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Fractionation and Determination of Antioxidant Activities, of the Leaves of *Senna Occidentalis* Ethanol Extract

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Abstract

Senna occidentalis has been reported to have certain medicinal properties in traditional settings. Extraction was carried out using absolute ethanol and elution was done with the following solvent; beginning from chloroform, ethyl acetate, methanol and finally water. The *in vitro* antioxidant activity, total flavonoid and total phenolic contents of nine (9) fractions of *Senna occidentalis* leaves ethanol extract were determined using spectrophotometric methods, the fractions obtained from the extract were concentrated based on the solvent combination for nine fractions, and the Total antioxidant capacity of the fractions expressed in mg/ml of trolox equivalent (TE) which ranged from 43 – 127 mg/ml, Total flavonoids content were expressed as quercetin equivalent (QE) ranging from 104 – 142 mg/ml, and Total polyphenol content expressed as Gallic acid equivalent (GAE) which ranged from 139 – 206. Water fraction possessed the highest antioxidant activity followed by methanol: water fraction. Linear correlation between TFC/TAC showed strongest positive correlation (R^2=0.8092) among all, followed by TPC/TAC (R^2=0.6938) and TPC/TFC (R^2=0.6284). This relationship indicates that the flavonoids content of the extract is basically responsible for the elicited antioxidant activities (Especially the methanol: water fraction).

Keywords

Fractionation, Antioxidant, Phenolics, Flavonoids, *Senna occidentalis*

1. Introduction

Plants are important source of drugs; especially in traditional medicine [1]. It is a common practice in Nigeria and other parts of the world to use plant in the form of crude extracts, decoction, infusion or tincture to treat common infection and chronic conditions. According to WHO, over 70% of the world population rely on medicinal plants for primary health care, and there are reports from various researchers on natural substances of plant origin which are biologically active, with desirable antimicrobial and antioxidant properties [2, 3]. Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health the impact is particularly high in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance [4]. The active principle of many drugs found in plants is phytochemicals [5]. The medicinal value of these phytochemicals is because of the presence of chemical substance that produces definite physiological action on the human body [6]. Some of the valuable ones include; alkaloids, tannins, saponins, glycosides, flavonoids, phosphorus and calcium for cell growth, replacement, and body building [6].

Medicinal plants have formed the basis of health care

throughout the world since the earliest days of humanity and are still widely used, and have considerable importance in international trade [7]. In developed countries such as United States, it is estimated that plant drugs constitute as much as 25% of the total drugs, whereas, in developing countries including China and India, the contribution is as much as 80% [8]. This underscores the increased research interest in medicinal plants and traditional medicine all over the world [8]. Plant derived natural products such as flavonoids, terpenoids, carbohydrates, tannins, saponins, steroids, proteins, amino acids [9] and Vitamin C [10] etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and hepatoprotective activity [11].

Continuous search by scientists have shown that some plant extracts have antioxidant properties that can help remove free radicals produced by oxidation. An example of such plant is *Senna occidentalis* which have been reported to have certain medicinal properties in traditional settings. This plant has been used locally in the treatment of ailments such as diabetes, malaria, typhoid, healing of wounds and hypertension. Hence, the fractionation and determination of antioxidant activities of the leaves of this plant.

2. Materials and Methods

2.1. Collection and Preparation of Sample

Fresh leaves of *Senna occidentalis* was collected from the vegetation within Federal University Wukari campus, Wukari LGA Taraba State, Nigeria. The leaves were examined to be free from diseases. Only healthy plant parts were used. The leaves were cut into pieces using a kitchen knife and dried under shade for 10 days to reduce moisture content and prevent enzyme action. The dry leaves were pulverized using a laboratory blender and kept in a clean and airtight container for subsequent use.

2.2. Sample Extraction

Exactly 400g of pulverized sample was soaked in absolute ethanol in the ratio 1:5w/v (100g: 500 ml) for exactly 48hrs. The extract was filtered out first using a clean white sieving mesh and then using the Watman No. 1 filter paper. The filtrate was concentrated using a thermostat water cabinet at 40°C for 7 days. The concentrated extract was then transferred to air-tight containers, corked and preserved in a refrigerator before analysis.

2.3. Fractionation

The ethanol extract was subjected to column chromatograph to separate the extract into its component fractions. Silica gel was used in packing the column while different solvent combinations based on increasing polarity were used as the mobile phase as described by Yakubu *et al*., [12].

2.3.1. Packing of Column

In the packing of the column, the lower part of the glass column was stocked with glass wool with the aid of glass rod. 75g of silica gel (G60-200 mesh size) was dissolved in 180 ml of absolute chloroform to make the slurry. The chromatographic column (30mm diameter by 40 cm height) was packed with silica gel and was allowed free flow of the solvent into a conical flask below. The set up was seen to be in order when the solvent drained freely without carrying either the silica gel or glass wool into the tap. At the end of the packing process, the tap was locked and the column was allowed 24 h to stabilize after which, the clear solvent at the top of the silica gel was allowed to drain down the silica gel meniscus.

2.3.2. Elution

The ethanol extract (5g) was dissolved in 5 ml absolute ethanol and the solution was applied unto a chromatographic column. Elution of the extract was done with solvent system of gradually increasing polarity, beginning from chloroform, ethyl acetate, methanol and finally water. The following ratio of solvent combination was sequentially used in the elution protocol:

i. Chloroform: ethyl acetate 100:0, 50:50, 0:100.
ii. Ethyl acetate: methanol 50:50, 0:100.
iii. Methanol: water 50:50 and 0:100.

A measured volume (400 ml) of each solvent combination was poured into the column each time using separator funnel. The eluted fractions were collected in aliquots of 400 ml in fraction collection tubes.

2.4. Determination of Total Antioxidant Capacity (TAC)

The scavenging action of the plant extracts and the resulting fractions from ethanol extract on 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined calorimetrically at 517 nm using Trolox as standard according to the method described by Singleton *et al.,*[13]. The absorbance was measured at 517nm in triplicate for each fraction. Total antioxidant capacity (TAC) was calculated as mg/ml of trolox equivalent (TE) using the regression equation from calibration curve.

2.5. Determination of Total Flavonoid Content (TFC)

Flavonoids were determined using the aluminium chloride colorimetric method of Chang *et al.,*[14]. Quercetin was used for derivation of the calibration curve and total flavonoids content was expressed as mg/ml quercetin equivalent (QE). The concentration of flavonoids in the sample was estimated using the calibration curve.

2.6. Estimation of Total Polyphenol Content (TPC)

Total polyphenol component was estimated colorimetrically at 765 nm as described by Lachman *et al.,* [15], using Follin-Ciocalteu reagent and expressed as gallic acid equivalent (GAE). The reactions were conducted in triplicates and absorbance of the sample was measured against the reagent blank at 765nm.

3. Results and Discussion

3.1. Results

Table 1 shows the results of the Total Antioxidant Capacity (TAC), Total Flavonoid Content (TFC) and Total Polyphenols Content (TPC) for ethanol extract.

The Total Antioxidant Capacity (TAC), Total Flavonoid Content (TFC) and Total Polyphenols Content (TPC) revealed that the methanol: water fraction has the highest total antioxidant activity and then continues as shown in the table below.

Table 1. *Showing the result from Senna occidentalis leaves ethanol extract.*

Fractions	solvent combination	TAC (mg/ml)	TFC (mg/ml)	TPC (mg/ml)
2	Chlo:Eth Acet (50:50)	43	106	150
3	Eth Acet:Eth (100:00)	44	107	139
4	Eth Acet:Eth (50:50)	49	107	140
5	Eth:Meth (100:00)	49	106	147
6	Eth:Meth (50:50)	47	104	172
7	Meth:Water (100:00)	50	107	164
8	Meth:Water (50:50)	48	121	169
9	Meth:Water (00:100)	127	142	206

Correlation

Correlation of TFC/TAC, TPC/TAC and TPC/TFC for *Senna occidentalis* leaves ethanol extract.

From the result it shows that all the correlations were strongly positive correlations but Flavonoid content is the strongest in Figure 1. (R^2=0.8092) with the antioxidant activity. And also strong positive correlation was observed in the correlation of TPC/TAC in Figure 2. (R^2=0.6958) and Figure 3. TPC/TFC (R^2=0.6284). Respectively as shown on the following figures of linear correlations.

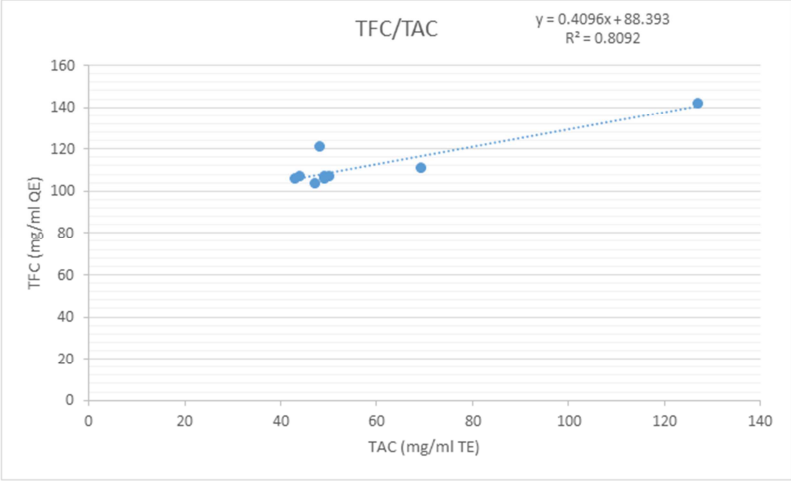

Figure 1. *Linear correlation between total flavonoids content and total antioxidant capacity of fractions obtained from ethanol extract of Senna occidentalis leaves.*

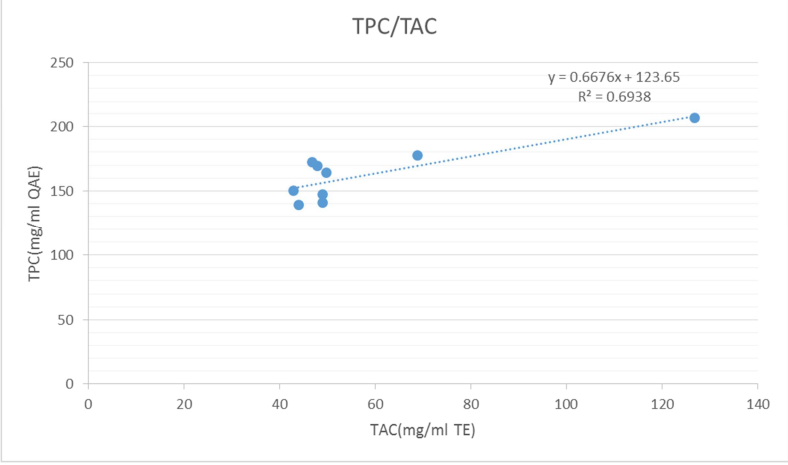

Figure 2. *Linear correlation between total polyphenolic content and total antioxidant capacity of fractions obtained from ethanol extract of Senna occidentalis leaves.*

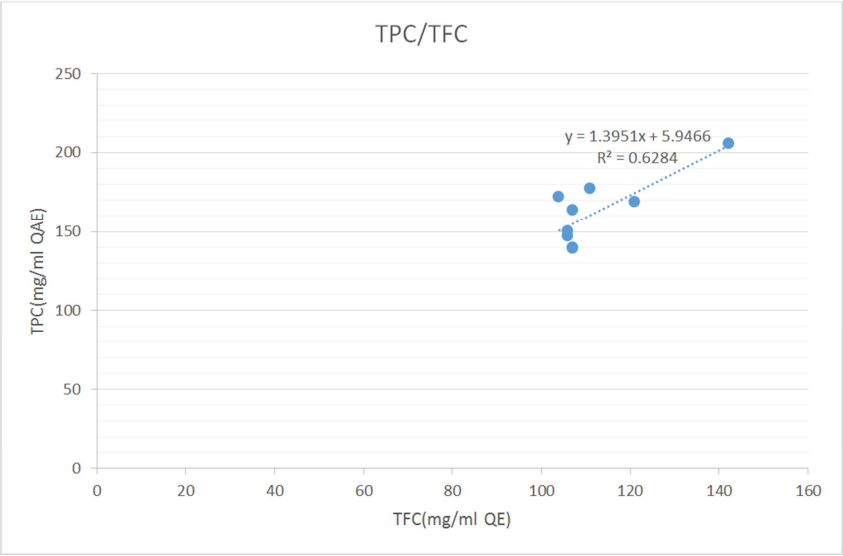

Figure 3. *Linear correlation between total polyphenolic content and total flavonoid content of fractions obtained from Senna occidentalis leaves ethanol extract.*

3.2. Discussion

The total Antioxidant capacity of ethanol extract of *Senna occidentalis* leaves ranges from 50-95 mg/ml. this may be due to the presence of flavonoids such as resversterol, catechins, anthocyanins, and isoflavones as well as phenolic acid and lignin [16], that play a major roles as antioxidants especially the flavonoids that can also play a role in phyto-preventive theraphies [17-21] the flavonoinds was correlated with the antioxidant activity, (R^2=0.8566) it has been clearly established that flavonoids have the strongest radical-scavenging power among all natural phenolic compound [22].

According to Velioglu *et al*, [23] there was strong relationship between the total phenol capacity and total antioxidant in selected fruits and vegetables, which is in tandem with our study using *Senna occidentalis* (R^2=0.6938) which could be based on total antioxidant capacity. And it was observed also a strong positive correlation (R^2=0.6284) which is based on the total phenolic content present in total antioxidant when correlated. Although it is consistent, the total antioxidant capacity of ethanol extract of *Senna oxidentalis* leaves depend on the polarity of the eluting solvent [24-26].

The total antioxidant of *Senna occidentalis* leaves ranged from 111-142 mg/ml QE and total phenol capacity ranged from 177-206 mg/ml GAE (table 1) from this report the total flavonoid capacity of Ethanol extract of *Senna occidentalis* may be responsible for Antioxidant activity since a strong positive correlation (R^2.0.8092) is observed with the total antioxidant capacity as shown in figure 1.

In the current study there was a strong relationship between Total antioxidant and total flavonoid content of *Senna occidentalis* leaves. Therefore it can be said that the total antioxidant capacity of a fraction is majorly dependent on their flavonoinds content.

Hence it can be deduced with certainty that the antioxidant capacity of the fraction Is dependent on the flavonoids content. The polarity and solubility these have been suggested to be some of the major factors responsible for variations in the contents of these different fractions from the same extract although there is a wide grade of variation between different phenolic compounds in their effectiveness as antioxidant [27, 28] which may possibly be a contributing factor to the moderately positive correlation.

3.3. Conclusion

The results of this study showed that the highest antioxidant activity and Total phenol capacity and total flavonoids content respectively of *Senna occidentalis* leaves ethanol extract was exhibited by the water fraction. Hence, there was strong positive correlation between the TAC and the TPC, indicating that the flavonoids content of the extract are to a larger extent responsible for the elicited antioxidant effects of the extract.

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