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Ferric Reducing Antioxidant Power Activity and Proximate Analysis of Root Bark Extracts of Caesalpinia Pulcherrima

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Abstract

Caesalpinia pulccherrima is a popular ornamental plant. The root bark extract of C. pulcherrima is employed as an anti-pyretic while the leaves are employed as purgative and mouthwash. It was established that various extracts of C. pulcherrima showed antimicrobial, anti-inflammatory and antioxidant activity. The present study investigated the antioxidant activity of the root bark (100% ethylacetate, 50% ethylacetate:50% n-hexane, 50% ethylacetate:50% methanol and crude) extracts of C. pulcherrima and determined the proximate parameters of C. pulcherrima. The method used was the FRAP assay which is based on the reduction of ferric tripyridyltriazine (Fe(III)- TPTZ) complex to ferrous tripyridyltriazine (Fe(II)- TPTZ) (coloured product) by an antioxidant, at low pH (3.6). Proximate analysis was carried out for the purpose of authentication of the crude powdered material. All the different extracts showed antioxidant activity with 50% ethylacetate: 50% methanol and 50% ethylacetate: 50% n-hexane showing the highest and lowest antioxidant activity respectively. The concentrations of the reduced product (ferrous tripyridyltriazine) of the extracts were: 0.4463±0.0036, 0.0354±0.0013, 2.7757±0.0866 and 0.7699±0.0040 for 100% ethylacetate, 50% ethylacetate:50% n-hexane, 50% ethylacetate: 50% methanol and crude extracts respectively . The results therefore suggest that C. pulcherrima root bark extracts have antioxidant capacity.

1.0 Introduction

Nature has made adequate provision for medicine to cure diseases in vast warehouses called plants [1,2]. These 'warehouses' (medicinal plants) contain chemicals which though do not have nutritive value, exhibit some characteristics that make them disease fighting agents [3,4,2].

The various organs of medicinal plants like leaves, roots, rhizomes, Stem barks, flowers, fruits, grains or seed contains certain therapeutic chemical constituent and consequently are used in therapy [5]. These therapeutic chemical constituents are called phytochemicals.

Oxidation is a very important process in a cell [6]. As important as oxidation reaction (or oxidative process) is, it has a down side which is the production of free radicals [7].Free radicals are responsible for oxidative stress that causes damages that might lead to diseases [7]. Oxidative stress occurs when there is an imbalance between oxidants and antioxidants [8].

Antioxidants are substances that are able to retard or stop the oxidation of other substances [7]. The antioxidant system of the body consists of endogenous antioxidants (Bilirubin, Uric acid, and enzymes), Dietary antioxidants (like vitamin C, vitaminE, beta-carotene, polyphenols), metal binding protein (like albumin, ceruloplasmin. ferritin, myoglobin) and many other phytochemicals present in different types of foods [9].

FRAP assay is based on the reduction of ferric tripyridyltriazine (Fe(III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)-TPTZ) (coloured product) by an antioxidant at low pH (about 3.6) [10,11]. The absorbance of Fe(II)-TPTZ (which has a strong blue colour) can be analyzed at 593.0nm(ultraviolet-visible region)[10].

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Caesalpiniapulcherrima also called red bird of paradise is a popular ornamental plant. It belongs to the family fabaceae. It is called eko-omode in Yoruba, akasi-ibieka in Edo and ofo in Igbo language. It is a shrub growing to 9.84 feet tall. In climates with little to no frosts, this plant will grow larger and is semi-evergreen. Grown in climates with light to moderate freezing, plant will die back to the ground depending on cold, but will re-bound in mid to late spring. This species is more sensitive to cold than others. The leaves are bipinnate, 20–40 cm long, bearing 3-10 pairs of pinnae, each with 6-10 pairs of leaflets 15–25 mm long and 10–15 mm broad. The flowers are borne in racemes up to 20 cm long, each flower with five yellow, orange or red petals. The fruit is a pod 6–12 cm long.

The root extract of C. *pulcherrima* is employed as an anti-pyretic while the leaves are employed as purgative and mouthwash [12]. It was established that various extracts of C. *pulcherrima* showed anti-microbial and anti-inflammatory activities and flavonoids and diterpenes were isolated from this plant (Leonido and [12]. The aqueous and ethanolic extract of the stem bark of C. *pulcherrima* showed antioxidant activity [13]. The methanolic root extract showed antibacterial activity [14].

2.0 Materials and Methods

2.1 Collection and Preparation of Plant Material

The fresh root bark of C. *pulcherrima* were collected from Ransome Kuti Road (main gate) in May, 2015 at the University of Benin. The plant sample was identified by Mr. Ugbogu O.A and Shasanya O.S of the Forestry Research Institute of Nigeria (FRIN), Ibadan with UCC voucher specimen number: FHI109969. The roots were rinsed with water and dried at ambient temperature (28-30^{0}C) and was ground to powder by means of mechanical grinder(British gallyhamp) at Phamacognosy Department, Faculty of Pharmacy, University of Benin. The powdered roots were stored in an air-tight container and kept for further analysis.

3.0 Proximate Analysis

3.1 Moisture Content Determination

Crucibles were washed properly and dried in oven at 105^{o}C for 20 minutes after which they were cooled in a dessicator for 10mins. The crucibles were weighed and the weights were recorded. 0.5g of the ground root bark of *C. pulcherrima* was transferred into the crucibles and the weight of the crucible + sample was noted. The samples were transferred into an oven for 2 hours to dry properly after which it was brought out and put into the dessicator to cool; they were weighed again after cooling and transferred back to the oven for some minutes. This process was repeated until a constant weight was reached.

$$\% \text{ moisture content} = \frac{weight\ loss}{initial\ weight} \times 100 \quad (1)$$

3.2 Total Ash Determination

Crucibles were washed and dried in oven at 100^{o}C for about 10mins, the crucibles were brought out and cooled in the dessicator for some minutes. They were weighed and the various weights were recorded. 3g of the pulverized root bark sample of *C. pulcherrima* was transferred to the already weighed crucibles. The samples were heated in a 500^{o}C muffle furnace till the sample was complete ash (5 hours). They were brought out, cooled in a dessicator and weighed after which percentage ash was determined.

$$\% \text{ total ash} = \frac{W_1 - Z}{N} \times 100 \quad (2)$$

W_1= weight of crucible + ash

Z= weight of empty crucible

N= weight of initial sample used

3.3 Acid Insoluble Ash Value Determination (AIAV)

The ash from the total ash determination was transferred into a beaker and dissolved using 25ml of dilute HCl. The mixture was boiled for 5mins, and was filtered using ashless filter paper. The filter paper + residue was transferred into an already weighed crucible and dried in oven after which it was ignited to ash. The crucible containing the residue was cooled in a dessicator and the weight of the crucible + residue was taken and subtracted from the weight of crucible then percentage of acid insoluble ash with reference to the air dried drug was then calculated.

$$\% \text{ AIAV} = \frac{W_2}{N} \times 100 \quad (3)$$

W_2= weight of Residue

3.4 Water Soluble Ash Value Determination (WSAV)

The ash obtained from the total ash determination was transferred to a beaker containing 25ml of distilled water and was boiled for 5mins. The resulting solution was filtered using ashless filter paper. The ashless filter paper containing the residue was transferred into a pre-weighed crucible after which it was dried in the oven and ignited. The crucible was cooled in a dessicator and weighed. The weight of residue was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

$$\% \text{ WSAV} = \frac{W_2}{N} \times 100 \quad (4)$$

W_2= weight of Residue

3.5 Water Soluble Extractive Values (WSEV)

2g of the dried powdered root sample was weighed into a 250ml stopper conical flask. 40ml of distilled water was added and the flask corked firmly. The mixture was shook for 5hours in a mechanical shaker then allowed to stand for 18hours. The extract was filtered through a filter paper and 20ml of the filtrate was measured into a previously weighed crucible. The filtrate was evaporated to dryness at 105^{o}C. The residue was dried to constant weight and the final weight recorded. The weight of the residue obtained from 20ml extract was determined in 3 replicates by subtracting the constant weight of the crucible from the residue and the percentage of extractive values with respect to amount of powdered material calculated.

$$\% \text{ WSEV} = \frac{W_3 - Z}{N} \times 100 \quad (5)$$

W_3= weight of crucible + Evaporated Filtrate

3.6 Alcohol (Ethanol) Soluble Extractive Values (ASEV)

4g of the powdered root sample of C. *pulcherrima* was weighed into a 250ml stopper conical flask. 90ml of 90% ethanol was added and the flask was corked firmly. This was shook using a mechanical shaker for 5hours and then allowed to stand for 18hours. The extract was filtered through a filter paper and 20ml of the filtrate was measured into a previously weighed crucible. The filtrate was evaporated to dryness at 100^{o}c. The residue was dried to constant weight. The weight of the residue obtained from 20ml extract and was determined in 3 replicates by subtracting the weight of crucible from the weight of the crucible + residue and the percentage of extractive values with respect to amount of powdered material calculated.

$$\% \text{ ASEV} = \frac{W_4 - Z}{N} \times 100 \quad (6)$$

W_4= weight of crucible + filtrate

3.7 Extraction of Crude Powdered Sample

The powdered plant material (600g) was extracted with 2.0L of methanol by maceration at room temperature for 3 days. The extract was concentrated to dryness using rotary evaporator at reduced pressure. The concentrated extract was weighed and the percentage yield calculated based on the initial weight of the crude powdered sample. The extract was stored in a refrigerator for further analysis.

3.8 Vacuum Liquid Chromatography Analysis (VLC)

40g of the methanol (crude) extract was measured and mixed thoroughly with silica gel until it became fine powder. It was then poured on a layer of pure silica gel in the column and covered with a layer of cotton wool. The extracting solvents were prepared and poured into the column to obtain different fractions of the extract. The following solvents in an array of increasing polarity were used:

(i) 50% ethyl acetate: 50% n-hexane; 500ml ethyl acetate:500ml n-hexane
(ii) 100% ethyl acetate; 1 litre of ethyl acetate
(iii) 50% ethyl acetate:50% methanol; 500ml ethyl acetate:500ml methanol

The various fractions were afterwards concentrated using the rotary evaporator. The fractions together with the crude extract were kept in sample bottles and stored in the refrigerator for ferric reduction antioxidant power (FRAP) assay analysis.

3.9 Procedure For Frap Test

The ferric reducing antioxidant power (FRAP) assay of root bark ectracts of *C.pulcherrima* was conducted according to the method of Benzie and Strain [10].

FRAP reagent is a straw coloured reagent prepared from the mixture of Acetate, TPTZ (2,4,6-tripyridyl-s-triazine), $FeCl_3$ in the ratio 10:1:1 (60ml:6ml:6ml).

FRAP solution (3.6ml) was added to distilled water (0.4ml) and incubated at ambient temperature about 37^{0}C for 5minutes. Then this solution was mixed with known concentrations of the plant extract (80µL) and incubated for 10mins at about 37^{0}C. The absorbance of the reaction mixture was measured at 593.0nm using the UV-Visible spectrometer. Ferrous sulphate standard was prepared which was used for the construction of calibration curve of absorbance against concentrations (0.1, 0.2, 0.4, 0.6, 0.8, 1.0) from which the various concentrations of the fractions of the root bark extract were determined at a known absorbance.

4.0 Results

Table 1: Proximate Analysis

Parameters	Value(%)
Moisture content	17.333 ±0.4108
Water soluble extractive value	0.233±0.023
Alcohol soluble extractive value	0.5916±0.000024
Total ash	5.813±1.243
Acid insoluble ash value	3.476 ±0.337
Water soluble ash value	7.044 ±0.536

Table 2: Vacuum Liquid Chromatography

Plant extract	Percentage Yield (%)
Crude	11.74
100% ethylacetate	20.00
50% ethylacetate:50% n-hexane	32.20
50% ethylacetate:50% methanol	29.50

Table 3: Frap Assay of $FeSO_4$ **(**Absorbance of blank (distilled water) = 0.088)

Concentration of Standard Solution	Absorbance (593.0nm)
0.1	0.164
0.2	0.282
0.4	0.377
0.6	0.379
0.8	0.612
1.0	0.759

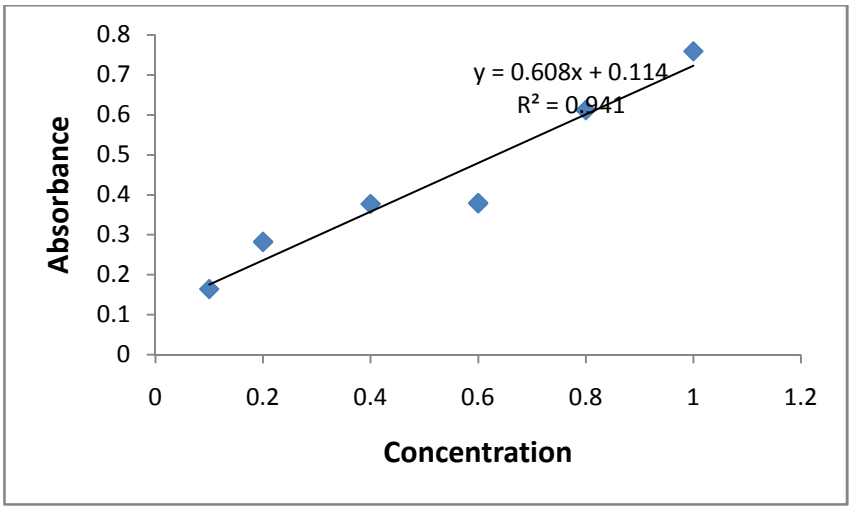

Fig. 1: Plot of Absorbance Vs Concentration of the Standard Solution ($FeSO_4$ Solution)

The concentration of the variousplant extracts were obtained from their absorbance using equation of the plot of absorbance vs concentration of the standard solution.

$$Y = MX + C \qquad (7)$$

Where
Y= Absorbance
M= Slope
X= Concentration
C= Intercept

$Y = 0.6086X + 0.1144$ (R^2=0.941)was obtained from the absorbance vs concentration plot of the standard solution. (Fig. 1)

Table 4: Frap Assay For The Different Extracts

Plant Extract	Concentration (%mmol)	Absorbance (593.0nm)
100% ethyl acetate	44.63^{a}±0.0036	0.3863 ± 0.0020
50% ethyl acetate/ 50% n- hexane	3.540^{b}± 0.0036	0.1360 ± 0.00081
50% ethyl acetate/ 50% methanol	277.57^{c} ± 0.0866	1.7656 ± 0.00124
Crude extract	76.99^{d} ± 0.0040	0.583 ± 0.00245

Values with different superscripts are significantly different (p<0.05).

5.0 Discussions

The result obtained from the proximate analysis is presented in Table 1.

The moisture content helps us to evaluate the vulnerability of a crude plant sample to microbial and hydrolytic degradation [15] The maximum permissible range for the moisture content (according to AOAC) of a crude drug sample is 6-8% [15].

The value obtained for moisture content is 17.333 ± 0.410 w/w %. This is well above the maximum permissible range for crude drugs. This implies that the phytochemicals present in the crude drug sample are susceptible to or may have undergone microbial and hydrolytic degradation. The total ash is a measure of the residue left after ashing. This residue is made up of non- volatile inorganic constituents [16]. The mean total ash value obtained from this analysis is (5.813 ± 1.243) w/w %. Acid insoluble ash is a measure of the sandy matter in the crude drug sample [17]. The mean acid insoluble ash value is (3.476±0.337) w/w %. Water soluble ash value was (7.044 ± 0.536) w/w %. The extractive values give information on which solvent is a better solvent extractor. From the result obtained (Table 1), alcohol is a better solvent extractor than water. The absorbance and concentration of the different extract – FRAP reagent mixture is given in Table 4.All the extracts showed antioxidant activity. The antioxidant capacity was measured based on the formation of Fe(II) –TPTZ which absorbs at 593.0nm. The 50% ethyl acetate:50% methanol showed the highest antioxidant activity and 50% ethyl acetate:50% n-hexane showed the least antioxidant activity. The antioxidant activity decreased according to decreasing polarity of the solvent extractor.

6.0 Conclusion

The proximate analysis shows that the sample susceptible to microbial and hydrolytic degradation due to high moisture content obtained from the results

This study also shows that the root bark extracts of C. *pulcherrima* have potent antioxidant activities, and therefore holds promise as a source of new lead drug candidates.However, it is recommended that further isolation and characterization work be carried out on the root bark extract of this plant in order to isolate the phytoconstituent(s) responsible for the antioxidant activity. . Also, this study will serve as a reference for future studies.

7.0 References

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