyranosyl units containing α-L-arabinofuranosyl, α-L-rhamnopyranosyl, β-D-glucuronopyranosyl and 4-Omethyl-β-D-glucuronopyranosyl units (Deckwer *et al*., 2006). It is fabricated from gummy exudates of Acacia senegal (Xiao *et al*., 2012). In the colon, GA is fermented by microorganisms to short chain fatty acids (Phillips, 1998; Alvarez-Lorenzo *et al*., 2013). It is considered one of the safest dietary fibers (Anderson, 1986) . In Middle Eastern countries GA is used in the treatment of patients with chronic kidney disease (Ali *et al.*, 2009; Ali *et al.*, 2013). GA increases fecal nitrogen excretion (Bliss *et al.*, 1996) and decreases the production of free oxygen radicals (Ali *et al*., 2009).

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Acacia polyacantha gum (APG), the common local name is Kakamut or Umsinina belongs to the acacia complex groups this according to earlier studies conducted by Karamalla (1998) have reported that, polyacantha species is distributed throughout of tropical and subtropical Africa, very rich in arabinose galactoprotein. In addition to soluble dietary fibers, APG contains also a high level of Na and Fe both of them ranged from (41.98 to 47.23) µg/g and (24.02 to 31.79) µg/g, respectively in two different areas compared with other types of acacia complex group of gum Arabic (Ahmed, 2015).

Obviously, so far, much potential has been demonstrated to extract and identify the antioxidant activity from gum Arabic sources. For this, the first step in the bioactive component isolation from plant materials is extracted defiantly. The main goal of an extraction process is to gain maximum value of required active compound that containing the greatest antioxidant capacity or activity of extractions (Musa *et al*., 2011). Also, the extraction yield is tent to be affected by the nature of the chemical compounds, the technical process of extraction and the interference presence substrate (Chirinos *et al*., 2007). One of the most convenient ways for extraction is that solvent extraction because it has been spectrum used to acquire active ingredient components from plants. Therefore, extraction of solvent defines as a process that implemented to isolate dissolved antioxidant ingredients through the diffusion phenomena from a hard or solid substance or materials (tissue of the plan), to achieve this liquid material (solvent) must be used. It is reported that solvent system for extracting is chosen regarding the aim of extraction, for example, preparation or analysis, the target compounds naturally, the physicochemical characterization of the matrix, the facility of instruments and chemical materials, safety financial aspect (Yu *et al.*, 2002).

The specific objectives of this study were to determine the influence of different extraction by organic solvents and techniques to evaluate the antioxidant capacity/activity of *A. polyacanthan* gum using different antioxidant assays with respects several factors affecting antioxidant extraction. However, the type of solvent has a high and positive impact on the efficiency of extraction. Therefore, solvent extraction is commonly tended to be used for separation of antioxidant and yield of extraction. This is dependent on the procedure of solvent and extraction technique, due to the different of antioxidant affords of compounds with different polarity (Goli *et al*., 2005).

The major extraction method used broadly is solvent extraction technique because of the extracting solvents such as acetone, ethanol and methanol or mixtures of these with water for the recovery of a wide range of polyphenols of diverse phenolic structures (Abad-García *et al*., 2007). Furthermore, when water was mixed with organic solvents can contribute to the creation moderately medium polar that ensures polyphenols extraction (Singh *et al*., 2011). However, gum Arabic completely dissolved in water (Heidelberger *et al*., 1929); complete solubility is not required for antioxidants capacity determination because of it is the importance of extractability rather than the solubility of extraction substrate as well as turbidity (Liyana-Pathirana and Shahidi, 2006).

The choice of organic solvent that can be used in plant extraction is generally quite restricted, normally; solvents are used with boiling points not exceeding 80^{o}C. For the current studies the extraction solvents were chosen taking into consideration the following factors: polarity; boiling point, latent heat of vaporization, reactivity, viscosity- must be low, stability to heat, oxygen and light, safety, cost, and suitability for re-use (Dai and Mumper, 2010).

In this study, there were nine different polar solvents chosen so as to extract the bioactive component from *Acacia polyacantha* gum exudates for the quantification of phenolic compounds and determent of antioxidant activity. From the perspective of choosing the polarities of the antioxidant compound from individual samples are likely to be different, the choice of extraction solvents is critical. Moreover, solvent extraction is frequently used for isolation of antioxidants and the antioxidant chemical activity of extracts is strongly dependent on the solvent due to the different antioxidant potentials of compounds with different polarity (Rababah *et al.*, 2011).

Materials and methods

Chemicals, solutions and instrument

Neocuproine (2,9-dimethyl-1,10 phenanthroline) and Folin–Ciocalteau phenol reagent (Sigma Chemical Company, Steinheim, Germany), TR [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] (Aldrich Chemicals Company, Steinheim, Germany), ABTS [2,2′- azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] (Fluka Chemicals, Steinheim, Germany), ammonium acetate, copper(II) chloride, potassium persulphate, sodium hydroxide, copper (II) sulphate, sodium carbonate, sodium potassium tartrate, 96% methyl alcohol and methanol (E. Merck, Darmstadt, Germany). All spectrophotometric measurements were made with microplate contained 96 micro - cuvettes using

Spectro - Star Nano spectrophotometer.

Samples collection and preparation

The *Acacia polyacantha* gum nodules were collected in November 2015 from Blue Nile State, Sudan. All samples were cleaned from impurities such as bark and sand. For homogeneity of the sample one nodule was selected randomly from the others nodules and divided into two parts. One part of it was ground and converted to mechanical powder through U.S.A standard testing sieve with opening 1.40 mm (0.0555 inches), Fisher Company. After that, 1 gram of *A. polyacantha* gum powder was weighed used Glossaries, DHAUS sensitive balance in each vial with a capacity of 20 mL. Then 10 mL of each aqueous solvents solution (Acetone, Ethanol, Methanol and Distil water) were added into each vial with a different concentration of 50% and 100% with respect to pure distilled water.

In addition to, all vials congaing (samples and aqueous solvents solution) were placed into magnetics stirrer model RT 15P, Serial no 2930700. The samples left to rotate for 24 hours, after that, all extracted samples were centrifuged using centrifuge (Mini, China) for 10 min at stirring speed of 1000 (rpm). Moreover, the clarified suspension was filtered again used Sartorius PTEF 0.45 µm filter. Finally, the supernatants were collected for further analysis.

Extraction solvent

The presence of organic solvents was attempted to assess their capability to extract phenolics as a convenient antioxidant from *A. polyacantha* gum nodules. Solvents included: acetone, ethanol, methanol, acetone and their mixture with water. All solvents and chemicals used were of analytical grade and obtained from either. The solvent extraction procedure was carried out according to the extraction procedures described by (Soares *et al*., 2009) , a required amount (1g) of *A. polyacantha* gum dry powder was weighed accurately using analytical balance (Glossaries, DHAUS) and each sample was mixed with 10 mL of methanol, ethanol, acetone, aqueous methanol (50 and 100%), aqueous ethanol (50 and 100%) and aqueous acetone (50 and 100%) to investigate the effect of solvent on the content of phenolic compounds in a beaker (which was wrapped with aluminum foil to prevent spilling of mixture and light exposure). The mixture was then shaken for 24 h, at ambient temperature. After extraction, the *A. polyacantha* gum extract was filtered using Sartorius PTEF 0.45 µm and the filtrate was placed in vials and kept in -20°C until used for further analysis studies.

Determination of total phenolic content (TPC)

Antioxidant activity was determined using TPC based on the method of (Musa *et al*., 2011). Approximately 0.4 mL distilled water and 0.5 mL diluted Folin–Ciocalteu reagent was added to 100 µL sample extracts. The samples (sample extracts with Folin–Ciocalteu reagent) were set aside for 5 min before 1 mL 7.5% sodium carbonate (w/v) was added. The absorbances were taken at 765 nm wavelength using a spectrophotometer after 2 h. The calibration curve of gallic acid (GA) was used for the estimation of sample activity capacity. The result was recorded in terms of mg of GA equivalents per 100 g of fresh sample (mg GA/100 g of FW).

Free radical-scavenging ability by the use of a stable (ABTS)

The ABTS radical cation (2, 2-azino-bis-3 ethylbenzothiazoline-6-sulfonic acid) was generated by the interaction of ABTS (250 µM) and K2S2O8 (40 µM). After the addition of 990 µL of ABTS solution to 10 ml of fruit extract, the absorbance at 734 nm was monitored. The percentage decrease of the absorbance was calculated and plotted as a function of the concentration of the extracts and Trolox for the standard reference data (Ozgen *et al.*, 2008). The following formula was used:

$$(\%)\text{of reduction power}=(A_{blank}- A_{sample})/A_{blank} \times 100$$

Where: (A) is the absorbance.

CUPRAC assay of total antioxidant capacity

Antioxidant activity was determined using CUPRAC, to a test tube were added 1 mL of $CuCl_2$ solution (1.0 × 10 2) m), 1 mL of neocuproine alcoholic solution (7.5 $\times 10^3$) m), and 1-mL NH4Ac buffer solution and mixed; 100 mL of (*A. polyacantha* gum samples) extract followed by 1.1 mL distil water were added (total volume 4.1 mL), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. As the molar absorptivity of Trolox (TR) in the CUPRAC method is $\varepsilon = 1.67 \times 10^4$ L mol^{-1}) 1 cm^{-1}) and the calibration curve for pure TR is a line passing through the origin, the TR equivalent molar concentration of the *A. polyacantha* gum extract sample in final solution was determined by dividing the observed absorbance to the ε for TR (microplate 1 cm). The TR equivalent antioxidant capacity may be traced back to the original extract considering all dilutions, and proportionated to the initial mass of *A. polyacantha* gum extracted sample taken to find a capacity in the units of micromoles TR per gram dry matter.

Statistical analysis

All assays were carried out in triplicates and the results are expressed as an average ± SD. One way ANOVA testing was used to analyze statistical differences amongst the various extracts for phenolic compound contents and different antioxidant assays with a least significance difference (LSD) ($P < 0.05$) as a level of significance. Correlations between the content of the components and antioxidant attributes were determined by linear regression analysis employing Mini tab software®(version 17).

Results and discussion

Extraction of total phenolics contents

The solvents percentage of the extracts of *A. polyacantha* gum was shown in Table 1. The extraction of antioxidant content of these samples from original dried gum powder with a descending order of acetone (100%) > acetone (50%) ethanol (100 %) > ethanol (50%) > methanol (100%) > methanol (50%). Thus, extraction with hydro-alcoholic solvents resulted in the highest amount of total extractable compounds. Whereas the extraction of antioxidant content yield with methanol 50%, was a significantly higher than all other solvents. The result was agreed with those findings reported in literature methods recorded that methanol was claimed to be a good extraction solvent for low-polymerized flavonols (Kallithraka *et al*., 1995). Methanol has also been selected as the hydrophilic extraction solvent for apricot, due to lipophilic contribution to overall antioxidant capacity is much lower (Scalzo *et al*., 2005).

Phenolics are plant secondary metabolites and are very important by virtue of their antioxidant activity by chelating redox-active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversions into reactive oxyradicals (Sahreen *et al.*, 2010). Table 1 summarizes the total phenolic compounds (TPC) in the extracts (expressed as gallic acid equivalents (GAE)). CUPRAC and ABTS in terms of total phenolics content in the extract (expressed as microgram of Trolox equivalents (TE). These varied between 4.26 mg and 34.65 mg/100g dry weight (DW) of extract for all solvent types. However, the methanolic extract at 50% showed the highest TPC about 60.7833 ± 0.0416 mg of GAE/100g DW. Whereas the content of TPC obtained with acetone and methanol 100% were much smaller ($P < 0.05$) values revealed 6.5567 ± 0.1504 mg and 5.3300 ± 0.0608 mg GAE/100g DW respectively.

On the other hand, CUPRAC expressed as Trolox equivalent, 7.6233 ± 0.0751 to 20.1367± 0.0153, mg TF/ 100g DW for acetone, ethanol, methanol and their aqueous solutions. Methanol (50%) extract showed the highest antioxidant activity determined by CUPRAC (34.6533 ± 0.0611 mg TF/ 100g). However, the acetone 50% and methanol 100% both of them revealed the lowest (7.6233 ± 0.0751 and 7.8000 ± 0.1249) mg TE/ 100g DW with a significant difference at $p < 0.05$. The antioxidant activity of phenolics content is shown in Table 1 and Figure 1, respectively.

Correlation

Correlation analysis for phytochemical contents with antioxidant activity of various extracts of *A. polyacantha* gum showed that the contents of phenolics exhibited a good correlation with TPC and CUPRAC only for some extracts: methanol 50% (TPC with CUPRAC), ethanol (TPC with ABTS and CUPRAC with ABTS.

Extraction yields

Extraction is the first step in the isolation of phenolic compounds from *A. polyacantha* gum. Extraction is influenced by the chemical nature of the compounds (simple and complex phenolics), the extraction method employed, the storage time and conditions, and the presence of interfering substances. Solvent extraction is a process designed to separate soluble phenolic compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent). This process is widely employed for phenolic extraction from various vegetable materials (Chirinos *et al*., 2007). The effects of different extracting solvents have been tested for the extraction of polyphenols from plant material. It is well known that the outputs yield of chemical extraction depends on the type of solvents with varying polarities, pH, extraction time and temperature, as well as on the chemical compositions of the sample. Under the same conditions of time and temperature, the solvent and the chemical properties of the sample are two most important factors (López *et al*., 2011). So, processing efficiency is quantitatively related to extraction yield (de Campos *et al.*, 2008). Variation in the antioxidant activity of various extracts is attributed to polarities of different compounds present in the plant and such differences have been reported in the literature (Hayouni *et al*., 2007).

In this study, different solvents with different polarities were used to determine which gave the greatest recovery of phenolic compounds. Nine solvents were tested: (1) Pure methanol, (2) methanol (50%), (3) pure ethanol, (4) ethanol (50%), (5) pure acetone and acetone (50%). The extraction of

Table 1. The values of antioxidant activity of phenolics contents compare to the effect of solvents polarity index on *Acacia polyacantha* gum extraction

Entry	Solvent types	Antioxidant activity			Polarity
		TPC a	CUPRAC b	ABTS c	
1	Acetone	06.5567 ± 0.1504	09.5000 ± 0.1000	4.2667 ± 0.0306	5.5
2	Acetone50 %	10.4833 ± 0.1041	07.6233 ± 0.0751	10.340 ± 10.01	7.2
3	Ethanol	9.13000 ± 0.0100	17.8833 ± 0.0764	18.600 ± 0.1000	5.2
4	Ethanol 50 %	28.5000 ± 0.0458	20.1367 ± 0.0153	34.4667 ± 0.0379	7.1
5	Methanol	05.3300 ± 0.0608	07.8000 ± 0.1249	10.9000 ± 0.0436	6.6
6	Methanol 50%	60.7833 ± 0.0416	34.6533 ± 0.0611	37.6467 ± 0.0808	7.8

[a] Total phenolic content.
[b] Cupric iron reducing capacity.
[c] 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)

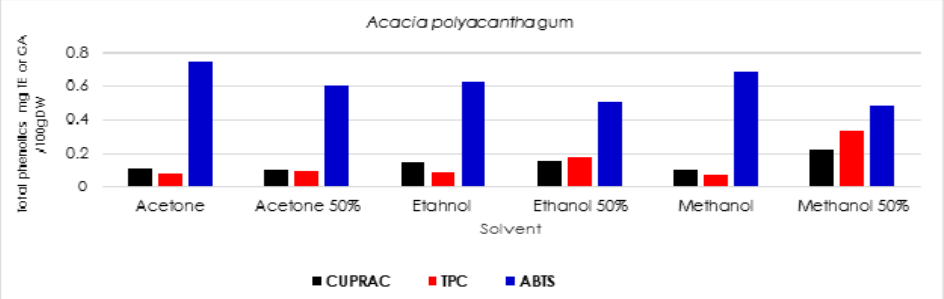

Figure 1. The influence of solvents on extraction efficiency of CUPRAC from *A. polyacantha* gum. Values are expressed as average ± SD of triplicate. DW = dry weight. TE=Trolox equivalent

antioxidant activity is dependent on the nature of the solvent used. Based on our results reported here, the highest extraction yield was found with hydro-alcoholic solvents. This indicates that most of the components in *A. polyacantha* gum are hydrophilic or water-soluble. The extract of antioxidant content with the solvent polarity as showed in Figure 1 and Table 1. Moreover, the addition of water into acetone and ethanol tremendously increases the extract of antioxidant compounds. It was also found that the highest antioxidant content can be achieved at 50% methanol solvent. These values were higher than those seen using pure solvents and dramatically higher than by using solvent at 100% in this study, since both the polar and less polar compounds were co-extracted together (Markom *et al.*, 2007).

In addition to, alcoholic solvents have been commonly employed to extract phenolics from natural sources products where they gave quite a high antioxidant activity / capacity of total extract even though they are not highly selective for phenols. Mixtures of alcohols and water have revealed to be more efficient in extracting phenolic constituents than compared to the mono-component solvent system (Spigno *et al*., 2007). The addition of small quantity of water to organic solvent usually creates a more polar medium which facilitates the extraction of polyphones as suggested by (Spigno *et al.*, 2007). By increasing the proportion of water, the polarity of the solvent also increases. When this is achieved, the solvent system is able to extract phenolic substances from both ends of the polarity range (highest polarity substances and low polarity substances), as well as those of moderate polarity (Uma *et al*., 2010).

Similar results were reported previously (Zhu *et al.*, 2011). Later reports studied extracts from the defatted wheat germ and noted that the yield of 50% ethanol extract did not show significant difference with the 30% and 70% ethanol extracts; but the yield of the aqueous extract was significantly ($P < 0.05$) different from the 100% ethanol extract. Moreover, these results agree with further studies which reported that aqueous ethanol (50%) showed the highest yield in extraction of phenolic compounds from *Phyllanthus niruri* (Markom *et al*., 2007). This is followed by methanol, ethanol, and acetone respectively.

The lowest extraction antioxidant activity was obtained by using acetone and methanol 100% (Table 1 and Figure 1) both were showed (4.2667 ± 0.0306 and 5.3300 ± 0.0608 for the TPC and ABTS) respectively with the polarity of 5.4 and 6.6. the result suggesting that polar compounds in the plant matrix would be easier to extract with a more polar solvent while lower polarity solvents enable to obtain the extracts with a higher concentration of bioactive

compounds (Bimakr *et al.*, 2011). Therefore, solvent polarity plays a crucial role in this and other extraction studies. The net molecular polarities of solvents are measured by their dipole moments. The polarities of solvents used are listed in Table 1. Nevertheless, the separation of components by solvents depends on the polarity of both the solvent and the component. According to (Markom *et al*., 2007), a single solvent might or might not be selective for the separation of two components.

Effect of extraction solvent on total phenolic and extractants

Methanol, ethanol and acetone separately or mixed with water are commonly used to extract phenolic compounds from sample (Uma *et al*., 2010). The ability of different solvents in extracting phenolic compounds was compared by performing Folin-Ciocalteu assay method. The results were expressed as gallic acid equivalents (mg GAE/g dry weight of spearmint leaves). Data show that extract of phenolic compounds is significantly affected by the extractant used. The concentration of polyphenols was determined by means of the Folin-Ciocalteou reagent, made from a mixture of phosphotungstic and phosphomolybdic acids. The addition of the Folin-Ciocalteou reagent to the polyphenol solution leads to the formation of chromophore compounds which have a maximum absorbance at wavelengths of 700 nm (Singleton and Rossi, 1965).

In this study, methanol-water at (50 %) extracted markedly greater amounts of phenolic compounds (60.7833 ± 0.0416, 34.6533± 0.0611 and 37.6467± 0.0808 mg GAE/100g DW for TPC, CUPRAC and ABTS respectively at polarity of 7.8) compared with pure methanol and pure acetone which presented the lowest contents for antioxidant activity showed ABTS and CUPRAC (9.5000± 0.1000, and 4.2667 ± 0.0306 mg TE /100g DW respectively). Whereas the value of antioxidant extracted by methanol revealed for ABTS and CUPRAC (10.9000 ± 0.0436 and 7.8000± 0.1249 mg TE /100g DW compared to only 5.3300 ± 0.0608 mg GAE/100g DW for TPC). Similar studies have that found that ethanol (50%) was examined the extraction of phenolics from citrus peels (Zahra'u *et al*., 2014). This study reported that the recovery of total phenolics increased with increasing methanol concentration until the concentration reached 85%; after which, the recovery reduced with the increase of ethanol concentration. Whoever, this may be not suitable for gum Arabic because gum completely dissolved in water into macro fragments such as protein and carbohydrates (arabino galactoprotein) AGP in other terms for phenolic determination extractability is more convenient than solubility. Our results are in accordance with those reported previously which has shown that methanol had better recoveries and is specifically effective in extracting polyphenols (Abaza *et al.*, 2011).

A recent report studying the influence of neat solvents on the extractability of total phenolics from the black cohosh matrix reported similar results. (Mukhopadhyay *et al*., 2006). According to (Anokwuru *et al.*, 2011) , the result of the total phenolic content of *Hibiscus sabdariffa* calyx in different solvents demonstrated that the methanol extract yield was higher than the yields both of ethanol and acetone. Similarly, it has been reported that the methanol extract of *Bridelia Retusa* Spreng Bark contained relatively higher levels of total phenolics than the other extracts (Banerjee and Bonde, 2011). Conversely, the aqueous mixtures of the ethanol and acetone tested in our study showed the same patent (Figure 4) of polyphenolic concentrations in contrast with the pure solvents. The findings were completely difference with other studies which reported that ethanol and acetone at 50% were the most efficient solvents compared to 100% ethanol and acetone for extracting phenolic compounds from black and black mate tea (Turkmen *et al.*, 2006).

In addition, similar results have been reported in studying the phenolic content of selected tropical fruits from Malaysia. (Alothman *et al*., 2009). Still in this context, (Mukhopadhyay *et al*., 2006), in studying the extraction efficiency of black cohosh have examined the aqueous effect by employing ethanol: water mixture (50:50, v/v) and obtained a high total phenolic content value compared with pure ethanol. Furthermore, the results with methanol – water 50: 50, % v/v was found to be similar to that with ethanol: water (50:50, v/v).

The high absorbance values indicated that the sample possessed significant antioxidant activity. In this study, maximum antioxidant capacities were observed in methanolic and ethanolic extracts without significant difference. This trend was similar to that observed in other studies examining the antioxidant capacity of Rio Red extracts (Jayaprakasha *et al*., 2008).

The differential responses between the methods used to evaluate the antioxidant activity in this study may partially be attributed to qualitative variations of the phenolic compounds as the structural differences may modify the antioxidant potential of the phenolic. Furthermore, some of the extracts have hydrophilic and hydrophobic compounds and those samples may not work efficiently in some in vitro model systems. (Casazza *et al.*, 2010).Therefore, the trend of

antioxidant activity of *A. polyacantha* gum extracts cannot be compared from one method to another method due to their different mechanisms involved in the assay.

Conclusion

In this study, the extraction solvent significantly affected total phenolics and antioxidant contents extracted from *A. polyacantha* gum collected from Sudan in season 2014/ 2015. Over all, the results showed that methanol at (50%) was the most efficient solvent for phenolic extraction. Further, antioxidant potential evaluation by this methods showed that methanol and ethanol extracts were promising. Nevertheless, according to the correlation study, the activity of the aqueous ethanolic extract is attributed to the phenolic contents. However, this is not the case for the aqueous acetonic extract. Moreover, acetone is regarded as a low-toxicity solvent. There are safety concerns associated with the use of aqueous ethanol for extraction of phenolic compounds from *A. polyacantha* gum. In cases where the extract is used for medicinal or ingestion purposes, pure ethanol or a mixture of ethanol and water has typically been used. Ethanol is also more acceptable for use in the food industry. From the results obtained in the extraction of the antioxidant compounds from *A. polyacantha* gum with different solvents at laboratory level, a mixture of methanol with water at a ratio of 50:50 (v/v) was the best choice among other solvent compositions evaluated in this study. So, the present results provide evidence for the potent antioxidative effect of methanolic at 50% extract under in vitro conditions. Consequently, the results of this study suggested that the extract can be utilized as an effective and safe antioxidant source. The phenolic compounds obtained for this procedure could be used as additives in food products as natural antioxidants to extend their shelf-life. The present research renews interest in the increased use of naturally occurring antioxidants. Furthermore, it can be concluded that *A. polyacantha* gum consumed as a foodstuff in different areas of Sudan as well as tropical Africa can be used as an accessible source of natural antioxidants with consequent health benefits. However, the components responsible for the antioxidative activity of aqueous methanolic extract are currently unclear. Therefore, it is suggested that further work could be performed in the isolation and identification of the antioxidative components in all *Acacia* complex group of gums.

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References

Abad-García, B., Berrueta, L., López-Márquez, D., Crespo-Ferrer, I., Gallo, B. and Vicente, F. 2007. Optimization and validation of a methodology based on solvent extraction and liquid chromatography for the simultaneous determination of several polyphenolic families in fruit juices. Journal of Chromatography A 1154(1): 87-96.

Abaza, L., Ben Youssef, N., Manai, H., Mahjoub Haddada, F., Methenni, K. and Zarrouk, M. 2011. Chétoui olive leaf extracts: influence of the solvent type on phenolics and antioxidant activities. Grasas y Aceites 62(1): 96-104.

Abdel Nour, H. 1999. Gum Arabic in Sudan: production and socio-economic aspects. Paper presented at the International Expert Meeting on Medicinal, Culinary and Aromatic Plants in the Near East., 19-21 May 1997. Cairo (Egypt).

Ahmed, O.A.A. 2015. The Thermodynamic Properties of Some Acacia Gums. Sudan: Sudan University of Science and Technology, PhD. Thesis

Ali, B. H., Al-Husseni, I., Beegam, S., Al-Shukaili, A., Nemmar, A., Schierling, S., Queisser, N. and Schupp, N. 2013. Effect of gum arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. PLoS One 8(2): e55242.

Ali, B. H., Ziada, A. and Blunden, G. 2009. Biological effects of gum arabic: a review of some recent research. Food and Chemical Toxicology 47(1): 1-8.

Alothman, M., Bhat, R. and Karim, A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry 115(3): 785-788.

Alvarez-Lorenzo, C., Blanco-Fernandez, B., Puga, A.M. and Concheiro, A. 2013. Crosslinked ionic polysaccharides for stimuli-sensitive drug delivery. Advanced Drug Delivery Reviews 65(9): 1148-1171.

Anderson, D. 1986. Evidence for the safety of gum arabic (Acacia senegal (L.) Willd.) as a food additive—a brief review. Food Additives and Contaminants 3(3): 225-230.

Anokwuru, C., Anyasor, G., Ajibaye, O., Fakoya, O. and Okebugwu, P. 2011. Effect of extraction solvents on phenolic, flavonoid and antioxidant activities of three nigerian medicinal plants. Natural Science 9(7): 53-61.

Banerjee, S.K. and Bonde, C. 2011. Total phenolic content and antioxidant activity of extracts of *Bridelia retusa* Spreng Bark: Impact of dielectric constant and geographical location. Journal of Medicinal Plants Research 5(5): 817-822.

Bimakr, M., Rahman, R.A., Taip, F.S., Ganjloo, A., Salleh, L.M., Selamat, J. and Zaidul, I. 2011. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. Food and Bioproducts Processing 89(1): 67-72.

Bliss, D.Z., Stein, T.P., Schleifer, C.R., and Settle, R.G. 1996. Supplementation with gum arabic fiber increases fecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet. The American Journal of Clinical Nutrition 63(3): 392-398.

Casazza, A.A., Aliakbarian, B., Mantegna, S., Cravotto, G. and Perego, P. 2010. Extraction of phenolics from *Vitis vinifera* wastes using non-conventional techniques. Journal of Food Engineering 100(1): 50-55.

Chirinos, R., Rogez, H., Campos, D., Pedreschi, R. and Larondelle, Y. 2007. Optimization of extraction conditions of antioxidant phenolic compounds from mashua (*Tropaeolum tuberosum* Ruíz and Pavón) tubers. Separation and Purification Technology 55(2): 217-225.

Dai, J. and Mumper, R.J. 2010. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules 15(10): 7313-7352.

de Campos, L.M., Leimann, F.V., Pedrosa, R.C. and Ferreira, S.R. 2008. Free radical scavenging of grape pomace extracts from Cabernet sauvingnon *(Vitis vinifera)*. Bioresource Technology 99(17): 8413-8420.

Deckwer, W.-D., Dill, B., Eisenbrand, E., Bornscheuer, U., Pühler, A., Heiker, F., Kirschning, A., Schreier, P., Fugmann, B. and Pohnert, G. 2006. Römpp online. Georg-Thieme-Verlag.

Frankel, E. N. 2014. Lipid oxidation. USA: Elsevier.

Glogauer, A., Martini, V.P., Faoro, H., Couto, G.H., Müller-Santos, M., Monteiro, R.A. and Krieger, N. 2011. Identification and characterization of a new true lipase isolated through metagenomic approach. Microbial Cell Factories 10(1): 1.

Goli, A.H., Barzegar, M. and Sahari, M.A. 2005. Antioxidant activity and total phenolic compounds of pistachio *(Pistachia vera)* hull extracts. Food Chemistry 92(3): 521-525.

Hayouni, E.A., Abedrabba, M., Bouix, M., and Hamdi, M. 2007. The effects of solvents and extraction method on the phenolic contents and biological activities *in vitro* of Tunisian *Quercus coccifera* L. and Juniperus phoenicea L. fruit extracts. Food Chemistry 105(3): 1126-1134.

Heidelberger, M., Avery, O.T. and Goebel, W.F. 1929. A" soluble specific substance" derived from gum arabic. The Journal of Experimental Medicine 49(5): 847-857.

Ibrahim, M. O. H. 2015. Assessment of Gum Arabic Marketing System in Main Auction Markets of North Kordofan State-Sudan. Sudan: Universtiy of Khartoum, MSc. Thesis.

Jayaprakasha, G., Girennavar, B. and Patil, B.S. 2008. Radical scavenging activities of Rio Red grapefruits and Sour orange fruit extracts in different *in vitro* model systems. Bioresource Technology 99(10): 4484-4494.

Kallithraka, S., Garcia-Viguera, C., Bridle, P. and Bakker, J. 1995. Survey of solvents for the extraction of grape seed phenolics. Phytochemical Analysis 6(5): 265-267.

Liyana-Pathirana, C.M. and Shahidi, F. 2006. Importance of insoluble-bound phenolics to antioxidant properties of wheat. Journal of Agricultural and Food Chemistry 54(4): 1256-1264.

López, A., Rico, M., Rivero, A. and de Tangil, M.S. 2011. The effects of solvents on the phenolic contents and antioxidant activity of Stypocaulon scoparium algae extracts. Food Chemistry 125(3): 1104-1109.

Markom, M., Hasan, M., Daud, W.R.W., Singh, H., and Jahim, J.M. 2007. Extraction of hydrolysable tannins from Phyllanthus niruri Linn.: Effects of solvents and extraction methods. Separation and Purification Technology 52(3): 487-496.

Mukhopadhyay, S., Luthria, D.L. and Robbins, R.J. 2006. Optimization of extraction process for phenolic acids from black cohosh *(Cimicifuga racemosa)* by pressurized liquid extraction. Journal of the Science of Food and Agriculture 86(1): 156-162.

Musa, K.H., Abdullah, A., Jusoh, K. and Subramaniam, V. 2011. Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. Food Analytical Methods 4(1): 100-107.

Ozgen, M., Durgaç, C., Serçe, S. and Kaya, C. 2008. Chemical and antioxidant properties of pomegranate cultivars grown in the Mediterranean region of Turkey. Food Chemistry 111(3): 703-706.

Ozgen, M., Reese, R.N., Tulio, A.Z., Scheerens, J.C., and Miller, A.R. 2006. Modified 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) methods. Journal of Agricultural and Food Chemistry 54(4): 1151-1157.

Phillips, G.O. 1998. Acacia gum (gum arabic): a nutritional fibre; metabolism and calorific value. Food Additives and Contaminants 15(3): 251-264.

Rababah, T.M., Al-Mahasneh, M.A., Kilani, I., Yang, W., Alhamad, M.N., Ereifej, K. and Al-u'datt, M. 2011. Effect of jam processing and storage on total phenolics, antioxidant activity, and anthocyanins of different fruits. Journal of the Science of Food and Agriculture 91(6): 1096-1102.

Renaud, S.C., Guéguen, R., Schenker, J. and d'Houtaud, A. 1998. Alcohol and mortality in middle-aged men from eastern France. Epidemiology 9(2): 184-188.

Sahreen, S., Khan, M.R. and Khan, R.A. 2010. Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chemistry 122(4): 1205-

1211.
Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B. and Battino, M. 2005. Plant genotype affects total antioxidant capacity and phenolic contents in fruit. Nutrition 21(2): 207-213.
Singh, A., Kuila, A., Yadav, G. and Banerjee, R. 2011. Process optimization for the extraction of polyphenols from okara. Food Technology and Biotechnology 49(3): 322.
Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16(3): 144-158.
Soares, A.A., de Souza, C.G.M., Daniel, F.M., Ferrari, G.P., da Costa, S.M.G. and Peralta, R. M. 2009. Antioxidant activity and total phenolic content of Agaricus brasiliensis (*Agaricus blazei* Murril) in two stages of maturity. Food Chemistry 112(4): 775-781.
Spigno, G., Tramelli, L. and De Faveri, D.M. 2007. Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering 81(1): 200-208.
Temple, N.J. 2000. Antioxidants and disease: more questions than answers. Nutrition Research 20: 449-459.
Tiss, A., Carrière, F. and Verger, R. 2001. Effects of gum arabic on lipase interfacial binding and activity. Analytical Biochemistry 294(1): 36-43.
Turkmen, N., Sari, F. and Velioglu, Y.S. 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. Food Chemistry 99(4): 835-841.
Uma, D., Ho, C. and Wan Aida, W. 2010. Optimization of extraction parameters of total phenolic compounds from Henna *(Lawsonia inermis)* leaves. Sains Malaysiana 39(1): 119-128.
Xiao, L., Xiao, M., Jin, X., Kawasaki, K., Ohta, N. and Sakaguchi, E. 2012. Transfer of blood urea nitrogen to cecal microbial nitrogen is increased by mannitol feeding in growing rabbits fed timothy hay diet. Animal 6(11): 1757-1763.
Yu, L., Perret, J., Davy, B., Wilson, J. and Melby, C. 2002. Antioxidant properties of cereal products. Journal of Food Science 67(7): 2600-2603.
Zahra'u, B., Mohammed, A., Ghazali, H. and Karim, R. 2014. Baobab Tree (*Adansonia digitata* L) Parts: Nutrition, Applications in Food and Uses in Ethno-medicine–A Review. Annals of Nutritional Disorders and Therapy 1(3): 1011.
Zhu, K.-X., Lian, C.-X., Guo, X.-N., Peng, W. and Zhou, H.-M. 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. Food Chemistry 126(3): 1122-1126.