



Phenolic Content, Flavonoid Content and Antioxidant Activity of Some Medicinal Plants Used for Traditional Maternal Healthcare in Katsina State, Nigeria

Sulaiman, S. K.

Department of Biology, Umaru Musa Yar'adua University P.M.B. 2218 Katsina, Nigeria

Email: sulaiman.kankara@umyu.edu.ng

ABSTRACT

An ethnobotanical study of medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria revealed that *Artemisia annua*, *Euphorbia balsamifera*, *Guiera senegalensis*, *Ipomoea asarifolia* and *Mitragyana inermis* are the most common herbs used by women to treat and prevent ailments associated with oxidative stress during pregnancy. In this study, the phenolic flavonoid content and antioxidant activities of those herbs were investigated to justify their folkloric use. Phenolic and flavonoid contents were respectively assessed by evaluating total phenolic content (TPC) and total flavonoid Content (TFC) while antioxidant activity was determined using ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assays. *G. senegalensis* showed the highest TPC, TFC, FRAP, TAC and lowest DPPH IC₅₀ (136.07mg GAE/g DW, 158.80mg QE/g DW, 34.25mg AAE/g DW 424.73mg AAE/g DW and 442.82µg/ml, respectively) followed by *M. inermis* (113.69mg GAE/g DW, 87.50mg QE/g DW, 19.37mg AAE/g DW, 332.30mg AAE/g DW and 609µg/ml, respectively). Correlation analyses revealed strong positive linear correlations between phenolic antioxidants (TPC and TFC) and antioxidant assays (FRAP, TAC and DPPH) with regression coefficient (R²) ranging from 0.695 to 0.867 implying that phenolic compounds are responsible for the antioxidant properties observed in these herbs.

Keywords: Antioxidant activity, Katsina state, maternal healthcare, medicinal plants, Nigeria, traditional.

INTRODUCTION

Oxidative stress has been linked with pathogenesis of adverse pregnancy outcomes including preeclampsia and fetal growth restriction (Mistry and Williams, 2011). Preeclampsia, characterized by hypertension, edema and/or proteinuria is one of the leading causes of maternal mortality and preterm deliveries in the world (Al-jameil *et al.*, 2014). About 790 maternal death per 100,000 live births have been attributed to preeclampsia (Wagner, 2004). Reactive oxygen species (ROS) have been identified as major key players in preeclampsia because of their involvement in lipid peroxidation, oxidative damage of biomolecules, cellular dysfunction and they are believed to initiate maternal vascular endothelial dysfunction (Padmini and Lavanya, 2012). Studies have revealed that reduced antioxidants and increased oxidative stress are associated with adverse birth outcomes in maternal complications such as preeclampsia and preterm deliveries (Kilari *et al.*, 2014). Free radicals are generated in the body as a result of normal metabolic processes, however, oxidative stress occurs when there is imbalance between the free radical and built-in antioxidants such as glutathione peroxidase, catalase, superoxide dismutase, uric

acid, bilirubin, vitamins A and E, etcetera (Sharma *et al.*, 2014).

To overcome oxidative stress, body cells are equipped with special machineries such as Heat Shock Proteins (HSPs) which coordinate mechanisms aimed at protecting cell from stressful environmental, pathological or physiological stimuli. During preeclampsia however, such proteins are compromised resulting in excessive oxidative stress (Padmini and Lavanya, 2012). Calcium, vitamins C and E are the common supplements employed to increase the level of endogenous antioxidants. However, studies revealed that the use of those supplements do not reduce the incidence of preeclampsia. As such, natural antioxidants capable of protecting cells from several diseases attributed to free radicals are much preferred. For this reason, antioxidants particularly from herbal origin may be of special benefit for women from developing countries like Nigeria who carry the greatest burden of morbidity and mortality associated with preeclampsia.

Many phenolic compounds and flavonoids possess antioxidant property because of their ability to scavenge free radicals thereby maintaining balance between oxidants and antioxidants (Sreeramulu *et al.*, 2013). Natural

antioxidants from herbs continue to receive attention in the scientific community because of safety issues associated with synthetic antioxidants some of which are believed to be carcinogenic (Djeridane *et al.*, 2006).

Women in Katsina State, Nigeria, often employ the services of medicinal plants for their traditional maternal health care because of their affordability, accessibility and fear of excessive side effects associated with orthodox drugs (Kankara *et al.*, 2015). In this study, we report the phenolic content, flavonoid content and antioxidant activity of some herbs used for traditional maternal health care in Katsina State, Nigeria. The herbs studied are *Artemisia annua*, *Euphorbia balsamifera*, *Guiera senegalensis*, *Ipomoea asarifolia* and *Mitragyana inermis*.

Artemisia annua L. (Asteraceae), commonly known as sweet annie or “Tazargade” in Hausa language, is endemic to the northern parts of Chahar and Suryuan Provinces of China but now wild to Europe, America and Africa (Bhakuni and Jain, 2001). *A. annua* has been used to treat various ailments especially those related to the treatment and prevention of fevers (van der Kooy and Sullivan, 2013). Pregnant women in Katsina State use this herb to cure fever symptoms and for general wellbeing.

Euphorbia balsamifera Aiton, commonly known as balsam spurge or “Aliyara” in Hausa language is an erect shrub branched from base up to 4m high. The plant is commonly used as a hedge and field boundary marker. Despite its toxic potential (Kheyrodin and Ghazvinian, 2013), it is used by women as laxative during postpartum period.

Guiera senegalensis J.F. Gmel (Combretaceae), locally known as “Sabara” or “Barbarta” in Hausa language of northern Nigeria is widely distributed in western Africa. Traditionally, *G. senegalensis* is used to treat various illnesses such as hypertension, malaria, cough, diabetes and many microbial infections. The plant is also widely used by women in Katsina State, Nigeria during postpartum period for general wellbeing (Kankara *et al.*, 2014).

Ipomoea asarifolia (Desr.) Roem. And Schult. (Convolvulaceae) locally known as “Duman Kada” in Hausa language, is a perennial creeping herb mostly found on sandy soils or waste areas. Traditionally, *I. asarifolia* is used to treat various disorders (Jegade *et al.*, 2009). It is also used as laxative by women during postpartum period in Katsina state.

Mitragyana inermis Wild Kuntze (Rubiaceae) commonly known as false Abura or “Giyayya” in Hausa language, is widely known and used in traditional medicine in West Africa to treat various diseases including fever, high blood pressure, dysentery, syphilis and epilepsy (Wakirwa *et al.*, 2013). In Katsina State, pregnant

women use *M. inermis* to treat edema (Kankara *et al.*, 2015).

Materials and Methods

Sample collection and identification

Artemisia annua leaves were graciously donated by Malam Musa, Director, Katsina Agricultural Project Unit (KTAPU). *Guiera senegalensis* leaves, *Euphorbia balsamifera* twigs and *Ipomoea asarifolia* leaves were collected from Umaru Musa Yar’adua University, Katsina campus. *Mitragyana inermis* leaves were purchased from *Filin Bugu* herbal market, Katsina. All species were identified and authenticated by Professor Munier Abdel-Ghani of Biology Department, Umaru Musa Yar’adua University, Katsina. Voucher specimens were prepared and deposited at the herbarium of Biology Department, Umaru Musa Yar’adua University Katsina State, Nigeria. Herbarium numbers for *A. annua*, *G. senegalensis*, *E. balsamifera*, *I. asarifolia* and *M. inermis* are SSK015, SSK009, SSK041, SSK108 and SSK030, respectively.

Samples preparation

Samples were air dried in laboratory and ground to powder using a mill (Retsch, SM100 comfort Hann, Germany). The powder obtained from different samples were individually packaged and stored in dark at an ambient temperature.

Extraction of plants material

About 60 g dried powder of each sample was put in 1 liter conical flask, covered with parafilm and wrapped with aluminium foil. Samples were then extracted with deionized water (600 cm³) in a temperature- controlled water bath shaker at a constant speed and temperature (40°C) for 1 hour. Crude extracts were then filtered through Whatmann No. 1 filter paper. Filtrates were collected and lyophilized using freeze dryer. The lyophilized extracts were used for the estimation of phenolic antioxidants and evaluation of antioxidant activities of each sample using various biochemical assays.

Determination of Total Phenolic Content (TPC)

Total Phenolic Content (TPC) was determined using Folin-Ciocalteu’s (FC) method as reported by Thoo *et al.*, 2010 with slight modifications. Briefly, 500 µL of dilute crude extract was mixed with 500 µL of Folin-Ciocalteu reagent (diluted 10 times). After 3 minutes, 400 µL of sodium carbonate anhydrous was added and vortexed. After 2 hours of incubation in the dark at room temperature, absorbance was determined at 765 nm against a blank (prepared by replacing plant extract with deionized water) using a UV/VIS spectrophotometer (Lambda 25, ParkinElmer, Singapore). Measurements were calibrated to a standard curve of prepared gallic acid solution (10 – 100 µg/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 100 g of dry weight (DW).

Determination of Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) was determined using aluminium chloride calorimetric assay reported by Kaur and Mondal, 2014 with slight modifications. Briefly, 125 μ L of crude extract was mixed with 625 μ L deionized water and 37.5 μ L of 5% sodium nitrite. The mixture was allowed to stand for 6 minutes and 75 μ L of 10% aluminium chloride-6-hydrate was added thereafter. After 5 minutes, 250 μ L sodium hydroxide solution was added. 137.5 μ L deionized water was added and mixed. Absorbance was measured immediately at 510 nm against a blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a standard curve of prepared quercetin solution (0 – 800 μ g/ml) with equation $y = 0.0000x + 0.003$ ($R^2 = 0.981$) and TFC was then expressed as milligram quercetin equivalent (QE) per 100 g dry weight (DW).

Ferric reducing antioxidant power (FRAP)

The FRAP assay was carried out using the protocols of Benzie and Strain, 1996. The stock solution included 300 mM acetate buffer (3.1g $C_2H_3NaO_2 \cdot 3H_2O$ and 16ml $C_2H_4O_2$), pH 3.6, 10 mM 2,4,6-Tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. Fresh FRAP working solution was prepared by mixing acetate buffer, TPTZ solution and $FeCl_3 \cdot 6H_2O$ solution in the ratio of 10:1:1 respectively. The FRAP solution was warmed at 37°C for 30 minutes in a water bath before being used. 100 μ L aqueous plant extract (1 mg/ml) was allowed to react with 1000 μ L FRAP solution in dark for 30 minutes. Absorbance of the colored product (ferrous tripyridyltriazine complex) was measured at 593 nm wavelength using UV/VIS spectrophotometer against a blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a linear standard curve of prepared ascorbic acid solution (5 – 35 mg/ml) with equation $y = 0,045x + 0.395$ ($R^2 = 0.996$) and results expressed as milligram ascorbic acid equivalent (AAE) per 100 g dry weight (DW).

Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using phosphomolybdate assay described by Mohammed *et al.*, 2014 with slight modifications. The working solutions consist of 600 mM sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate. 1000 μ L working solution was mixed with 100 μ L of the aqueous plant extract (1 mg/ml) and incubated in a water bath at 95°C for 90 minutes. The mixture was allowed to cool to room temperature before absorbance was

measured at 695 nm using UV/VIS spectrophotometer against a blank (prepared by replacing plant extract with deionized water). Measurements were calibrated to a linear standard curve of prepared ascorbic acid solution (100 – 700 μ g/ml) with equation $y = 0.0016 + 0.0222$ ($R^2 = 0.999$) and results expressed as milligram ascorbic acid equivalent (AAE) per 100 g dry weight (DW).

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

Antioxidant capacity through DPPH scavenging activity was determined according to the experimental protocol reported by Tan *et al.*, 2013 with slight modifications. Methanolic DPPH stock solution was prepared by dissolving 4mg 2,2'-diphenyl-1- picrylhydrazyl (DPPH) powder into 100 mL absolute methanol. Working solution was obtained by mixing 50ml stock solution with 20 mL methanol in order to obtain absorbance of 1.00 ± 0.02 unit at 517 nm wavelength. 100 μ L of various concentrations of plant extracts were mixed with 900 μ L methanolic DPPH solution and allowed to stand in the dark, at room temperature for 30 minutes. After 30 minutes incubation, absorbance was measured at 517 nm using UV/VIS spectrophotometer against a blank (methanol). Percentage DPPH scavenging activity was determined using the relation:

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

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

Statistical analysis

Results were analyzed using SPSS software (version 20) and expressed as mean \pm standard deviation of 3 replicate. One- way analysis of variance (ANOVA) with Duncan's test was carried out to test significant difference between levels of treatments. Significant levels were defined using value $P < 0.05$. Pearson correlation between variables were also established using the SPSS.

RESULTS AND DISCUSSION

Total phenolic content (TPC)

The Folin-Ciocalteu method is widely used to determine the total phenolic content from plants' extracts because of its simplicity and reproducibility. The assay is based on phenolic compounds' ability to transfer electron to the Folin-Ciocalteu reagent in alkaline medium, forming a dark blue complex (Li *et al.*, 2013). Total phenolic content (TPC) of the studied plant extracts is presented in figure 1. *Guiera senegalensis* had significantly ($P < 0.05$) higher TPC value (136.07 ± 21.68 mg GAE/g DW) followed by *Mitrigyana inermis* (113.69 ± 7.80 mg GAE/g DW). Then followed by *Ipomoea asarifolia* (97.02 ± 8.98 mg GAE/g DW) which is not significantly different from *Artemisia annua* (94.40 ± 8.69 mg GAE/g DW) while *Euphorbia balsamifera* had the least

TPC value (64.64 ± 1.55 mg GAE/g DW). Sombie *et al.*, 2011 reported higher total phenolic content values (19.87 ± 2.68 mg GAE/100 mg DW to 73.90 ± 2.34 mg GAE/100 mg DW) from the galls of *G. senegalensis* extracted with different solvents of varied polarity. The higher TPC values reported in their study could be attributed to the different part of the plants and/or the solvents they used for extraction. The TPC value of *G. senegalensis*

reported in this study is however, higher than that of all the 223 medicinal plants of China which ranges from 0.19 mg GAE/DW to 101.33 mg GAE/g DW (Li *et al.*, 2013). Higher TPC values observed in *G. senegalensis* may be responsible for the various biological activities attributed to this plant (Sombie *et al.*, 2011; Yagana *et al.*, 2012; Sombié *et al.* 2013).

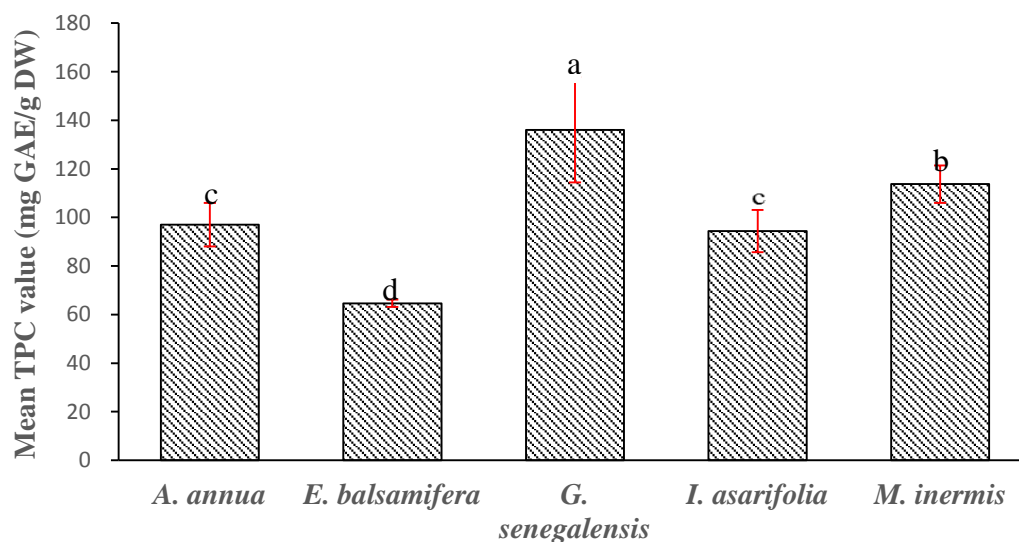


Figure 1: Total phenolic content (TPC) of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria. Error bars represent standard deviation (n = 3) and values with same superscripts are not significantly different (P < 0.05).

Total Flavonoid Content (TFC)

Total flavonoid content is expressed as quercetin equivalents (QE). Significant difference (P < 0.05) exists among the tested plant extracts (Figure 2). Similar pattern was observed where *G. senegalensis* showed the highest TFC (158.80 ± 11.32 mg QE/g DW) followed by *M. inermis* (87.50 ± 10.01 mg QE/g DW). The latter being not significantly different from the results obtained in respect to *I. asarifolia* and *A. annua* (75.00 ± 5.01 mg QE/g DW and 72.22 mg QE/g DW, respectively). *E. balsamifera* showed the least TFC (35.18 ± 0.80 mg QE/g DW) content. The total flavonoid content observed in *G.*

senegalensis is higher than those of all the 20 Indian medicinal plants reported by Sulaiman and Balachandran 2012 (0.08 – 8.54 mg QE/100 mg DW) and all the 31 medicinal plants of Taiwan (3.11 ± 0.04 to 71.89 ± 0.42 μ g RE/ mg DW) (Ho *et al.*, 2012). Although *E. balsamifera* appeared to have least TFC in this study, its TFC value is higher than those of the 4 medicinal plants reported from Indonesia (Suhartono *et al.*, 2012). Flavonoids are polyphenolic compounds associated with antioxidant activity because of their ability to induce lipid oxidation. Flavonoids were reported to possess anti-inflammatory effects, anti-carcinogenic effects and activity against many other diseases which are commonly associated with oxidative stress (Bhadwaj *et al.*, 2014).

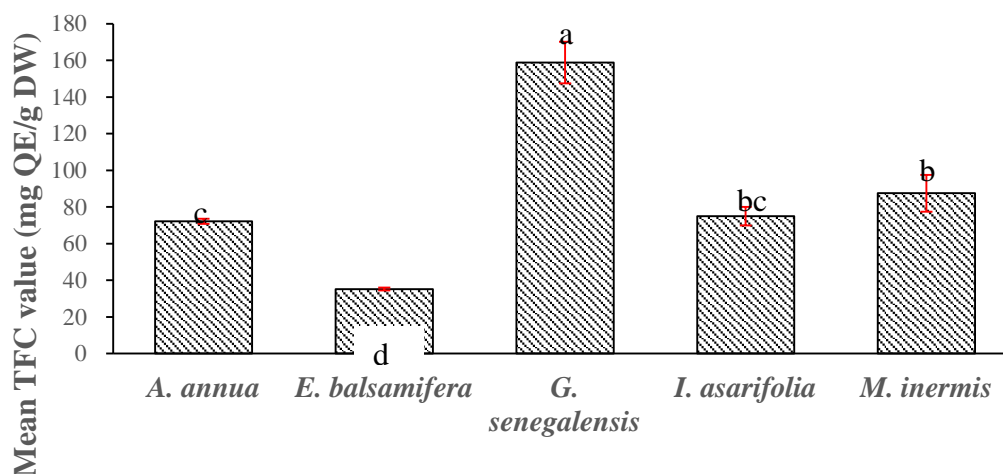


Figure 2: Total flavonoid content (TFC) of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria. Error bars represent standard deviation (n = 3) and values with same superscripts are not significantly different (P< 0.05).

The Ferric reducing antioxidant power (FRAP) is a simple and widely used analytical assay used to assess the antioxidant property of samples. The assay relies on the ability of an antioxidant compound to reduce ferric tripyridyltriazine (Fe^{3+} -TPTZ) complex to a colored ferrous tripyridyltriazine complex (Bothon *et al.*, 2013). Ferric reducing power in this study is expressed as ascorbic acid equivalent and sample values were compared to that standard antioxidant (trolox). Tested plants showed significant difference in their ferric reducing ability (figure 3). Like in phenolic antioxidants, *G. senegalensis* appeared to have the highest ferric reducing ability among all the tested plant extracts, having 34.24 ± 1.46 mg AAE/g DW

which is close to that observed with regard to trolox (41.07 ± 3.11 mg AAE/g DW). These patterns are followed by *A. annua* (19.40 ± 4.1 mg AAE/g DW) which is not significantly different from *M. inermis* (19.37 ± 1.64 mg AAE/g DW). Unlike in phenolic antioxidant assays (TPC and TFC), *E. balsamifera* showed higher FRAP value (10.94 ± 0.97 mg AAE/g DW) than *I. asarifolia* (9.18 ± 1.53 mg AAE/g DW). The FRAP values reported in this study are relatively lower than those of the majority of selected medicinal plants of India (Arasu, 2014). The FRAP value of *G. senegalensis* observed is higher than those of hexane and chloroform extracts of *Rumex acetosella* (Baig *et al.*, 2011).

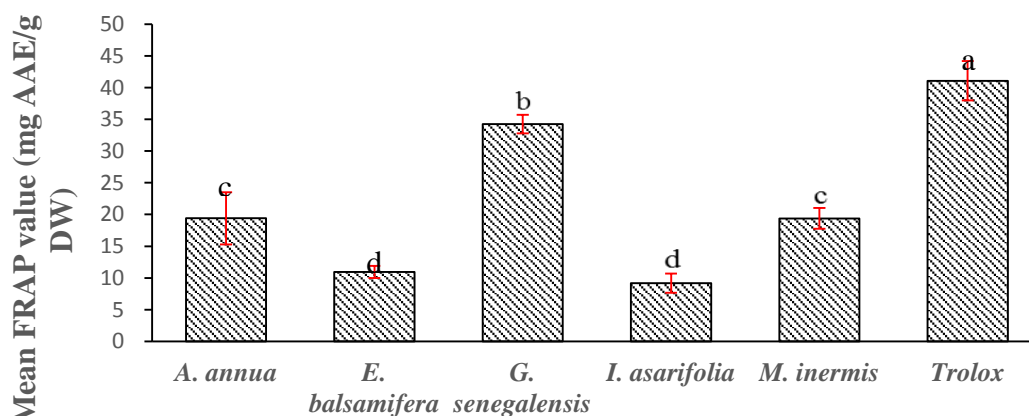


Figure 3: Ferric reducing antioxidant power (FRAP) of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria. Error bars represent standard deviation (n = 3) and values with same superscripts are not significantly different (P< 0.05).

Total antioxidant capacity (TAC)

The total antioxidant capacity of the various plant extracts was evaluated using phosphomolybdate assay which is based on the reduction of molybdate (VI) to molybdate (V) by an antioxidant forming a green phosphomolybdate (V) complex whose formation can be monitored spectrophotometrically (Ahmed and Tariq, 2012). All the tested plants showed considerably high total antioxidant capacity (figure 4). Among the tested plants, *G. senegalensis* still showed the highest TAC value (424.73 ± 46.31 mg AAE/g DW) followed by *M. inermis* (332.30 ± 10.27 mg AAE/g DW) whose TAC value is not significantly ($P < 0.05$) different from that of *I. asarifolia* (294.20 ± 16.63 mg AAE/g DW). *E. balsamifera*

however, showed higher TAC value (259.90 ± 11.95 mg AAE/g DW) in comparison to *A. annua* (196.37 ± 24.56 mg AAE/g DW). The TAC values reported in this study are higher than those of *Rumex hastatus* irrespective of the extraction solvent (Sahreen *et al.*, 2011). TAC values reported herein are also higher than those of all the tested solvent extracts of *Melilotus indicus* in both flowering and fruiting seasons (Ahmed *et al.*, 2012), as well as those of all extracts and fractions of *Vernonia blumeoides* (Aliyu *et al.*, 2011). Phosphomolybdate assay is readily employed for antioxidant activity evaluation because of its simplicity and the fact that it targets both fat and water soluble antioxidants.

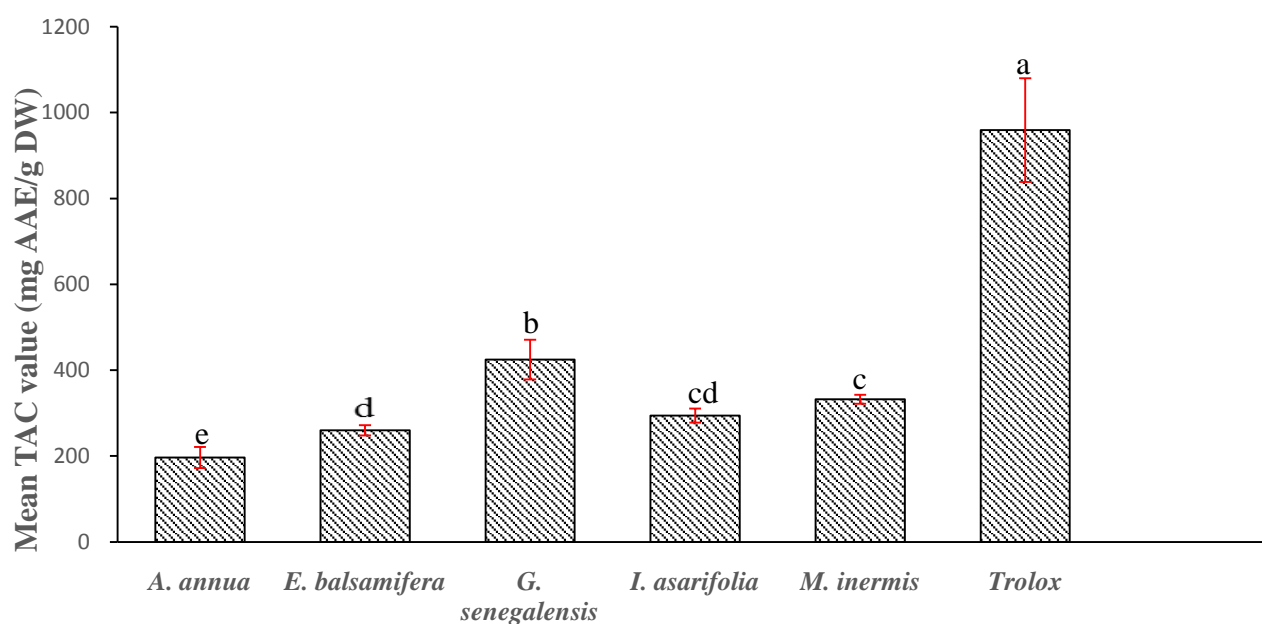


Figure 4: Total antioxidant capacity (TAC) of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria. Error bars represent standard deviation (n = 3) and values with same superscripts are not significantly different ($P < 0.05$).

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

The result of 2,2'- diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability is presented in figure 5. From the figure, it can be seen that all the tested plant extracts differ significantly ($P < 0.05$) in their DPPH- radical scavenging activity. The figure also revealed that DPPH radical scavenging among all the tested plant extracts is concentration dependent, whereby the sample concentration is proportional to the DPPH radical scavenging activity. *G. senegalensis* showed higher scavenging activity in all concentrations except in 1000 $\mu\text{g/ml}$ where it was superseded by *M. inermis*. The higher scavenging activities observed in *G. senegalensis* and *M.*

inermis may be attributed to their higher phenolic and flavonoid contents. *E. balsamifera* showed the lowest activity. The DPPH scavenging activity of *G. senegalensis* and *M. inermis* reported in this study is higher than those of *Bacopa monniera*, *Aloe vera*, *Moringa oleifera* and *Zingiber officinale* reported by Padmanabhan and Jangle (2012). DPPH radical scavenging has been widely used to assess the antioxidant activity of plant samples (Sahu *et al.*, 2013; Asadujjaman *et al.*, 2013). This assay procedure is rapid, simple and inexpensive. It depends on the ability of an antioxidant to reduce DPPH radical whose color changes from purple to yellow, when its odd electron receives hydrogen from the antioxidant to form reduced DPPH-H radical. This color change is monitored

spectrophotometrically at 517 nm (Kedare and Singh, 2011). The DPPH scavenging results were used to determine the IC₅₀ of the tested plants. The IC₅₀ is the concentration required to scavenge 50% of the DPPH radical and it is used to determine the

efficacy of antioxidants whereby the lower the IC₅₀, the better the DPPH scavenging. In this study, the IC₅₀ of the tested plant extracts is in the order *E. balsamifera* > *I. asarifolia* > *A. annua* > *M. inermis* > *G. senegalensis* (Figure 6).

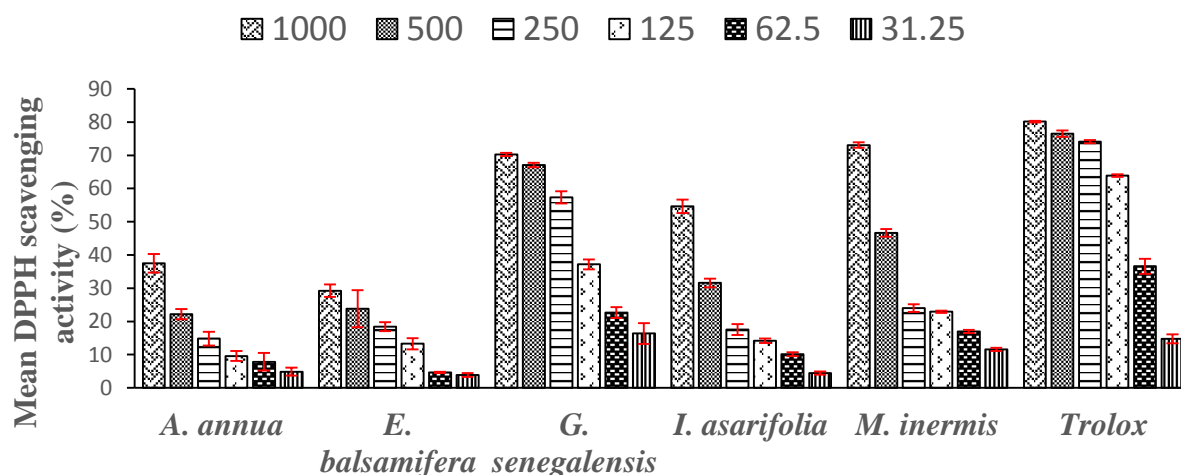


Figure 5: 2,2'- diphenyl-1- picrylhydrazyl (DPPH) radical scavenging activity of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria. Error bars represent standard deviation (n = 3) and values with same superscripts are not significantly different (P < 0.05).

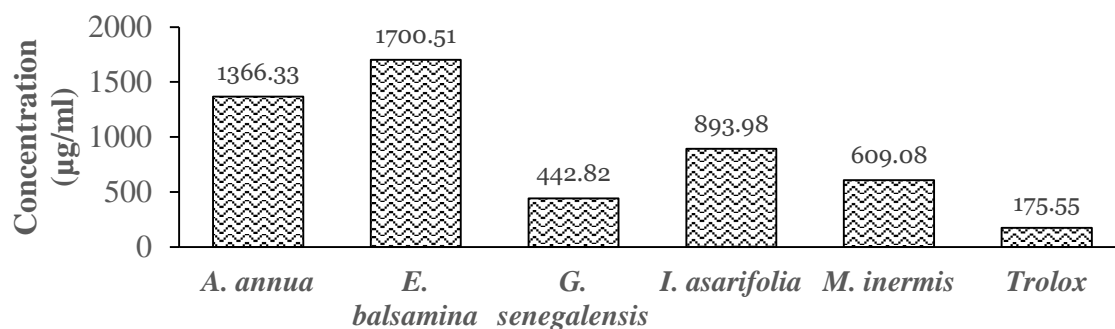


Figure 6: IC₅₀ (based on DPPH radical scavenging) of some medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria.

Pearson’s correlation analysis

Pearson’s correlation between the phenolic antioxidants (TPC and TFC) and their antioxidant activities (FRAP, TAC and DPPH) is presented in table 2. From the table, it can be seen that there is highly significant (P < 0.01) positive correlations among all the assays. Highest correlations (r² = 0.883) was observed between TPC and TFC. TFC displayed higher correlations with the antioxidant assays (r² = 0.867 and 0.799 for FRAP and TAC respectively) except in DPPH, where higher correlation was observed with TPC (r² = 0.750). In general, there is strong relationship between phenolic compounds (TFC and TPC) and antioxidant properties (FRAP, TAC and DPPH) in the tested plants hence it is assumed that the

phenolic compounds are responsible for the antioxidant properties observed in the tested plants. Although phenolic compounds are believed to be responsible for antioxidant properties, other chemicals such as vitamins and minerals also contribute to the antioxidant properties of medicinal plants (Ogbonnaya and Chinedum, 2013). Correlation analysis results reported in this study agree with the findings of Diaz *et al.* (2012) who reported a positive strong linear correlation between phenolic compounds (TPC and TFC) and antioxidant activity (FRAP) in some selected Chinese medicinal plants. Adriana *et al.* (2012) however, reported weak correlation between phenolic compounds and antioxidant activities of *Sida rhombifolia* and *Herissantia crispa*.

Table 1: Correlation analysis between phenolic antioxidants (TPC and TFC) and antioxidant activities (FRAP, TAC and DPPH) of medicinal plants used for traditional maternal healthcare in Katsina State, Nigeria.

	TPC	TFC	FRAP	TAC
TFC	0.883**			
FRAP	0.768**	0.867**		
TAC	0.722**	0.799**	0.650**	
DPPH	0.750**	0.695**	0.487*	0.717**

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 $P < 0.01$

CONCLUSION

In conclusion, medicinal plants that are commonly used for traditional maternal healthcare in Katsina State, Nigeria especially *Guiera senegalensis* and *Mitragyana inermis* have shown considerably high antioxidant activity which could be attributed to their high phenolic content. This study therefore validates the folkloric use of these medicinal plants to alleviate ailments associated with oxidative stress, within the concentrations covered. Considering the fact that *G. senegalensis* is in most cases prepared as decoction in combination with *A. annua*, *E. balsamifera* and/or *I. asarifolia*, further studies aimed at evaluating the effects of herbal combination on the phenolic compounds and antioxidant properties would be ideal. In addition, influence of other cultural practices on the medicinal properties of these plants should be assessed.

REFERENCES

- Adriana, M. F. O., L.S., L., Souto Pereira, C., Neves Matias, W., Albuquerque Gomes, R., Souza Chaves, O., Simões de Assis, T. (2012). Total Phenolic Content and Antioxidant Activity of Some Malvaceae Family Species. *Antioxidants*, 1(1), 33–43. doi:10.3390/antiox1010033
- Ahmed, D., Baig, H., and Zara, S. (2012). Seasonal variation of phenolics, flavonoids, antioxidant and lipid peroxidation inhibitory activity of methanolic extract of *Melilotus indicus* and its sub-fractions in different solvents. *International Journal of Phytomedicine*, 4, 326–332.
- Ahmed, D., and Tariq, S. A. (2012). In vitro study of antimicrobial and antioxidant activities of methanolic extracts of leaves, fruits and bark of *Ficus glomerata*. *International Journal of Medicinal and Aromatic Plants*, 2(1), 30–33.
- Aliyu, A.B., Ibrahim, M.A., Musa, A.M., Bulus, T. and Oyewale, A. O. (2011). Phenolic Content and Antioxidant Capacity of Extracts and Fractions of *Vernonia blumeoides* (Asteraceae). *International Journal of BiologicaChemistry*, 5(6), 352–359.
- Al-jameil, N., Tabassum, H., Al-mayouf, H., Aljohar, H. I., and Alenzi, N. D. (2014). Analysis of serum trace elements-copper, manganese and zinc in preeclamptic pregnant women by inductively coupled plasma optical emission spectrometry: a prospective case controlled study in Riyadh, Saudi Arabia. *International Journal of Clinic Experimental Pathology*, 7(5), 1900–1910.
- Arasu, P. T. (2014). Quantification of total phenol, flavonoid contents and antioxidant. *International Journal of Current Research in Chemistry and Pharmaceutical Sciences*, 1(7), 73–80.
- Asadujjaman, M., Hossain, M.A., Karmakar, U. K. (2013). Assessment of DPPH free radical scavenging activity of some medicinal plants 1. *Pharmacology Online*, 1, 161–165.
- Baig, H., Ahmed, D., and Zara, S. (2011). In vitro Evaluation of Antioxidant Properties of Different Solvent Extracts of *Rumex acetosella* Leaves. *Oriental Journal of Chemistry*, 27(4), 1509–1516.
- Benzie, I. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. *Analytical Biochemistry*, 239(1), 70–6. doi:10.1006/abio.1996.0292
- Bhadwaj, R., Yadav, A., Sharma, R. A. (2014). *Tecomella undulata*-Phenolic Compounds and Antioxidant Activities. *Research Journal of Medicinal Plants*, 8(5), 223–230.

- Bothon, F. T. D., Debiton, E., Avlessi, F., Forestier, C., Teulade, J.-C., and Sohounhloue, D. K. C. (2013). In vitro biological effects of two anti-diabetic medicinal plants used in Benin as folk medicine. *BMC Complementary and Alternative Medicine*, 13(1), 51. doi:10.1186/1472-6882-13-51
- Diaz, P., Jeong, S. C., Lee, S., Khoo, C., Koyyalamudi, S. R., Bo, M., and Gan, S. (2012). Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. *Chinese Medicine*, 7(26), 1–9.
- Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P. and Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry*, 97(4): 654-660.
- Ho, Y., Huang, S., Deng, J., Lin, Y., Chang, Y., and Huang, G. (2012). In vitro antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan. *Botanical Studies*, 53, 55–66.
- Jegade, I. A., Nwinyi, F. C., Ibrahim, J., Ugbabe, G., Dzarma, S., and Kunle, O. F. (2009). Investigation of phytochemical , anti inflammatory and anti nociceptive properties of Ipomoea asarifolia leaves. *Journal of Medicinal Plant Research*, 3(3), 160–165.
- Kankara, S. S., Ibrahim, M. H., Mustafa, M., and Go, R. (2015). Ethnobotanical survey of medicinal plants used for traditional maternal healthcare in Katsina state, Nigeria. *South African Journal of Botany*, 97, 165–175. doi:10.1016/j.sajb.2015.01.007
- Kankara, S. S., Mustafa, M., Ibrahim, H. M., Nulit, R. and Go, R. (2014). 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. *American Journal of Phytomedicine and Clinical Therapeutics*, 2(12), 1378–1392.m
- Kaur, S. and Mondal, P. (2014). Study of Total Phenolic and Flavonoid Content , Antioxidant Activity and Antimicrobial Properties of Medicinal Plants. *Journal of Microbiology and Experimentation*, 1(1), 1–6.
- Kedare, S. B., and Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*, 48(4), 412–22. doi:10.1007/s13197-011-0251-1
- Kheyrodin, H., and Ghazvinian, K. H. (2013). The Toxicity Material Extraction From Euphorbia Species. *Iran Agricultural Research*, 31(2), 66– 73.
- Kilari, A., Deshpande, M. and Joshi, S. (2014). A Novel Molecular Mechanistic Hypothesis to Validate the Therapeutic Effects of Tribulus terrestris (Gokshur) to Ameliorate Pathophysiology and Improve Pregnancy and Fetal Outcome in Preeclampsia. *Gynecology and Obstetrics*, 04(01), 1–7. doi:10.4172/2161-0932.1000195
- Li, S., Li, S.-K., Gan, R.-Y., Song, F.-L., Kuang, L., and Li, H.-B. (2013). Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. *Industrial Crops and Products*, 51, 289–298. doi:10.1016/j.indcrop.2013.09.017
- Mistry, H. D., and Williams, P. J. (2011). The importance of antioxidant micronutrients in pregnancy. *Oxidative Medicine and Cellular Longevity*, 2011, 1– 12. doi:10.1155/2011/841749
- Mohammed, C. B., Chahid, B., Fatima, B., Fatima-Zohra, S., Meriem, B., and Farid, C. (2014). 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*, 13(3), 486–491. doi:10.5897/AJB2013.12833
- Ogbonnaya, E. C., and Chinedum, E. K. (2013). Health promoting compounds and in vitro antioxidant activity of raw and decoctions of *Gnetum africanum* Welw. *Asian Pacific Journal of Tropical Disease*, 3(6), 472–479. doi:10.1016/S2222-1808(13)60103-6
- Padmanabhan, P., and Jangle, S. N. (2012). Evaluation of DPPH Radical Scavenging Activity and Reducing Power of Four Selected Medicinal Plants and Their Combinations. *International Journal of Pharmaceutical Science and Drug Discovery*, 4(2), 143–146.
- Padmini, E. and Lavanya, S. (2012). Effect of Mint-Tea on HSP Mediated Survival. *International Journal of Biological and Pharmaceutical Research*, 3(5), 703–712.
- R. S. Bhakuni, D. C. Jain*, R. P. S. and S. K. (2001). Secondary metabolites of *Artemisia annua* and their biological activity. *Current Science*, 80(1), 35– 48.
- Sahreen, S., Khan, M.R., Khan, R. A. (2011). Phenolic compounds and antioxidant activities of *Rumex hastatus* D . Don . leaves. *Journal of Medicinal Plant Research*, 5(13), 2755–2765.
- Sahu, R. K., Kar, M., and Routray, R. (2013). Journal of Medicinal Plants Studies DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribals of Odisha , India. *Journal of Medicinal Plants Studies*, 1(4), 21–27.

- Sharma, A., Tiwari, A., Kiran, P., and Meera, A. (2014). FRIOS Parameters , Key to Reduce Feto-Maternal Mortality in Pre-Eclampsia. *Open Journal of Obstetrics and Gynecology*, (April), 321–325.
- Sombié, P. A. E. D., Konate, K., Youl, E., Coulibaly, A. Y., Kiendrébéogo, M., and Muhammad, I. (2013). GC-MS analysis and antifungal activity from galls of *Guiera senegalensis* J . F Gmel (Combretaceae). *Journal of Applied Pharmaceutical Sciences*, 3(12), 6–12. doi:10.7324/JAPS.2013.31202
- Sombie, P.A.E.D, A. Hilou, C. Mounier, A.Y. Coulibaly, M. Kiendrebeogo, J. F. M. and O. G. N. (2011). Antioxidant and Anti-inflammatory Activities from Galls of *Guiera senegalensis* J.F. Gmel (Combretaceae). *Research Journal of Medicinal Plants*, 5(4), 448–461.
- Sreeramulu, D., Reddy, C. V. K., Chauhan, A., Balakrishna, N., and Raghunath, M. (2013). Natural antioxidant activity of commonly consumed plant foods in India: effect of domestic processing. *Oxidative Medicine and Cellular Longevity*, 2013, 369479. doi:10.1155/2013/369479
- Suhartono, E., Viani, E., Rahmadhan, M. A., Gultom, I. S., Rakhman, M. F., and Indrawardhana, D. (2012). Total flavonoid and Antioxidant Activity of Some Selected Medicinal Plants in South Kalimantan of Indonesian. *APCBEE Procedia*, 4, 235–239. doi:10.1016/j.apcbee.2012.11.039
- Sulaiman, CT. and Balachandran, I. (2012). Total Phenolics and Total Flavonoids in Selected Indian Medicinal Plants. *Indian Journal of Pharmaceutical Sciences*, 74(3), 258– 260.
- Tan, E. S., Abdullah, A., and Maskat, M. Y. (2013). Effect of drying methods on total antioxidant capacity of bitter melon (*Momordica charantia*) fruit, 710(2013), 710–716. doi:10.1063/1.4858738
- Thoo, Y. Y., Ho, S. K., Liang, J. Y., Ho, C. W., and Tan, C. P. (2010). Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (*Morinda citrifolia*). *Food Chemistry*, 120(1), 290–295. doi:10.1016/j.foodchem.2009.09.064
- Van der Kooy, F., and Sullivan, S. E. (2013). The complexity of medicinal plants: the traditional *Artemisia annua* formulation, current status and future perspectives. *Journal of Ethnopharmacology*, 150(1), 1–13. doi:10.1016/j.jep.2013.08.021
- Wagner, L. K. (2004). Diagnosis and Management of Preeclampsia. *American Family Physician*, 70(12), 2317–2324.
- Wakirwa, J. H., Yawate, U. E., Zakama, S. G., Muazu, J., and Madu, S. J. (2013). Phytochemical and Antibacterial Screening of the Methanol Leaf Extract of *Mitragyna inermis* (Wild). *International Journal of Pharmaceutical Research and Innovation*, 6, 1–6.
- Yagana, S. A., Sodipo O. A. Yagana, K. and Modu, S. (2012). Antibacterial activity of the extracts of the root of *Guiera senegalensis* J. F. Gmel. *Journal of Pharmaceutical and Biomedical Sciences*, 23(26), 1– 7.