Original Article

Effect of Drying Methods, Solid-Solvent Ratio, Extraction Time and Extraction Temperature on Phenolic Antioxidants and Antioxidant Activity of *Guiera senegalensis* J.F. Gmel (Combretaceae) Leaves Water Extract

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ABSTRACT

Hot water extract of *Guiera senegalensis* leaves is used traditionally by women in Katsina State, Nigeria during postpartum period for general wellbeing. However the preparation of this beverage has not been optimized so far, which may underestimate its health benefit potentials. In this study, the effects of drying methods (air, oven and sun), solid- solvent ratio (1:10- 1:30), extraction time (30- 180 min) and extraction temperature (25- 85°C) on phenolic antioxidants and antioxidant activity of *G. senegalensis* were studied using Single Factor Experiment (SFE). Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) assays were used to determine the phenolic antioxidants. Antioxidant activity was evaluated by measuring scavenging effect on 2,2'- diphenyl-1- picrylhydrazyl (DPPH) radical, Ferric Reducing Antioxidant Power (FRAP) and Total Antioxidant Capacity (TAC) using phosphomolybdate assay. Results showed that extraction conditions significantly ($P<0.05$) affect phenolic antioxidants extraction and antioxidant properties of *G. senegalensis* leaves water extract. The optimal conditions were air drying using 1:10 solid – solvent ratio for 60 minutes at 40°C with values of 2720.37 mg QE/g DW for TFC, 1431.74mg GAE/100g DW for TPC, 96.73% for DPPH scavenging, 2797.04mg AAE/100g DW for FRAP and 1144.49mg AAE/100g DW for TAC. TFC was found to be significantly correlated with TAC ($r^2= 0.851$; $P<0.01$) and TPC with DPPH ($r^2= 0.732$: $P<0.05$) under the influence of drying methods. TFC was also significantly correlated with TAC ($r^2= 0.683$; $P<0.01$)

and DPPH (r^2= 0.538; P<0.01) under the effect of solid- solvent ratio. Negative significant correlations were found between TFC and FRAP (r^2= -0.369; P<0.01), DPPH and FRAP (r^2= -0.591; P<0.01) under the influence of extraction temperature. However, poor correlations were observed among all variables under the influence of extraction time.

Keywords: Antioxidant activity, Extraction, *Guiera senegalensis*, Phenolic antioxidant.

INTRODUCTION

Reactive Oxygen Species (ROS) produced as a result of normal physiological body functions or acquired from environment are directly or indirectly associated with cellular and metabolic injury, accelerated aging, cancer cardiovascular diseases neurodegenerative diseases and inflammation[1]. Many exogenous factors contribute to the "over production" of ROS as a result of which the in- built antioxidant system is not sufficient enough to counteract the excess radicals generated thereby creating oxidative stress in the body system[2]. An antioxidant is therefore "any substance that, when present at low concentration compared with those of an oxidisable substrates significantly delays or prevents oxidation of that substrate[3]." Synthetic antioxidants such as butylated hydroxy- anisol (BHA) and butylated hydroxyl toluene (BHT) have been in use for quite long time to alleviate oxidative stress. However, indications showed that they are linked with number of diseases as such their use is restricted and eventually banned in many developed countries[4]. Natural antioxidants (mostly from plant source) are considered safer hence many antioxidants were examined and isolated from plant materials[5,6].

Guiera senegalensis J.F. Gmel (Combretaceae), locally known as "*sabara"* or *"barbarta"* in *hausa* language of Northern Nigeria is widely distributed in western Africa[7]. Traditionally, *G. senegalensis* is used to treat various illness such as hypertension, malaria, cough, diabetes and many microbial infections[8]. The plant is also widely used by women in Katsina State, Nigeria during postpartum period for general wellbeing. Many researchers have reported various medicinal properties of *G. senegalensis* for example antimicrobial[8,9], antiulcer[10], antimalarial[11,12] and antiviral[13] to mention but few. Although some researchers reported antioxidant properties of *G.* senegalensis[7,14,15], to the best of our knowledge on available literature no study is reported on the optimization of protocol for the extraction of natural antioxidant from this plant.

A universal extraction protocol for natural antioxidant from plant source is not possible taking into consideration the diverse composition of natural phenolic compounds, their structure and physico-chemical properties. A unique extraction protocol for individual antioxidant from individual source should therefore be designed and optimized. It is against this background this study is designed to optimize the protocol for extraction of antioxidant from *G. senegalensis* using Single Factor Experiment (SFE). Despite being more tedious and unable to provide basic information on the interaction among different extraction factors, single-factor experiment is considered classical optimi-

zation method as it provides fundamental information on the ranges of factors that show significant effects and results generated could be used for Response Surface Methodology (RSM) to obtain a central composite design[16].

MATERIAL AND METHODS

Chemicals

Aluminium chloride, iron III chloride, sodium acetate, gallic acid, Folin and Ciocateu's phenol reagent, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), sodium hydrogen carbonate, sulphuric acid, hydrochloric acid and methanol were purchased from R&M marketing, Essex, United Kingdom. Ammonium molybdate, sodium hydroxide pellets, ascorbic acid, quercetin and 2, 4, 6-Tripyridyl-s-triazine (TPTZ) were purchased from BDH Limited Poole, England. Sodium nitrate and Sodium phosphate were purchased from Sigma-Aldrich Inc St. Louis, USA. All reagents were analytical grade and all stock solutions were prepared using purified deionized water (ELGA, OPTION- R 15B, Veolia water system United Kingdom).

Plant material

Guiera senegalensis leaves were collected from Umaru Musa Yar'adua University Campus Katsina, Katsina State, Nigeria. The plant was authenticated by Professor Munier Abd el Ghani of the Department of Biology, Umaru Musa Yar'adua University, Katsina and a voucher specimen was deposited in the Herbarium of the same Department.

Drying processes

For air drying, leaves were evenly spread on a tray and kept in the laboratory at a temperature of approximately 36°C until completely dry for 5 days. For sun drying, the leaves were exposed to direct sunlight and were left to dry for 3 days while for the oven drying the leaves were placed in an oven (Memmert GmbH Model 600 Schwabach, Germany) and the temperature adjusted to 75°C for 12 hours.

Grinding of plant material

The dried leaves were ground using a mill (Retsch, SM100 comfort Hann, Germany). The powder obtained was packaged and stored in dark at an ambient temperature.

Extraction of plant material

Dried leaves of *G. senegalensis* obtained from different drying methods were put in 50ml conical flask covered with parafilm (Pechiney plastic packaging Menasha, Wisconsin U.S.A) and wrapped with aluminium foil (Diamond Reynolds, Richmond, U.S.A.) and extracted with deionized water in a temperature- controlled water bath shaker (WNB 7-45, Memmert, Germany) at a constant speed for the various solid-solvent ratio and extraction time at required temperature. Crude extracts were then filtered through Whatman No. 1 filter paper (Whatman International Ltd, England). Filtrates were collected in amber bottles and used directly for the estimation of phenolic antioxidant and evaluation of antioxidant activities using various bio-chemical assays[17].

Experimental design

Single factor experiments were used in this study. A total of four factors (drying method, solid- solvent ratio, extraction time and extraction temperature) were studied. The levels for each independent variable were chosen based on responses of the crude extract to Total Phenolic Content (TPC) Total Flavonoid Content (TFC) as priority followed by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) radical- scavenging activity, Ferric Reducing Antioxidant Power

(FRAP) and Total Antioxidant Capacity (TAC).

In the first experiment effect of drying method on the extraction of phenolic antioxidant and antioxidant capacity of *G. senegalensis* leaves water extract was investigated by extracting the dried leaves obtained from three drying methods (air, oven and sun) using 1:10 solid- solvent ratio at 25°C for 120 minutes. The best drying method was chosen based on the five responses.

In the second step, dried leaves obtained from the best drying methods according to step 1 were extracted using varied solid- solvent ratio (1:10, 1:15, 1:20, 1:25, and 1:30) while the extraction time and the extraction temperature were kept constant at 120 minutes and 25°C respectively. The best solid- solvent ratio was again chosen based on the five responses.

Using the best drying method and solid-solvent ratio, phenolic antioxidants were extracted at various extraction temperatures ranging from 25 to 85°C while holding the extraction time constant at 120 minutes. The best extraction temperature was also chosen based on the five responses.

To determine the optimum extraction time, leaves were extracted using best drying method, solid- solvent ratio and temperature obtained from previous steps while the extraction time was varied from 30 to 180 minutes. Best extraction time was also selected based on the five responses.

Determination of total Phenolic content (TPC)

Total Phenolic Content (TPC) was determined using Folin-Ciocalteu's (FC) method as reported by Thoo *et al*[17], with slight modifications. Briefly, 500µL of dilute crude extract was mixed with 500µL of 10-fold diluted Folin-Ciocalteu reagent. After 3 minutes, 400µL of sodium carbonate anhydrous was added and vortexed. After 2 hours of incubation in dark at room temperature, absorbance was determined at 765nm against a blank (prepared by replacing plant extract with deionized water) using a UV/VIS spectrophotometer (Lambda 25, PerkinElmer, Singapore). Measurements were calibrated to a standard curve of prepared gallic acid solution (10-100µg/ml) with equation $y = 0.01x – 0.009$ ($R^2 = 0.999$) and TPC was then expressed as milligram of gallic acid equivalent (GAE) per 100g of dry weight (DW).

Determination of total flavonoid content (TFC)

Total Flavonoid Content (TFC) was determined using modified aluminium chloride calorimetric assay reported by Kaur and Mondal[18]. Briefly, 125µL of crude extract was mixed with 625µL deionized water and 37.5µL of 5% sodium nitrite. The mixture was allowed to stand for 6 minutes and 75µL of 10% aluminium chloride-6-hydrate was added thereafter. After 5 minutes, 250µL sodium hydroxide solution was added. 137.5µL deionized water was added and mixed. Absorbance was measured immediately at 510nm against a blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a standard curve of prepared quercetin solution (0 – 800µg/ml) with equation $y = 0.0000x + 0.003$ ($R^2 = 0.981$) and TFC was then expressed as milligram quercetin equivalent (QE) per 1g dry weight (DW).

Evaluation of antioxidant activity

2, 2'- diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity

Antioxidant capacity through DPPH scavenging activity was determined according to the protocol reported by Zothanpuia *et al*[19], with slight modifications. Methanolic DPPH stock solution was

prepared by dissolving 4mg 2, 2'- diphenyl-1-picrylhydrazyl (DPPH) powder into 100ml absolute methanol. Working solution was obtained by mixing 50ml stock solution with 20ml methanol to obtained absorbance of 1.00±0.02 unit at 517nm wavelength. 100µL plant extract was mixed with 900µL methanolic DPPH solution and allowed to stand in dark at room temperature for 30 minutes. After 30 minutes incubation, absorbance was taken at 517nm using UV/VIS spectrophotometer (Lambda 25, PerkinElmer, Singapore) against blank (methanol). Percentage DPPH scavenging activity was determined using the relation:

DPPH scavenging activity (%) = $[(A_0 - A_{30})/A_0] * 100$.

Where A_0 = Absorbance at time 0; A_{30} = Absorbance after 30 minutes.

Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out using the protocols as reported by Thaipong *et al*[20]. The stock solution included 300mM acetate buffer (3.1g $C_2H_3NaO_2$.$3H_2O$ and 16ml $C_2H_4O_2$), pH 3.6, 10mM 2,4,6-Tripyridyl-s-triazine (TPTZ) solution in 40mM HCl and 20mM $FeCl_3$. $6H_2O$ solution. Fresh FRAP working solution was prepared by mixing acetate buffer, TPTZ solution and $FeCl_3$. $6H_2O$ solution in the ratio of 10:1:1 respectively. The FRAP solution was warmed at 37°C for 30 minutes in water bath (GFL 1004 Burgwedel, Germany) before using. 100µL plant extract was allowed to react with 1000µL FRAP solution in dark for 30 minutes. Absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at 593nm wavelength using UV/VIS spectrophotometer (Lambda 25, Perkin Elmer, Singapore) against blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a linear standard curve of prepared ascorbic acid solution (5–35mg/ml) with equation y = 0,045x + 0.395 (R^2 = 0.996) and results expressed as milligram ascorbic acid equivalent (AAE) per 100g dry weight (DW). Additional dilution was needed if the FRAP value measured is over the linear range of the standard curve.

Total antioxidant capacity (TAC)

Total antioxidant capacity was determined using phosphomolybdate assay described by Mohammed *et al*[21], with slight modifications. The assay was based on the reduction of the molybdenum (VI) to molybdenum (V) by antioxidants and the subsequent formation of a green phosphate/molybdenum complex at acidic pH. The working solutions consist of 600mM sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate. 1000µL working solution was mixed with 100µL plant extract and incubated in a water bath (GFL 1004 Burgwedel, Germany) at 95°C for 90 minutes. The mixture was allowed to cool to room temperature before absorbance was measured at 695nm using UV/VIS spectrophotometer (Lambda 25, PerkinElmer, Singapore) against blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a linear standard curve of prepared ascorbic acid solution (100 – 700µg/ml) with equation y = 0.0016 + 0.0222 (R^2 = 0.999) and results expressed as milligram ascorbic acid equivalent (AAE) per 1g dry weight (DW). Additional dilution was needed if the FRAP value measured is over the linear range of the standard curve.

Statistical analysis

Results were expressed as mean ± standard deviation of replicate extraction and triplicate of assays and analysed using SPSS software (version 20). One- way analysis of variance (ANOVA) with Duncan's test was carried out to test significant difference between levels of

treatment. Significant levels were defined using value $P < 0.05$. Pearson correlation between variables were also established using SPSS software (version 20).

RESULTS AND DISCUSSION

Effect of drying methods

Results showed that drying methods significantly ($P<0.05$) affects phenolic antioxidants extraction and antioxidant activities in *G. senegalensis*. It could be seen that highest TPC and TFC were observed in air drying (figure 1a, b). Highest DPPH radical scavenging activity and total TAC were also exhibited by air dried leaves (figures 1c and e). Although no significant difference observed among drying methods, air drying still exhibited highest ferric reducing antioxidant power (figure 1d). Sun drying resulted in least variables except TFC and TAC. Loss of phenolic antioxidants and antioxidant properties in thermal drying could be attributed to enzymatic processes that occurred during sun drying as degradative enzymes such as phenolic oxidases which degrade phenolic compounds are not deactivated[22]. Solar radiation was also reported to have caused degradation of phenolic compounds[23]. This result agrees with the findings of previous researchers such as Kade *et al*[24], who reported that sun drying diminishes the antioxidative potentials of *Eugenia uniflora*. Higher losses of β-carotene and ascorbic acid in green leaves as a result of sun drying was also reported[25].

Effect of solid- solvent ratio

Phenolic antioxidants and anti-oxidant activities of *G. senegalensis* are significantly ($P<0.05$) affected by solid-solvent ratio. 1:10 appeared to be the best solid- solvent ratio for extracting antioxidants and antioxidant activity in *G. senegalensis* as it exhibit the best TFC and TAC (figures 2a and e). Although the best TPC was observed in 1:25, it is not significantly ($P<0.05$) different from 1:10 (figure 2b). 1:15 that showed the best DPPH radical scavenging activity was also not significantly different from 1:10 (figure 2c). 1:10 solid solvent ratio however exhibited the least ferric reducing power (figure 2d). According to mass transfer principle, high concentration gradient as a result of high solid solvent ratio increases diffusion rate and consequently promotes grater extraction of solids by the solvent[26]. However, this phenomenon was not observed in this study as low solid- solvent ratio (1:10) showed best TFC and TAC. This may be explained by the fact the interaction among bioactive compounds coming into contact with extracting solvent expands as a result of high extraction solvent and this brings about increase leaching out of phenolic components[27].

Effect of extraction temperature

Effect of extraction temperature on the recovery of phenolic antioxidant and antioxidant activity in *G. senegalensis* was evaluated using temperature ranges from 25 to 85°C. Results showed that extraction temperature significantly ($P<0.05$) affects all the variables investigated. From the results, it could be seen that total phenolics were best extracted at 40°C while total flavonoids were best extracted at 55°C (figures 3a and b). Best DPPH radical scavenging was also achieved at 40°C (figure 3c). Least FRAP and TAC were, however observed in 40°C and 55°C, respectively (figures 3d and e). Phenolic compounds are believed to be more extracted at elevated temperatures as increased temperatures promote extraction by enhancing both diffusion coefficients and solubility of polyphenols[17]. Elevated temperatures also increase cell membrane permeability following the breakdown of cellular constituents thereby setting more polyphenols free to be extracted[28]. In

addition, coagulation of polyphenols with lipoprotein is reduced by the release of bound polyphenols and this go a long way in enhancing solubility of the polyphenols and diffusion hence polyphenols yield increased[29]. High temperatures are, however, not always suitable for extracting all sorts of phenolic compounds as the less soluble antioxidants extracted at low temperature decompose before the extraction of thermo stable antioxidants and this could lead to loss in antioxidant activity[30]. As such, only samples with higher proportions of thermo stable compounds should be extracted at high temperatures. In this study, it can be deduced that *G. senegalensis* contains both thermo stable and thermo labile polyphenols in varied proportions as higher phenolic contents and antioxidant activity were observed in both low and high temperatures. 40°C was chosen as the optimum temperature in this study considering the fact that it gives best TFC and DPPH radical scavenging activity. More so, the best TPC and TAC observed respectively at 55°C and 25°C are not significantly different with the ones observed at 40°C.

Effect of extraction time

Extraction time plays significant role in optimizing the recovery of polyphenols and their antioxidant capabilities. Although longer extraction time could lead to increase exposure to temperature, light and oxygen which eventually lead to oxidation of phenolic compound[31] as well as enzymatic degradation, different bioactive compound with diverse structures require different time of extraction. Effect of extraction time on the recovery of phenolic antioxidant an antioxidant activities are shown in figures 4a to e. from the figures it can be shown that extraction time significantly affects TPC, DPPH- radical scavenging and FRAP but not TFC and TAC. Highest TPC was recovered after 60 minutes of extraction. The highest TFC recorded at 150 minutes is not significantly different with that recorded at 60 minutes. Highest DPPH- radical scavenging observed at 180 minutes is also not significantly different with that recorded at 60 minutes. It can also be observed that phenolic antioxidants were best extracted in both shorter and longer times of extraction (60 minutes for TPC, and 150 minutes for TFC). This can be ascribe to the fact that *G. senegalensis* contains diverse phenolic compounds with varied stability and resistance to atmospheric conditions. It is interesting however, to note that optimum antioxidant activities were achieved at longer times (180 minutes for DPPH- radical scavenging; 120 minutes for FRAP and TAC). This may be attributed to the fact that estimation of antioxidant ability is not solely dependent on a particular group of phenolic antioxidant, in fact it depends on the presence of any antioxidant compound[32]. Hence it can be deduced that phenolic compounds are not the only antioxidants present in *G. senegalensis*. Taking into consideration economic point of view, 60 minutes is considered optimum time for the extraction of phenolic antioxidant from *G. senegalensis* as it gave highest TPC and it is not significantly different from other times where maximum TFC, DPPH- radical scavenging, FRAP and TAC were observed.

Pearson correlation analysis

A correlation analysis shows that relationships exist between phenolic antioxidant and antioxidant assays in *G. senegalensis* leaves water extract under the influence of different extraction parameters (Table 1). Under the influence of drying method, a moderately strong significant ($P<0.05$) correlation was observed between TPC and DPPH ($r = 0.732$), likewise a strong, highly significant ($p<0.01$) correlation exists between TFC and TAC (r

= 0.851). A moderate, though not significant correlation was also observed between FRAP and TAC (r = 0.609). From this experimental result, we can deduce that phenolic antioxidant may play a role in antioxidant activities of *G. senegalensis* leaves water extract. This is in agreement with previous studies for example Ibrahim *et al*[33], observed strong correlation (r = 0.929) between phenolic compound and antioxidant activity in *Streblus asper*. Besides, significant correlation (r = 0.684) between phenolic compounds and antioxidant activity in some rhizomes was also reported[34]. High correlation between phenolic and DPPH/FRAP activities (r = 0.996 and r = 0.985, respectively) was also observed in *Boerhavia diffusa*[35]. A moderately significant correlation (r = 0.538) was observed between TFC and DPPH under the influence of solid- solvent ratio. Another moderate but highly significant correlation (r = 0.683) was also observed between TFC and TAC as affected by solid- solvent ratio. From this it can be inferred that total flavonoids contribute more to antioxidant activity in *G. senegalensis* than total phenolics. However, with respect to extraction temperature a weak, significant negative correlation between TFC and FRAP (r = -0.309) as well as between FRAP and DPPH (r = -0.591). This shows that antioxidant activity in *G. senegalensis* are not only governed by phenolic compound. Ogbonnaya and Chinedum[36] also reported that beside phenolic compounds, vitamins, proteins and minerals contribute to antioxidant capacity of *Gnetum africanum*. Significant antioxidant activity in *Costus speciousus* was also attributed to flavonoid and mineral contents[37]. Clarke*et al*[38], (2013) however, reported highly significant (P<0.0001) correlation between DPPH and FRAP (r = 0.852). With respect to extraction time, phenolic antioxidants are weakly correlated with antioxidant assays in both positive and negative directions. Based on this, we suggest that phenolic compounds in *G. senegalensis* are of low molecular weight as such more susceptible to degradation if exposed to temperature and other environmental conditions for longer period of time. A weak correlation between phenolic antioxidants and antioxidant assays was also observed in *Morinda citrifolia*[17].

CONCLUSION

Optimum extraction parameters for extracting phenolic antioxidants from *G. senegalensis* were determined using single factor experiment. The best extraction condition based on the compromise between the yield of phenolic antioxidants (TPC and TFC) and their antioxidant properties (DPPH, FRAP and TAC) are air drying using 1:10 solid- solvent ratio at 40°C for 60 minutes. Significant correlations were found between phenolic antioxidant and antioxidant assays under the influence of all the extraction parameters except extraction time. This study provides baseline data based on which further optimization using response surface methodology (RSM) could conducted. It is also evident that *G. senegalensis* possesses different phenolic compounds with diverse chemical properties. Assessment of these compounds using qualitative and quantitative analytical methods such as high performance liquid chromatography (HPLC) is hereby recommended to ascertain the specific compounds responsible for antioxidant property in this plant.

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Conflict of interest

We hereby declare no conflict of interest.

REFERENCES

1. Rop, O., Ercişli, S., Mlcek, J., Jurikova, T., Hoza, I. Antioxidant and radical scavenging activities in fruits of 6 sea buckthorn (*Hippophae rhamnoides* L.) cultivars. *Turkish Journal of Agriculture and Forestry*2014; 38: 224-232.
2. Iqbal, S., Younas, U., Chan, K. W., Zia-Ul-Haq, M., Ismail, M. Chemical composition of *Artemisia annua* L. leaves and antioxidant potential of extracts as a function of extraction solvents. *Molecules* 2012; 17(5): 6020–32.
3. Halliwell, B. Gutteridge,J.M.C.Free radicals in Biology and medicine, 3rd ed.,1999 pp. 617-783. Oxford university press, Newyork, USA.
4. Yingming, P., Ying, L., Hengshan, W. Min, L. Antioxidant activities of several Chinese medicine herbs. *Food Chem.*2004; 88: 347–350.
5. Velioglu, Y.S., Mazza, G., Gao, L., Oomah, B.D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural food and chemistry*1998; 46: 4113-4117.
6. Rubilar, M., Pinelo, M., Ihl, M., Scheuermann, E., Sineiro, J., Nunez M.J. Murta leaves (*Ugni molinae* Turcz) as a source of antioxidant polyphenols. *Journal of Agricultural and food chemistry* 2006; 54: 59-64.
7. Sombie, P.A.E.D, Hilou, A., Mounier, C., Coulibaly,A.Y., Kiendrebeogo, M., Millogo, J. F., Nacoulma, O.G.. Antioxidant and anti-inflammatory activities from the galls of *Guiera senegalensis* J.F. Gmel (Combretaceae) *Research Journal of Medicinal Plants*2011; 5(4): 448- 461.
8. Fiot, J., Sanon, S., Azas, N., Mahiou, V., Jansen, O., Angenot, L., Ollivier, E. Phytochemical and pharmacological study of roots and leaves of Guiera senegalensis J.F. Gmel (Combretaceae). *Journal of Ethnopharmacology*2006; 106(2): 173–8.
9. Kudi, A.C., Umoh, J.U., Eduvie, L.O. Gefu, J. Screening of some Nigerian medicinal plants for antibacterial activity. *Journal of Ethnopharmacology*1999; 67: 225–228.
10. Aniagu, S.O., Binda, L.G., Nwinyi, F.C., Orisadipe, A., Amos, S., Wambebe, C., Gamaniel, K. Anti-diarrhoeal and ulcer-protective effects of the aqueous root extract of *Guiera senegalensis* in rodents. *Journal of Ethnopharmacology*2005; 97: 549–554.
11. Benoit, F., Valentin, A., Pelissier, Y., Diafouka, F., Marion, C., Kone-Bamba, D., Kone, M., Mallie, M., Yapo, A. Bastide, J.M. In vitro antimalarial malarial activity of vegetal extracts used in West African traditional medicine. *American Journal of Tropical Medicine and Hygiene* 1996; 54: 67–71.
12. Ancolio, C., Azas, N., Mahiou, V., Ollivier, E., Di Giorgio, C., Keita, A., Timon-David, P., Balansard, G. Antimalarial activity of extracts and alkaloids isolated from six plants used in traditional medicine in Mali and Sao Tome. *Phytotherapy Research* 2002; 16: 646–649.
13. Lamien, C.E., Meda, J., Mans, M., Romito, Nacoulma, O.G., Viljoen, G.J. Inhibition of fowlpox virus by an aqueous acetone extract from galls of *Guiera senegalensis* J.F. Gmel (Combretaceae) *Journal of Ethnopharmacology* 2005; 96: 249- 253.
14. Mariod, A., Matthäus, B., Hussein, I. H. Antioxidant activities of extracts from Combretum hartmannianum and Guiera senegalensis on the oxidative stability of sunflower oil. *Emir Journal of Agricultural Science* 2006; 18(2): 20–28.
15. Abubakr, M., Sirag, N., Osman, I., Abakar, S., Aboul Enien, A.M. Anticancer and antioxidant activities of *Guiera senegalensis*. *Sudan Journal of Medical Sciences*2013; 8(3): 135-140.
16. Chen, X., Wu, X., Chai, W., Feng, H., Shi, Y., Zhou, H., Chen, Q. Optimization of extraction of phenolics from leaves of Ficus virens. *Journal of Zhejiang University. Science. B*, 2013; 14(10): 903–15.

17. Thoo, Y.Y., Ho, S.K., Liang, J.Y., Ho, C.W. and Tan, C.P. Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chemistry* 2010; 120(1): 290–295.
18. Kaur, S., Mondal, P. Study of Total Phenolic and Flavonoid Content, Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. *Journal of Microbiology and Experimentation* 2014; 1(1): 00005.
19. Zothanpuia, Lalawmpuii, P.C., Kakoti, B.B. Free radical scavenging and preliminary phytochemical screening of methanolic extract of the bark of *Dillenia pentagyna* Roxb. *American Journal of Phytomedicine and Clinical Therapeutics* 2014; 2(7): 910-918.
20. Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J. Food Compos Anal*2006; 19: 669–675.
21. Mohammed, C.B., Chahid, B., Fatima, B., Fatima-Zohra, S., Meriem, B., Farid, C. Antioxidant activity, phenolic and flavonoid content in leaves, flowers, stems and seeds of mallow (*Malva sylvestris* L.) from North Western of Algeria. *African Journal of Biotechnology* 2014; 13(3): 486–491.
22. Zhang, Z., Lv, G., Pan, H., Wu, Y. and Fan, L. Effects of Different Drying Methods and Extraction Condition on Antioxidant Properties of Shiitake (*Lentinus edodes*). *Food Science and Technology Research* 2009; 15(5): 547–552.
23. Mueller-Harvey I. Analysis of hydrolysable tannins, *Anim, Feed Sci, Technol*, 2001; 91: 3-20.
24. Kade, I.J., Ibukun, E.O., Nogueira, C.W. Da Rocha, J.B. Sun-drying diminishes the antioxidative potentials of leaves of *Eugenia uniflora* against formation of thiobarbituric acid reactive substances induced in homogenates of rat brain and liver. *Experimental and Toxicologic Pathology* 2008; 60(4-5): 365-371.
25. Negi, P.S., Roy, S.K. Effect of drying conditions on quality of green leaves during long term storage. *Food Research International*2001; 34(4): 283-28.
26. Tan, P.W.; Tan, C.P.; Ho, C.W. Antioxidant properties: Effect of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*). *International Food Research Journal*2011; 18:553–558.
27. Zhang, Z.S., Li, D., Wang, L.J., Ozkan, N., Chen, X.D., Mao, Z.H. Optimization of ethanol–water extraction of lignans from flaxseed. *Separation and Purification* 2007; 57(1): 17–24.
28. Wang, J., Sun, B., Cao, Y., Tian, Y. Li, X. Optimization of ultrasound-assisted extraction of phenolic compounds from wheat bran. *Food Chemistry* 2007; 106(2): 804–810.
29. Al-Farsi, M.A., Lee, C.Y. Optimization of phenolics and dietary fibre extraction from date seeds. *Food Chemistry*2008; 108(3): 977–985.
30. Liyana-Pathirana, C., Shahidi, F. Optimi-zation of extraction of phenolic compounds from wheat using response surface methodology. *Food Chemistry* 2005, 93(1), 47–56.
31. Tay, P., Tan, C., Abas, F., Yim, H. and Ho, C. Assessment of Extraction Parameters on Antioxidant Capacity, Polyphenol Content, Epigallocatechin Gallate (EGCG), Epicatechin Gallate (ECG) and Iriflophenone 3-C-β-Glucoside of Agarwood (*Aquilaria crassna*) Young Leaves. *Molecules* 2014; 19(8): 12304–12319.
32. Prior, R.L., Wu, X., Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*2005; 53(10): 4290–4302.
33. Ibrahim, N.M., Mat, I., Lim, V., Ahmad, R. Antioxidant Activity and Phenolic Content of *Streblus asper* Leaves from Various Drying Methods. *Antioxidants*2013; 2: 156–166.
34. Yan, S.W., Asmah, R. Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *Int. Food Res. J.* 2010; 17: 417–423.
35. Avasthi, A.S., Jawaid, S.A., Jain, S., Bhatnagar, M., Purkayastha, S., Ghosal, S.

Free radical scavenging and antioxidant impact of Indian medicinal plants extracts on H_2O_2 mediated oxidative stress on human erythrocyte. *American Journal of Phyto-medicine and Clinical Therapeutics* 2014; 2(9): 1052-1069.

36. Ogbonnaya, E.C., Chinedum, E.K. Health promoting compounds and *in vitro* antioxidant activity of raw and decoctions of *Gnetum aficanum* Welw. *Asian Pacific Journal of Tropical Disease* 2013; 3(6): 472–479.
37. Jagtap, S. Satpute, R. Phytochemical Screening and Antioxidant Activity of Rhizome Extracts. *Journal of Academia and Industrial Research*2014; 3(1): 40–47.
38. Clarke, G., Ting, K.N., Wiart, C., Fry, J. Plants from the Malaysian Rainforest. *Antioxidants* 2013; 2: 1–10.

Table 1. Correlation analysis between assays as affected by different extraction conditions

	Drying method				Solid- solvent ratio				Temperature				Time			
	TPC	TFC	DPPH	FRAP	TPC	TFC	DPPH	FRAP	TPC	TFC	DPPH	FRAP	TPC	TFC	DPPH	FRAP
TFC	0.157				0.084				0.209				-0.111			
DPPH	0.732*	0.203			0.313	0.538			0.129	-0.195			0.009	-0.082		
FRAP	0.357	0.609	0.555		-0.053	-0.223	0.215		-0.369*	0.022	-0.591*		-0.041	0.102	-0.013	
TAC	0.081	0.851**	0.131	0.604	0.094	0.683**	0.376*	-0.308	-0.085	-0.085	-0.025	-0.221	-0.247	0.165	0.016	-0.034

TPC, total phenolic content; TFC, total flavonoid content; DPPH, DPPH-radical scavenging activity, FRAP, ferric reducing antioxidant power, TAC, total antioxidant capacity.
*Significant level at P< 0.05
** Highly significant level at P< 0.01

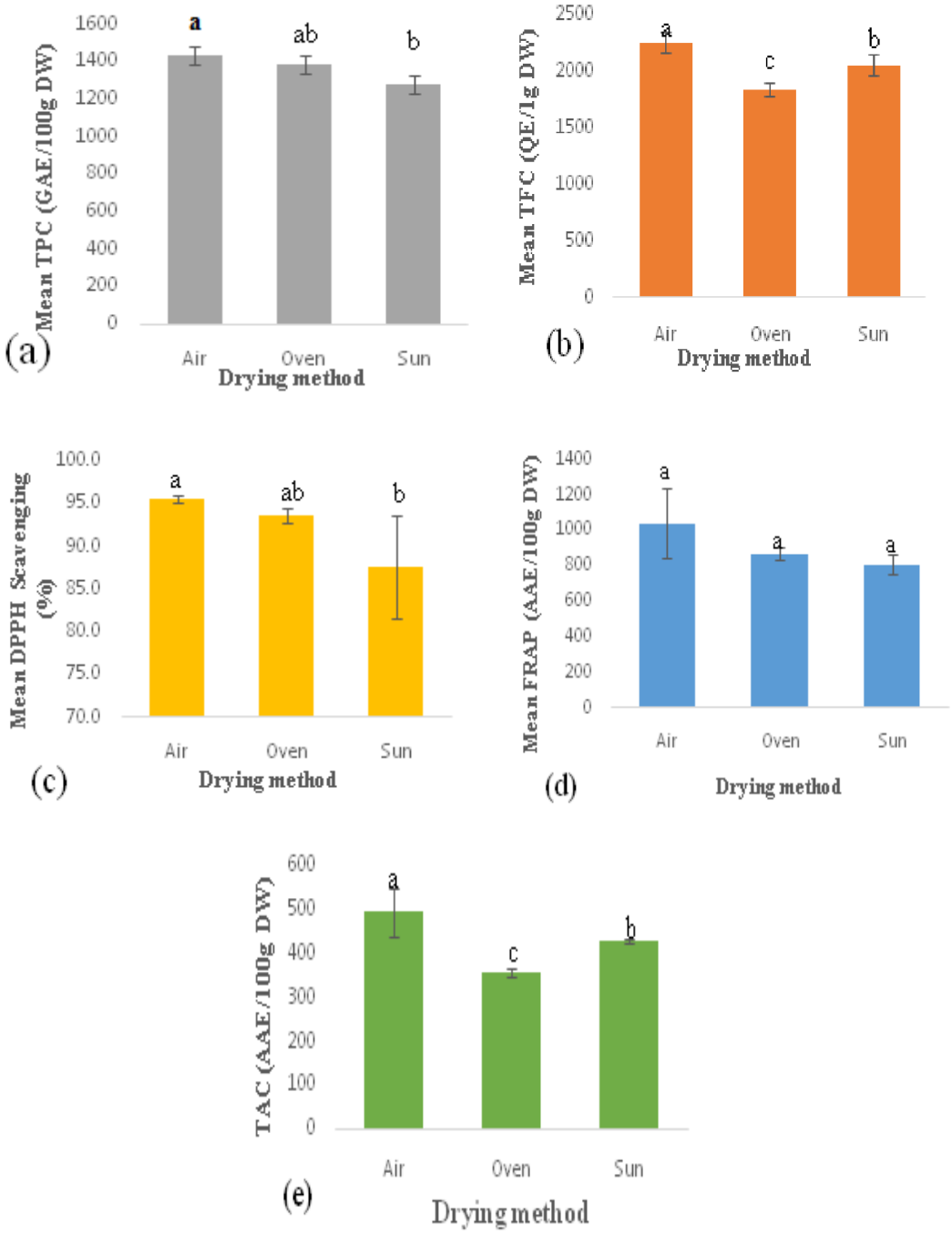

Figure 1. Effect of Drying methods on (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) DPPH- radical scavenging, (d) Ferric reducing antioxidant power (FRAP) and (e) total antioxidant capacity (TAC) in *Guiera senegalensis* leaves water extract. Note: values are means of 6 replicates, error bars represent standard deviation and values with same superscript are not significantly different (P< 0.05)

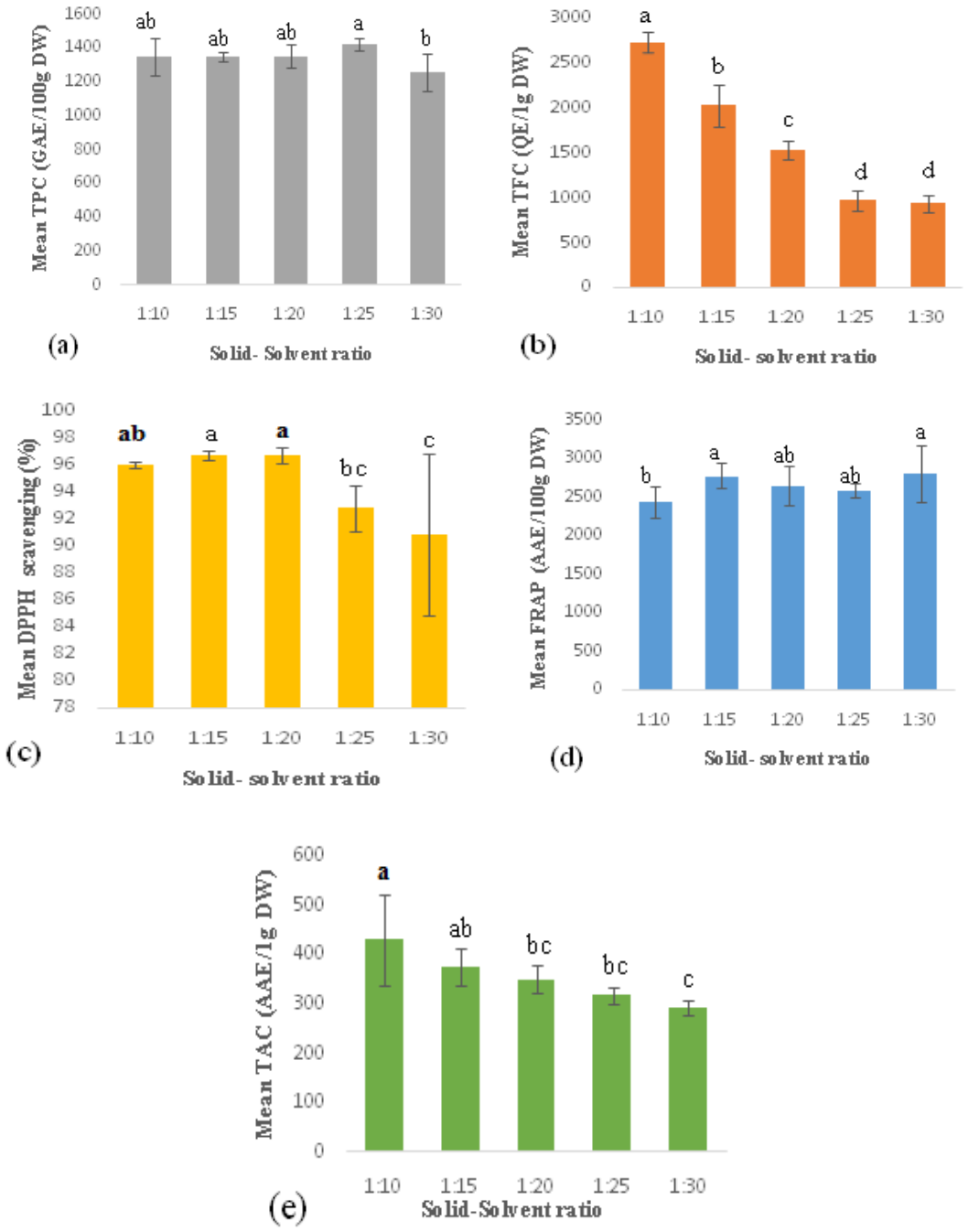

Figure 2. Effect of solid- solvent ratio on (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) DPPH- radical scavenging, (d) Ferric reducing antioxidant power (FRAP) and (e) total antioxidant capacity (TAC) in *Guiera senegalensis* leaves water extract. Note: values are means of 6 replicates, error bars represent standard deviation and values with same superscript are not significantly different (P<0.05)

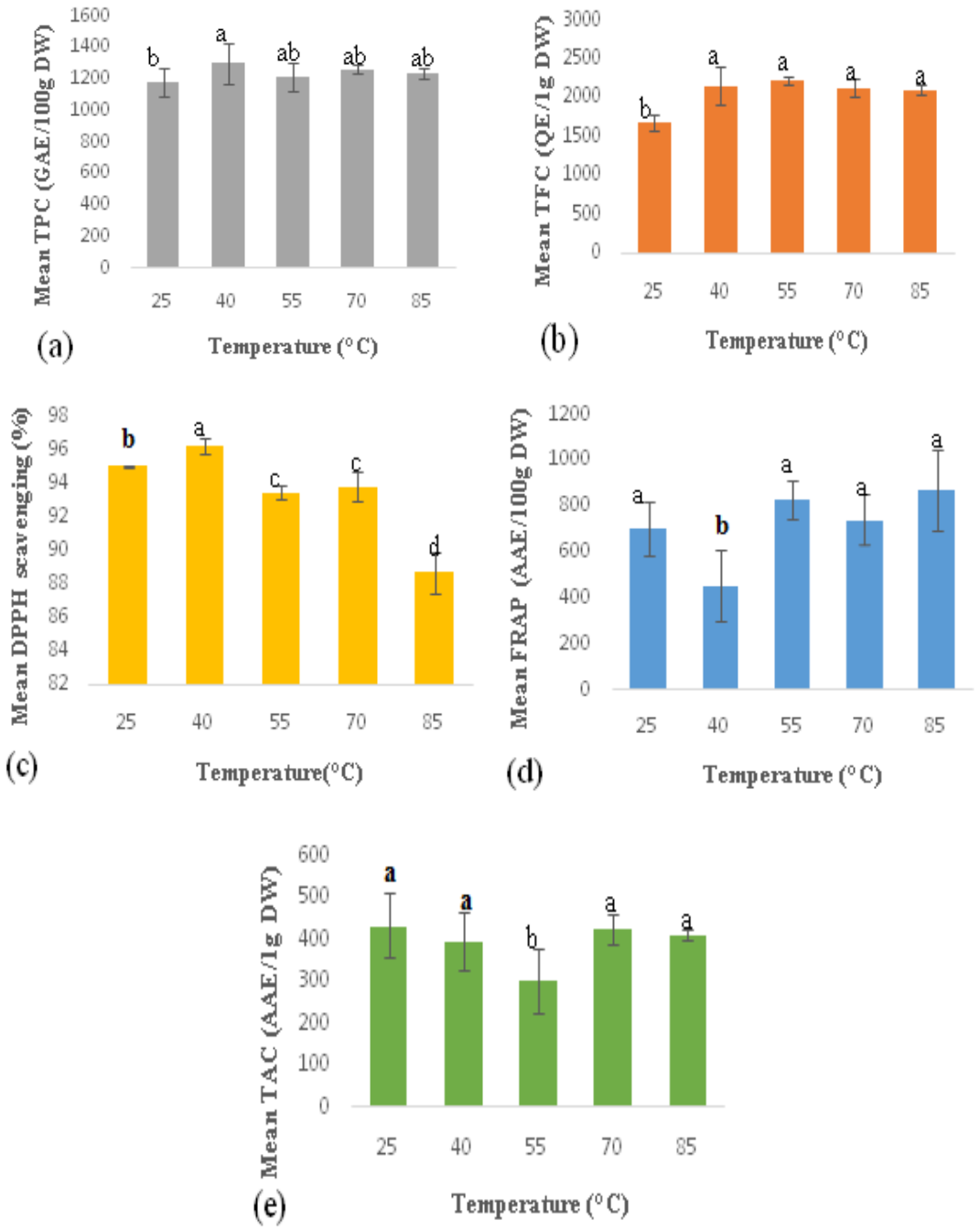

Figure 3. Effect of extraction temperature on (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) DPPH- radical scavenging, (d) Ferric reducing antioxidant power (FRAP) and € total antioxidant capacity (TAC) in *Guiera senegalensis* leaves water extract. Note: values are means of 6 replicates, error bars represent standard deviation and values with same superscript are not significantly different (P< 0.05)

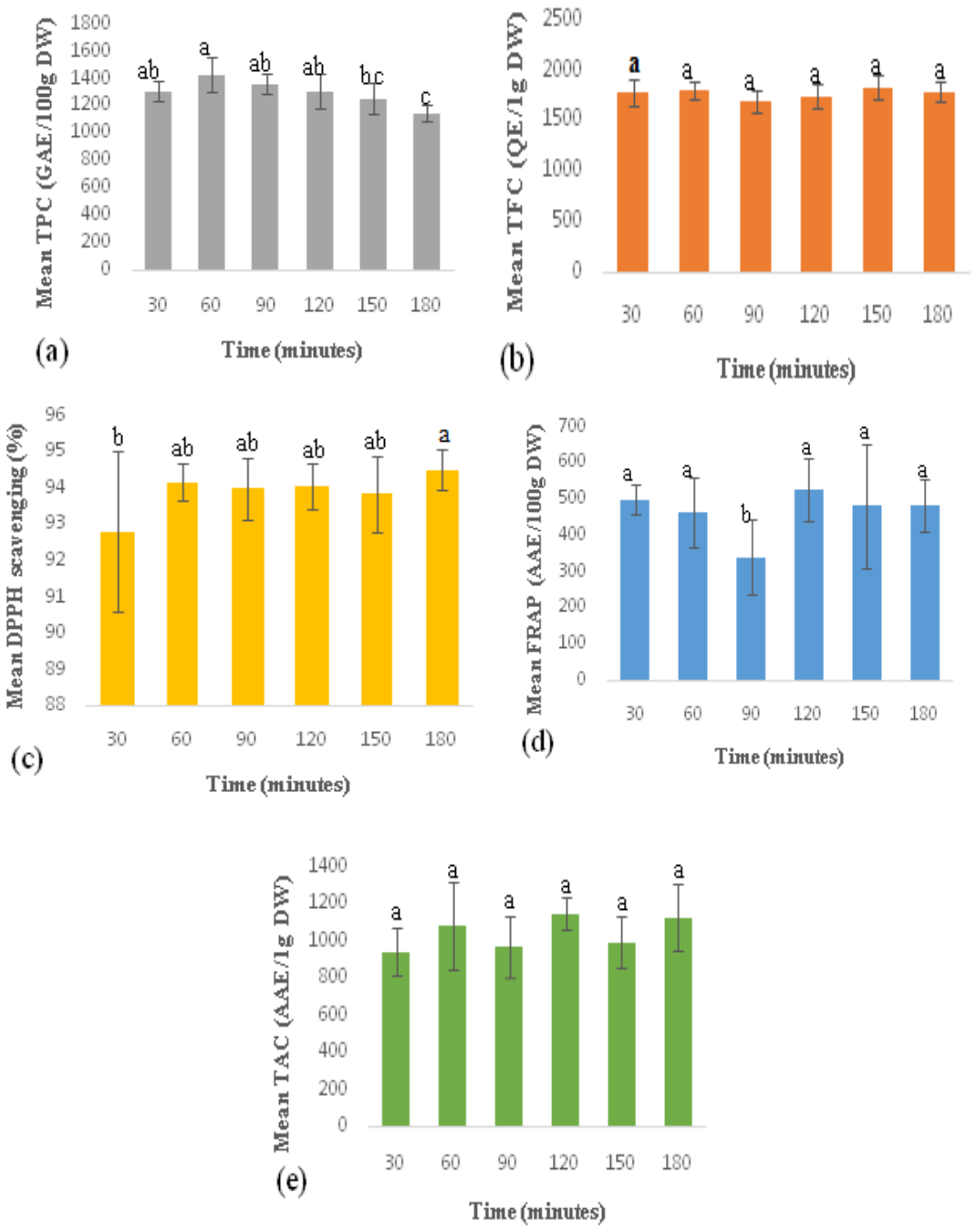

Figure 4. Effect of extraction time on (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) DPPH- radical scavenging, (d) Ferric reducing antioxidant power (FRAP) and € total antioxidant capacity (TAC) in *Guiera senegalensis* leaves water extract. Note: values are means of 6 replicates, error bars represent standard deviation and values with same superscript are not significantly different (P< 0.05)