DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF DICHROSTACHYS CINERA, GUIERA SENEGALENSIS AND VITEX DONIANA

Mohamed Abubakr[1], Mahomed Osman Babiker[2]
Nizar Sirag[3] N. abdAlla[4]

ABSTRACT

The present work was undertaken to determine the phenolic content and antioxidant capacity of three plants used in Sudanese folk medicine.

The total phenolic content was measured by Folin Ciocalteau method using gallic acid as standard while the antioxidant capacity was based on the ability of these plant extracts to scavenge DPPH radical.

High total phenolic content was detected in *Dichrostachys cinera* followed by *Guiera senegalensis* and *Vitex doniana. Dichrostachys cinera* exhibited the strongest antioxidant capacity with EC_{50} 6.63 µg/ml, followed by *Guiera senegalensis* (7.74 µg/ml) and *Vitex doniana (*22.72 µg/ml*)*. A significant linear correlation between total phenolic content and antioxidant capacity(R2=0.9957) confirmed that the phenolic phytoconstituents are responsible for the antioxidant activity.

It can be concluded that the three surveyed plants could be potential sources of antioxidant agents.

Keywords: Phenolic compounds; Antioxidant activity; Sudanese medicinal plants.

INTRODUCTION

Polyphenolic compounds are commonly found in both edible and inedible plants, and they have been reported to have multiple biological effects, including antioxidant activity (Karhkoinen *et al*., 1999). Herbs are used in many domains, including medicine, nutrition, flavouring, beverages, dyeing, repellents, fragrances and cosmetics (Djeridane *et al*., 2006). Many species have been recognized to have medicinal properties and beneficial impact on health, e.g. antioxidant activity, digestive stimulation action, anti-inflammatory, antimicrobial, hypolipidemic, antimutagenic effects and anticarcinogenic potential (Aaby *et al*., 2004).

Reactive oxygen species (ROS) which include the oxygen free radicals; superoxide anion (O^{-}_{2}), hydroxyl radical (OH·) and some non-radical hydrogen peroxide (H_2O_2) derivatives of oxygen are normally produced in living organisms with the potential of reacting with almost all types of molecules in living cells (Mensor *et al*, 2001). The harmful effects of free radicals are neutralized by the enzymatic antioxidant defenses including the superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). However, overproduction of the ROS arising from either mitochondrial electron transport chain, excessive stimulation of NAD(P)H, exposure to environmental pollutants, cigarette smoke, ultraviolet rays, some parasitic infections, radiation and toxic chemicals results in oxidative stress- a phenomenal disturbance in the equilibrium status of pro-oxidant/antioxidants reactions in living systems, which mediates damage to cell structures, including lipids and membranes, proteins, and DNA (Valko *et al*., 2006). There has been an increased interest in oxygen containing free-radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic and ageing diseases, including heart disease, stroke, arteriosclerosis, diabetes mellitus, cancer, malaria, rheumatoid arthritis, neurodegenerative diseases (Alzheimer's and Parkinson's diseases) and AIDS (Olukemi *et al*., 2005). Hence, therapy using free radical scavengers (antioxidants) has the potential to prevent, delay or ameliorate many of these disorders (Delanty and Dichter, 2000). Numerous natural antioxidants have already been isolated from different varieties of plant material such as leafy vegetables, fruits, seeds, cereals and algae (Pokorny, 1991). They have been shown to have ROS scavenging and lipid peroxidation preventive effects (Atawodi, 2005; Aqil *et al.*, 2006). The protection can be explained by the capacity of the antioxidants phenolics, flavonoids and polypropanoids in the plants and plant products to scavenge free radicals, due to its proton donating ability.

An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 2, 2 diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva *et al*., 2002).In particular, despite widespread use of wild and edible plants as medicines in Sudan, the literature contains few reports of antioxidant activity and the chemical composition of these plants.*Dichrostachys cinera*, (Kadad*), Guiera senegalensis (*Gibech), and *Vitex doniana* (Umtululkul) are widely used in Sudanese Folk medicine.The objectives of this study were to: (1)) determine the phenolic contents of the tested species; (2) evaluate the antioxidant activity of these plants , (3) determine the relationship between

antioxidant activity and phenolic compounds of species extracts to confirm that phenolic constituents are responsible for antioxidant activity of the plants.

MATERIALS AND METHODS

MATERIALS

Chemicals and reagents:

2, 2 diphenyl-2-picryl hydrazyl (DPPH), gallic acid and rutin were purchased from Sigma –Aldrich company (UK). Folin Ciocalteau reagent and methanol were purchased from Merck company (Germany).Chemicals and reagents used were of the highest commercially available purity.

Plant materials:

The three plants were collected from West Sudan. They were taxonomically identified and authenticated by taxonomy expert at Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan where the voucher specimens had been deposited (Table 1).

Table 1: Plants screened for phenolic content and antioxidant capacity.

Botanical name/ family	Common/vernacular name	Plant part tested
1. *Dichrostachys cinera* Family: Fabaceae	Sickle bush (Kadad)	Leaves
2. *Guiera senegalensis* Family: Combretaceae	Moshi medicine (Gibech)	Leaves
3. *Vitex doniana* Family: Verbenaceae	African oak (Umtululkul)	Leaves

METHODS

Extraction of plant materials:

The plant samples were washed and dried at room temperature for one week. The dry samples were ground and extracted separately by maceration using methanol
in a conical flask for 48 hours, filtered and dried at room temperature and kept in a refrigerator until use.

Determination of total phenolic content:

The total phenolic content in the methanolic extracts of the three plants was determined with Folin Ciocalteau reagent according to the method described by Chinedu *et al*.,(2011).The crude extract (50 mg) was mixed with Folin Ciocalteau reagent (1ml) and deionized water (7.5 ml).
The mixture was kept at room temperature for 5 minutes and then 10 ml of 7% sodium carbonate was added to the mixture and then incubated for 90 minutes at room temperature. After incubation the absorbance against the reagent blank was determined at760 nm using UV-Visible spectrophotometer. The total phenolic content of the plants was expressed as mg/g Gallic acid equivalent. All samples were analyzed in triplicates.

Free radical scavenging activity:

This method was carried out according to that described by Mensor *et al*.,(2001).Sample stock solution (1mg/ml) was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol. One ml of a 0.3 mM DPPH in methanol solution was added to a 2.5 ml solution of the different concentrations of the extracts and allowed to react at room temperature for 30 minutes. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage antioxidant activity (AA %), using the formula below:

$$AA\% = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as control. Stock solution (1 mg/ml) of rutin was diluted to final concentrations of 250, 125, 50, 10 and 5 µg/ml in methanol used as a positive control.

A freshly prepared DPPH solution exhibits a deep purple colour with a maximum absorbance at 518 nm. The purple colour disappears when an antioxidant is present in the medium. Thus, the change in the absorbance of the reduced DPPH was used to evaluate the ability of test compound to act as free radical scavenger. Furthermore, the "efficient concentration" or EC_{50} value (the concentration of antioxidant that causes 50% loss of the DPPH activity (colour) was also used to assess the antioxidant activity of the plant extract compared to the standard drug. The higher the antioxidant activity, the lower is the value of EC_{50} (Songklanakarin, 2004).

The EC_{50}values were calculated by linear regression of plots where the abscissa represented the concentration of the tested plant extracts and the ordinate the average percentage of antioxidant activity from three separate tests.

RESULTS AND DISCUSSION

Total phenolic content:

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl group (Hatano *et., al* 1989). As shown in Table 2, the phenolic content varied from 62.5 to 48 mg gallic acid/g of dry materials. High total phenolic content was detected in *Dichrostachys cinera* as 62.5 mg gallic acid/g while that of *Guiera senegalensis* and *Vitex doniana* was estimated as 58 mg gallic acid/g and 48 mg gallic acid/g respectively.

Table (2): Total phenolic content of plant extracts.

No	Plant	Phenolic content(gallic acid /g)
1.	*Dichrostachys cinera*	62.5 mg
2.	*Guiera senegalensis*	58 mg
3.	*Vitex doniana*	48 mg

Antioxidant activity:

The antioxidant capacity of the selected plants determined by the DPPH method is expressed in units of EC_{50} values. High antioxidant capacity was produced by *Dichrostachys cinera* with EC_{50} (6.63), followed by *Guiera senegalensis* and less effect was developed by *Vitex doniana. Dichrostachys cinera* and *Guiera senegalensis* were found to be more active than the standard antioxidant agent (rutin) (Table 3).

Table (3): DPPH scavenging activity of plant extracts.

No	Plant	EC_{50}
1.	*Dichrostachys cinera*	6.63
2.	*Guiera senegalensis*	7.74
3.	*Vitex doniana*	22.72
4.	Rutin (standard)	12.00

Table 4 and Figure 1 confirmed a very interesting correlation between the antioxidant capacity and the total phenolic content with $R^2 = 0.995$. Hence, the antioxidant activity of these plant extracts may probably depends mainly on the content of total phenolic compounds present in such plant extracts.
These findings agreed with pervious research workers (Mahnaz, *et. al*. 2009; Rohman *et a*l., 2010).

Table (4): Correlation between total phenolic content and antioxidant capacity of plant extracts.

No	Plant	Total phenolic	EC_{50}	$1/EC_{50}$
1.	*Dichrostachys cinera*	62.5	6.36	0.16
2.	*Guiera senegalensis*	58	7.79	0.13
3.	*Vitex doniana*	48	22.72	0.04

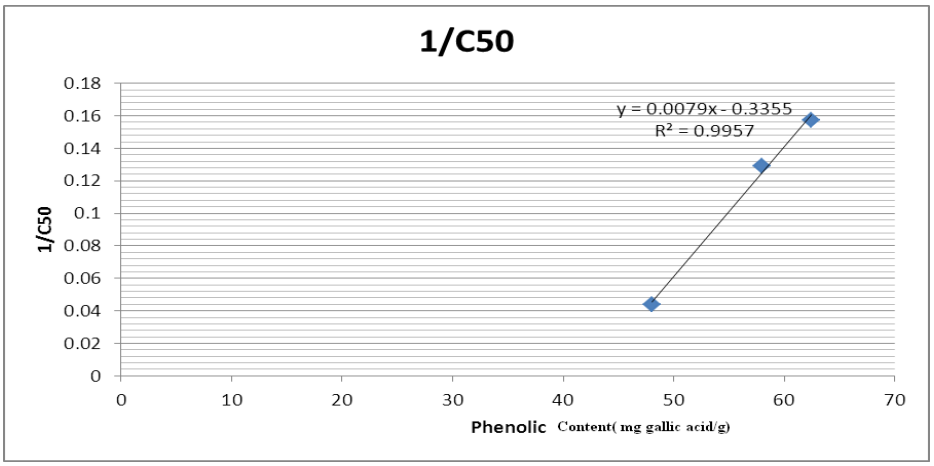

Figure 1: Correlation between total phenolic content and antioxidant capacity of plant extracts.

It can be concluded that the three surveyed plants could be potential sources of antioxidant principles. Further studies should be conducted to verify the biological activities of these plants.

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النشاط المضاد للأكسدة لثلاث نباتات طبية سودانية تحديد محتوي الفينولات الكلية

محمد أبوبكر [1],محمد عثمان بابكر[2] , نزار سراج النعيم عبدالله[4] [3]
1 . قسم الكيمياء الصيدلانية -كلية الصيدلة- جامعة الجزيرة.
2 . قسم الكيمياء –كلية الهندسة والتكنولوجيا- جامعة الجزيرة.
3. قسم العقاقير الطبية-كلية الصيدلة-جامعة الجزيرة.
4. هيئة البحوث الزراعية.

الملخص

اجريت هذه الدراسة لتحديد محتوي الفينولات الكلية و النشاط المضاد للأكسدة لثلاث نباتات طبية تستخدم في الطب الشعبي السوداني. تم تقدير المحتوي الفينولي الاجمالي بواسطة طريقة فولين كيوكالتيو باستخدام حمض قاليك كمادة مرجعية بينما تم تحديد السعة المضادة للأكسدة اعتماداً علي كنس المستخلص النباتي لجذر DPPH الحر.

وجد ان مستخلص نبات الكداد يحتوي علي اعلي محتوي فينولي اجمالي ويليه مستخلصي الغبيش وام تكلكل. اظهر مستخلص الكداد اقوي نشاطا مضادا للأكسدة بتركيز كفاءة 6,36 ميكروغرام لكل مليميتر ويليه مستخلص الغبيش و ام تكلكل. اكدت العلاقة الخطية الواضحة بين محتوي الفينول الاجمالي و النشاط المضاد للأكسدة (R2= 0.9957) أن المركبات الفينولية مسؤولة عن النشاط المضاد للأكسدة.

يمكن أن نستنتج أن النباتات لثلاثة المختبرة يمكن ان تمثل مصادرا متوقعة لمواد مضادة للأكسدة .