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Evaluation of antioxidant activities and total phenolic contents of selected Sudanese medicinal plants

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Abstract:
Antioxidant activities of methanol and water extracts of *Cymbopogon proximus* aerial parts, *Ocimum basilicum* leaves and *Tribulus terrestris* fruits were evaluated using free radical scavenging activity of 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) and total antioxidant capacities (TAC) by the phosphomolybdenum method. The total phenolic, flavonoid and tannin contents for these extracts were estimated and thereafter were analyzed for their correlation with antioxidant activities. Correlation analysis between the values of DPPH% and TAC revealed a significant correlation with (r=0.96; P=0.002) indicates the viability of the two models for evaluating antioxidants from medicinal plants. It was also observed that there were strong relationships between antioxidant activities and total phenols with higher values observed with water extract(r=0.994 and r= 0.996) with RSA% and TAC respectively. A significant correlation between total phenolic contents and flavonoids was found with (r=0.996; P=0.000). The study findings revealed that *O. basilicum* methanol extract of the leaves displayed powerful antioxidant activity in the two assay models with (482.25±0.08mg AA Eq/l) for TAC and (80±0.01%) for RSA%, followed by the plant water extract, while *C. proximus* showed moderate activities for its two extracts compared to pyrogallate(PRG) as standard compounds, whereas *T. terrestris* showed the lowest antioxidant activity. Among screened plant materials, remarkable high phenolic and flavonoid contents were found in *O. basilicum* leaves methanol extract (14.56±0.071mg Gallic acid Eq/g) and (19.03±0.03 mg Querectin E/g) on the basis of dry matter respectively, which contributed to its high antioxidant activity. These findings may indicate that the plant can provide protection against free radicals induced disorders and oxidative stress.

Key words: *Ocimum basilicum,* DPPH, Total phenolic content, Total antioxidant capacity .

Introduction:

During metabolic process and exposure to external environment, a large amount of free radicals are generated in human body which pose a major influence on biological macromolecules. Numerous disorders like rheumatoid arthritis, cardiovascular disorders, cystic fibrosis, metabolic disorders, neurodegenerative diseases and gastrointestinal ulcerogenesis are reported as reactive oxygen species mediated(Muller *et al*, 2007). The elimination of free radicals by the intake of anti-oxidative agents is important to reduce the oxidative stress and hence for the prevention of chronic diseases(Stahl-Biskup *et al*, 2004).

Cymbopogon proximus (Fig:1) is one of the important plants in African and Sudanese folk medicine. Traditional applications of *Cymbopogon* genus in different countries shows high applicability as a common tea, medicinal supplement, anti-inflammatory and analgesic(Avoseh, 2015). The plant has antioxidant, anti-inflammatory, detoxification, and chemoprotective properties(Al Haznawi *et al*, 2007).

Ocimum basilicum (Fig: 2) contains bioactive compounds and minerals that could enhance the curative process of health(Daniel *et al*, 2011). Many studies have established that basil leaves extracts have potent antioxidant, anti-aging, anticancer, antiviral, and antimicrobial properties (Bozin *et al*, 2006).

Tribulus terrestris fruits(Fig: 3) has an ancient tradition in folk medicine and in ayurveda as a diuretic, antiseptic, mood enhancer and anti-inflammatory agent(Sasikumar *et al*, 2014). Since ancient times it is regarded as an aphrodisiac in addition to its beneficial claims on various ailments such as urinary infections, inflammations, oedema and ascites(Gauthaman and Ganesan, 2008). The present study was undertaken to evaluate *in vitro* antioxidant and antiradical activities of water and 80% methanol extracts of three selected Sudanese medicinal plants. Total Antioxidant Capacity (TAC) using phosphomolybdenum method and antiradical scavenging activity using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) methods and Total Phenolics (TP), Total Flavonoids (TF) contents and total tannins of the extracts were assessed.

Fig(1): *Cymbopogon proximus*

Fig(2): *O. basilicum* leaves(Prasath, *et al,* 2019).

Fig(3): *Tribulus terrestris* fruits (Shama *et al*, 2019).

Material and methods:

Chemicals and Reagents

Folin-Ciocalteu reagent, querecetin, tannin, gallic acid (Fluka, UK), 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) (Sigma-Aldrich Co.), aluminum chloride, anhydrous sodium carbonate, ascorbic acid and all other chemicals were of analytical grade.

Plant materials:

Cymbopogon proximus aerial parts and *Ocimum basilicum* leaves were obtained from horticulture section, ministry of agriculture, Khartoum state, whereas *Tribulus terrestris* fruits were obtained from Om durman market. All these plants were identified in the Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, where antioxidant activities and phytochemical screening carried out. The Scientific name, family, local name, plant part used and medicinal uses of the selected plants have been presented in Table (1). Selected parts of *C. proximus* and *O. basilicum* were separated and washed thoroughly in running tap water and left until dried at room temperature. Dried plant materials were milled, to make a fine powder, in a grinder.

Table (1): Selected medicinal plants

Scientific name	Family/ Part used	Arabic name	Medicinal uses	References
Cymbopogon proximus	Poaceae/ Gramineae Aerial parts	Mahareeb	In Sudan for gout, renal colic, helmenthiasis, diuresis, antipyretic and inflammation of the prostate.	Eltohami, 2012
Ocimum basilicum	Lamiaceae Leaves	Rihan	Basil leaves are used in folk medicine for cancer, convulsion, epilepsy, gout, nausea, sore throatand bronchitis	Khalid *et al*, 2006.
Tribulus terrestris	Zygophyllaceae Fruits	Derisa	Roots and fruits are useful in rheumatism, piles, renal vesicle calculi and menorrhagia. The seeds are used to treat kidney stone and gout	Kardy, 2010; Naira *et al*, 2017.

Preparation of extracts

Preparation of methanol extract

500 g of each plant were extracted by maceration with sufficient quantity of 80% (v/v) methanol at room temperature for 48h. The process of extraction was repeated twice to maximize the extraction process. The extracts were filtered through Whatman No1, then concentrated using a rotary evaporator under reduced pressure at 40°C. The dry extracts obtained were weighed (Yanti *et al*, 2015).

Preparation of the aqueous extract:

About 100 g of each plant sample was soaked in 500 ml hot distilled water, and left till cooled down with continuous stirring at room temperature. Extract was then filtered and freeze dried. Yield percentage was calculated.

Phytochemical analysis:

The extracts were analyzed for their phyto-constituents according to (Harborne, 1998).

Determination of total phenolics content (TPC)

Total phenolic content was determined with Folin-Ciocalteu colorimetric modified method of Ainsworth and Gillespie, (2007). The total phenolic content was determined using a calibration curve prepared with gallic acid standard (0–100 mg/L) as a reference. The standard curve was calculated from the blank-corrected A765 of the gallic acid standards, which conformed Beer's Law at 510 nm to a regression coefficient (R2) = 0.998. The equation of standard curve is y = 0.005x + 0.000. The total phenolic contents were expressed as milligrams of gallic acid per gram dry weight of residues (mg GAE/g of dry matter).

Determination of the total flavonoids content (TFC):

Total flavonoid contents were measured according to the aluminum chloride modified colorimetric assay as described by Wulandari *et al*, (2017). The total flavonoid content was determined using a calibration curve prepared with quercetin standard (0-100 mg/L) as a reference, which conformed Beer's Law at 510 nm with a regression co-efficient (R2) = 0.9915. The equation of standard curve is y = 0.005x + 0.0958. The total flavonoids were determined as mg of quercetin per gram dry weight.

Determination of the total tannins content (TTC):

To estimate the total tannin content of the extracts, the Prussian blue's modified method(Margraf *et al*, 2015) was followed. The total tannin content was determined using a calibration curve prepared with tannic acid standard (0-800 mg/L) as a reference, which conformed Beer's Law at 510 nm with a regression co-efficient (R2) = 0.997. The equation of standard curve is y = 0.002x + 0.591. The total tannin content was expressed as mg of tannic acid per gram dry weight of residues.

Evaluation of free radical scavenging activity:

DPPH scavenging activity was determined according to the modified method of Shimada *et al*, (1992). It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in methanol and the ability to scavenge the stable free radical of DPPH was measured in the absorbance at 517 nm. 5 mg of the plant crude extracts samples were dissolved in 1 ml of Dimethyl Sulphoxide (DMSO) while DPPH was prepared in ethanol (300µM). 10µl of plant extracts was added to 90 µl of DPPH solution; in 96-wells plate, the test samples were allowed to react with 2.2 Di (4-tert-octylphenyl)-1-picryl-hydrazyl stable free radical (DPPH) for half an hour at 37ºC. After incubation, decrease in absorbance was measured at 517nm using multiplate reader spectrophotometer. Percentage radical scavenging activity by samples was determined in comparison with a DMSO treated pyrogallate as standard.

The scavenging percentage was calculated according to:

$$\% \text{ Scavenging} = (A_{control} - A_{sample})/A_{control} \times 100$$

where Acontrol is the absorbance at 516 nm of DPPH solution without addition of the extract, Asample is the absorbance at 516 nm of DPPH with the sample.

Evaluation of total antioxidant capacity (TAC):

The total antioxidant capacity of the extracts was evaluated by the phosphomolybdenum method according to Prieto *et al*, (1999). The method is based on the reduction of Mo (VI) to Mo (V) by the action of antioxidant compounds and the formation of a green phosphate - Mo(V) complex with a maximal absorption at 695 nm. 0.3 ml extract was mixed with 3 ml of reagent solution (0.6 Msulphuric acid, 28 mM

sodium phosphate and 4 mMammonium molybdate). The tubes containing the reaction solutions were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in place of extract was used as blank. The antioxidant activity was expressed as ascorbic acid equivalent (mg AAE/g extract) which served as a positive control. Mean values from three independent samples were calculated for each extract.

Statistical analysis:

Assays were performed in triplicate and results are shown as mean ± standard deviation. Linear correlation and coefficient of determination (R2) were obtained (Paixao et al, 2007) using Microsoft excels 2007. Pearson's correlation coefficient was calculated and statistical significance was determined among various treatments with one way ANOVA test (Teixeira *et al*, 2017) using SPSS 22.0. A statistical significance of $p < 0.05$ was considered to be significant.

Results and Discussions:

The usage of medicinal plants presents a very important aspect of the traditional medicine which is imbedded in the culture of people of developing countries (Kloucek *et al*, 2005). Although, there is wide experience among the people of Sudan in the employment of medicinal plants as a part of the health care system, this experience passed from one generation to another without documentation (El Ghazali *et al*, 1997). Experimental research must be carried out to provide scientific validation of this traditional knowledge in Sudan(Shama *et al*, 2019). This study aimed to: - investigate chemical constituents, estimation of total phenolic contents, flavonoids and tannins; and determination antioxidant activity of three selected Sudanese medicinal plants. Phytochemical screening of the three plant extracts revealed the presence of various bioactive components of which phenolics, saponins, alkaloids, flavonoids, carbohydrates and tannins are the most prominent components; the result of phytochemical test was presented in Table (2). Among these phytochemical tests, *O. basilicum* was found to contain maximum alkaloids content along with flavonoids and tannins, which act as primary antioxidants or free radical scavengers. All these phytochemicals possess good antioxidant activities and have been reported to exhibit multiple biological effects including anti-inflammatory and antitumor activities(Sharma and Paliwal, 2013).

The phenolic contents expressed as gallic acid equivalent per gram (GAE /g) of dry matter in the order *O. basilicum* methanol extract(OBM) was found to be the highest (14.56 ± 0.071mg GAE/g dry weight) followed by *O. basilicum* water extract (OBW) (4.48 ±0.188mg GAE/g), *C. proximus* water extract (CPW) (4.22 ± 0.056 mg GAE/g), *T. terrestris* methanol extract (TTM) (2.69±0.0125), *T. terrestris* water extract (TTW)(2.16±0.21) and *C. proximus* methanol extract (CPM) (1.69 ± 0.109mg GAE/g). The contents of total flavonoids were measured and expressed in terms of querectin equivalent (QE)/g dry matter as follow OBM> CPW>OBW> TTM> TTW and CPM , whereas with the following order for total tannins OBM> TTM> OBW>TTW> CPW and CPM(Table 3). Table(4) represents the percentage of DPPH free radical scavenging activity and TAC for the tested plant extracts. *O. basilicum* methanol extract showed the highest antioxidant activity, followed by the plant water extract, while *C. proximus* showed moderate activities for its two extracts compared to

pyrogallate(PRG) as standard compounds, whereas *T. terrestris* showed the lowest antioxidant activity. Pearson' correlation coefficients and coefficient of determination (R2) between the total phenol contents(TPC) and antioxidant activity revealed a strong relationship for both [r = 0.994, R2=0.988 Fig(1-a)] with the free radical scavenging activity (RSA%) and TPC with TAC [r=0.996; R2=0.991 Fig(1-b)] of the water extracts, while the same relationship with the methanol extracts was found to be considerably lower [r = 0.741, R2=0.743 Fig(2-a)] with RSA% and [r=862; R2=0.38 Fig(2-b)] with respect to TAC. These strong correlations confirmed that the antioxidants found in plants are capable of both free radical scavenging and antioxidant reduction(Li *et al*, 2008). The results of this study are consistent with those found in the literature that recorded high correlations within the range of 0.7–0.9 were reported for DPPH and TPC values (Kim *et al*, 2011). Typical phenolics that possess antioxidant activity are known to be mainly phenolic acids and flavonoids(Pitchaon *et al*, 2007). Correlation analysis between the values of DPPH and TAC for water and methanol extracts showed nearly the same correlation coefficients(r=0.98 and r=0.979) and coefficients of determination [R2= 0.959 and 0.958], respectively, while for both solvent extracts together (r=0. 960; R2=0.973 with p=0.002) indicate the viability of the two models for evaluating antioxidants from medicinal plants Fig(3-a). Correlation analysis between the values of total phenolic contents and total flavonoids (r=0.996 R2=0.992 with p=0.000) Fig(3-b), indicates that total phenols increased with flavonoids, which is positively correlated with antioxidant activities. Hence, that antioxidant activity increased with increase in both total phenols and flavonoids. While total tannins is negatively correlated with total phenolic contents[r= -0.06; R2=0.51]. Also total tannin is negatively correlated with RSA% and TAC(r=-0.757 and r=-0.786) respectively. The study results coincidence with pervious findings of; Thirugnanasampandan, Jayakumar, 2011, who demonstrated that *O. basilicum* extract possess high content of phenols and maximum levels of free radical scavenging activity. Khalifa *et al*, (2013) showed that methanolic extract of *O. basilicum* plant is a potent antioxidant with 91% activity at 100 ppm and 25.7% at 50 ppm. Amrani *et al*, (2006) found that aqueous extract of *O. basilicum* displayed very high antioxidant power. The antioxidative effect of basil is mainly due to its content of phenolic components, such as flavonoids, phenolic acids, rosmarinic acid and aromatic compounds (Gulcin *et al*, 2007). The flavonols (e.g., quercetin) and hydroxyicinnamic acids (e.g., caffeic and ferulic acids) were determined to be more potent antioxidants than ascorbic acid (Martin-Sanchez *et al*, 2014).

The current results also in accordance with those of Selim (2011) who reported that *C.proximus* was shown to possess good antioxidant activity; the total antioxidant capacity of *C. proximus*, expressed as the number of equivalents of ascorbic acid, was found to be 48.66±3.1. Methanolic extracts of *C. proximus* also showed a highly effective free radical scavenging in the DPPH (Selim, 2011). The plant contains the flavonoids rutin and quercetin (Heiba and Rizk, 1986), which are well known antioxidants.

T. terrestris contains high tannin contents which are negatively correlated with total phenols and low flavonoids which positively correlated with total phenolic contents and antioxidant activity. Rutin has been reported to be one of the important bioactive phytoconstituents in *T. terrestris*(Ivanova *et al*, 2010). The aglycones have stronger

antioxidant activity than glycoside forms. The weakening of the antioxidant activity of flavonoids after glycosylation may be due to removing hydroxyl groups by conjugated glycosides, and thereby inhibiting them from scavenging reactive oxygen species or chelating transition metals (Akhlaghi and Foshati, 2017).

Conclusion:

The study findings revealed that methanol extract of *O. basilicum* possess interesting antioxidant activity, which may provide protection against free radicals induced damage to biomolecules. The antioxidant activity of medicinal plants could be attributed to its flavonoid content. Flavonoid act as scavengers of various oxidizing species i.e. superoxide anion, hydroxyl radical or peroxy radicals, they also act as quenchers of singlet oxygen (Ratty and Das, 1988). Also *C. proximus* presented good activity, while *T. terrestris* showed weak antioxidant activity.

Table (1): Preliminary phyto-chemical screening of tested plants

Plant extract	Alk	Fla	Phen	Tannins	Gly	Ster	Trite	Sap	Car	AA
OBM	+	++	++	+++	-	+	-	-	+	-
OBW	+++	+	+	+++	-	-	-	+	+	-
CPM	+	+	+	++	+	++	+++	+	++	+
CPW	++	+	+	+++	-	+++	++	++	+++	+
TTM	-	++	+	+	-	-	-	+++	+	+
TTW	++	+	++	++	-	+	+	+++	++	+

OBM: *O. basilicum* methanol extract; OBW; *O. basilicum* water extract; CPM: *C. proximus* methanol extract; CPW: *C. proximus* water extract; TTM: *T. terrestris* methanol extract; TTW: *T. terrestris* water extract; +++: Present in high concentration ++: moderate +: low : Not detected ALK: alkaloids Fla: flavinoids Phe: phenols Gly: glycosides Ster: steroids Trite: triterpenes Car: carbohydrates AA: aminoacids.

Table (2): Total phenol contents, total flavinoids and tannins of plant extracts

Plant extract	Phenolic content (mg GAE/ g dry matter) ± SD	Total Flavinoids (mg QUE/mg dry matter) ± SD	Total Tannins (mg of TAE/ g dry matter) ± SD
OBM	14.56±0.071	19.03±0.03	26.55±0.068
OBW	4.48±0.0188	3.68±0.010	15.03±0.047
CPM	1.69±0.1098	1.22±0.012	6.64±0.061
CPW	4.22±0.056	4.91±0.017	7.69±0.224
TTM	2.69±0.0126	1.81±0.001	22.07±0.093
TTW	2.16±0.210	1.64±0.006	11.21±0.321

OBM: *O. basilicum* methanol extract; OBW; *O. basilicum* water extract; CPM: *C. proximus* methanol extract; CPW: *C. proximus* water extract; TTM: *T. terrestris* methanol extract; TTW: *T. terrestris* water extractGAE: Gallic acid equivalent; QUE: Querectin equivalent; TAE: Tannic acid equivalent.

Table (3): Anti-oxidant activity of tested plant extracts

Extract	TAC (mg Ascorbic acid E /L) of extract ± SD	%RSA ±SD (DPPH)
OBM	482.25±0.08	80±0.01
OBW	456.25±1.03	73±0.02
CPM	252±0.55	56±0.01
CPW	451.75±0.91	62±0.04

TTM	66.5±1.42	16±0.07
TTW	31.75±0.78	23±0.09
Propyl gallate	-	92±0.01

OBM: *O. basilicum* methanol extract; OBW; *O. basilicum* water extract; CPM: *C. proximus* methanol extract; CPW: *C. proximus* water extract; TTM: *T. terrestris* methanol extract; TTW: *T. terrestris* water extract

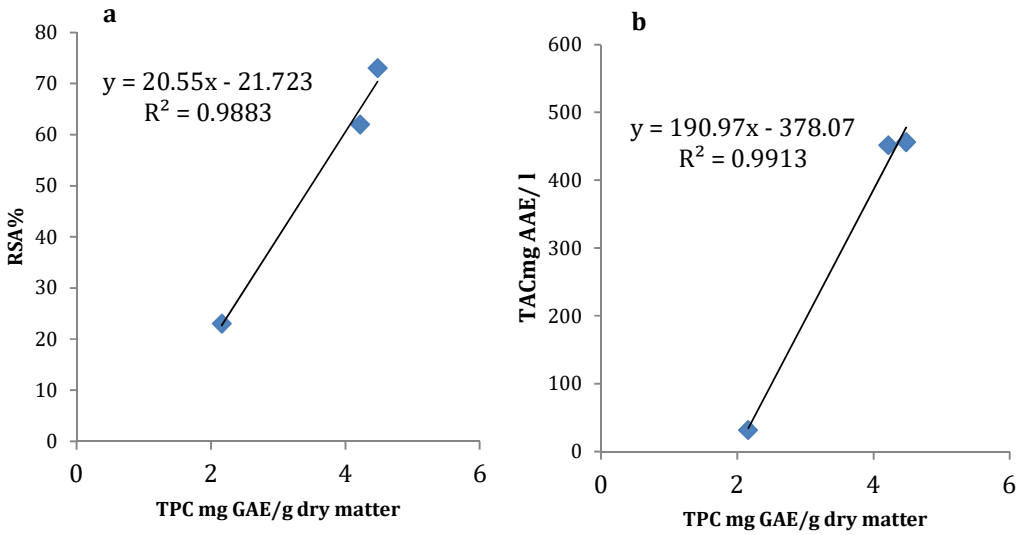
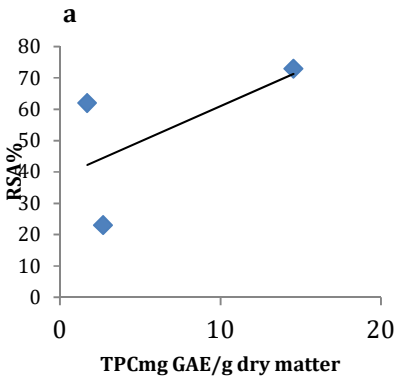
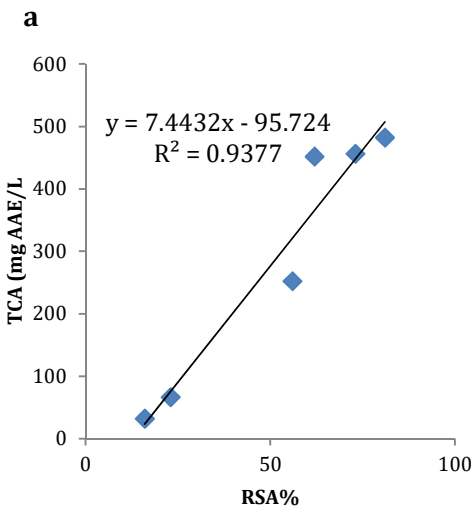


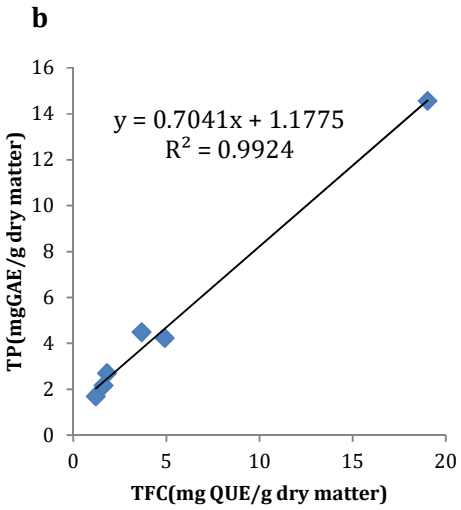
Fig (1): Linear correlation of the total polyphenol content (TPC) of water extracts as a function of (a) Radical scavenging activity (RSA %), and (b) total antioxidant potential (TAC). AAE; Ascorbic acid equivalents; GAE Gallic acid equivalents



y = 2.2634x + 38.377
R^2 = 0.3804

Fig(2): Linear correlation plot of the total polyphenol content (TPC) of methanol extracts as a function of (a) Radical scavenging activity (RSA%), and (b) total antioxidant potential(TAC).





Fig(3-a): Linear correlation between RSA% and TAC Fig(3-b): Linear correlation between TPC and TFC; AAE: Ascorbic acid equivelant; QUE: Querectin equivelant; GAE: Gallic acid equivelant

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