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RESEARCH ARTICLE

EVALUATION OF IN VITRO ANTIOXIDANT POTENTIAL OF *Mucana flagellipes.*

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Abstract

Mucana flagellipes also known as 'Agbara' leave in Eastern Nigeria is a very strong forest climber that is normally squeezed in water and taken orally to boost the blood level. The study appraises the antioxidant activities of the leave. In addition to the estimation of the total phenol content, total flavonoid content, anthocyanin content and ascorbic acid, the antioxidant activity of the extract was evaluated by measuring the reducing power and the hydrogen peroxide scavenging activity. The leave extract contained 11% flavonoid, 0.00243mg/g anthocyanin, 1.13 %phenol and 46.3% ascorbic acid. For the scavenging assay, the reducing power of the leave and the standard at concentrations of 30mg/m1, 50mg/^{m1} and 70mg/ml are 94.7%, 110.1% and 120.8%, for the standard 98.6%, 115.0% and 129.4%. For the hydrogen peroxide scavenging activity of the leave and standard at concentrations of 30mg/ml, 50mg/ml and 70mg/ml are 13.9%, 15.6% and 19.7% for the standard 9.4%, 15.1% and 17.8%. The presence of these anti-oxidative parameters in the leave extract showed the ability of the leave to prevent free radicals which causes damage or death of cells. The result of this work showed that the leave extract can be utilized as an effective and safe antioxidant source.

Introduction:-

Plants as a valuable source of medicine that helps in the maintenance of human health cannot be over emphasized. They constitute an important source of active natural products which differ widely in terms of structure, biological properties and mechanisms of action.

Free radicals are fundamental to anti biochemical process and represent an essential part of aerobic life and metabolism (Tiwari, 2001).Over the past decades natural antioxidants of both nutritive and medicinal plants have been of significant interest to the pharmaceutical and food industries due to their roles of combating myriads of oxidative damages incurred by living cells from free radical activities. Antioxidants offer resistance against oxidative stress by scavenging the free radicals and by many other mechanisms and thus prevent the disease progression (Braugghler *et al*., 1999*).* The most commonly used synthetic antioxidants at present are butylated hydroxyanisole, propyl gallate, and tertbutylhydroquinonc. However they are suspected of being responsible for liver damage and acting as carcinogens in laboratory animals (Anagnostopou *et al., 2006).* the search for new products with anti oxidative properties and fewer side effects is very active domain of research. Therefore, the development and utilization of more effective antioxidants of natural origin is desirable (Sagakami *et* al., 1991). Since ancient times, many official herbs have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of any infection and preservation of food from toxic effects of oxidants (Tatjana *et al.,* 2005). Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidants and radical scavenging properties (Sangameswaran *et al.2008).* The aim of this present study is to evaluate the antioxidant properties of *Mucana*

flagellipes inorder to encourage its usage.

Materials and methods

Sample collection and preparation:-

The freshly harvested *Mucuna flagellipes* leaves were purchased from local farmers at Umuerim Nekede, Owerri West L.G.A in Imo state Nigeria. Immediately after purchasing the leaves, they were rinsed in clean water to remove dirts and then cut into smaller pieces and dried at room temperature. The dried leaves were crushed using the blender and then stored for use.

Determination of total flavonoid content by bohn and kocipai (1994).

5g of the blended sample was weighed and dissolved in 20m1 of 40% methanol at room temperature for 30minutes. The whole solution was filled with Whatman filter paper into beaker. The filtrate was evaporated to dryness over a water bath and then dried in an electric oven to a constant weight. The flavonoid content was expressed as % f1avonoid.

$$\% \text{ flavonoids} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of anthocyanin content

Theanthocyanin content in the leave was determined using the method described by Ebrahimzadeh *et al.(20*09). 1g of the blended sample was soaked in 5ml of acidified methanol (79ml methanol, 20ml water and 1ml HCL) for 24hours in darkness at 4°C with occasional shaking. 2ml of distilled water and 4.8ml of chloroform were mixed and added to the extract. The absorbance of the upper phase was determined at 530nm and 657nm. The concentration of the anthocyanin as mg dry weight of the treated plant was expressed using the following equation;

$$\text{Anthocyanin} = \frac{[OD530 - 0.25\ OD657] \times TV}{[dwt \times 1000]}$$

Where:

OD =Optical density

TV = Total volume of the extract (ml)

DWT= Weight of the dry leaf (g)

DETERMINATION OF PHENOL CONTENT

The concentration of the phenol in the leave was determined by the method described by Pearson (1976). 0.2g of the powdered sample was weighed in triplicate into a test tube and 10ml of methanol was added to each of the samples and was thoroughly shaken. The mixtures were left to stand for 15minutes before being filtered using Whatman's (N01) filter paper. 1ml of the extract was placed in each test tube and 1ml of Folin Dennis reagent in 5ml of distilled water was added to each test tube and was allowed to stand for 2hours at room temperature. The absorbance of the developed colour in each tube was measured at 760nm wavelength. The phenolic content of the leave was calculated thus:

$$\% \text{ phenol} = \frac{100}{W} \times \frac{AU \times}{AS} \quad \frac{C \times}{100} \quad \frac{VF \times D}{VA}$$

Where:

W= Weight of sample analyzed

AU= absorbance of the test sample

AS= Absorbance of the Standard solution

C= Concentration of the standard solution

VF= Total filtrate volume

VA= Volume of filtrate analyzed

D =Dilution factor where applicable

Determination of ascorbic acid (vitamin c) by voogt osbong (1978).

25ml of 0.5% oxalic acid was poured in 2g of the blended sample and 100ml of water was added to it. After that, 2.5ml of acetone was added to the sample. The mixture was filtered and 100ml of the filtrate was titrated against 2.6dichlorophenol indophenol. Amount of ascorbic acid was calculated thus:

Vitamin C (mg/100ml) = 20 x V x C

Where:

V= Titre Value

C = 2g concentration volume.

Determination of reducing power

The reducing power of the leave extract was determined according to the method described by Yen *et al*. (2000) with slight modification. 1 ml of the extract with a varying concentrations of 30mg/ml, 50mg/ml and 70mg/ml. 5ml of 0.2ml of sodium phosphate buffer (PH 6.6) and 5ml of 1.0% potassium ferricyanide were mixed and incubated at 50°*C* for 20minutes. Then 5ml of 10% trichloroacetic acid was added and the mixture centrifuged at 980g for 10minutes. The upper layer of the solution (5.0ml) was taken and diluted with 5ml of distilled water and ferric chloride (1.0ml, 0.1%) and absorbance was noted at 700nm using a spectrophotometer,

$$\text{Reducing Power (RP)} = AM \quad \frac{X}{AB\text{-}1} \quad \frac{100}{1}$$

Where: AM = Absorbance of the reaction mixture

AB = Absorbance of the control mixture

Determination of hydrogen peroxide scavenging activity by Ruch*et al., (1987).*

A solution of hydrogen peroxide (2.5ml) was prepared in phosphate buffer (22.5ml) at pH 7.0. Hydrogen peroxide concentration was determined trophotometrically at 250nm. 1ml of the extract was added to 5ml of hydrogen peroxide. The absorbance of hydrogen peroxide was determined at 250nm after 10 minutes against a blank solution (phosphate buffer). Ascorbic acid was used as positive control.

$$\%\ \text{Hydrogen Peroxide} = \frac{AO - AS\ X}{AO} \quad \frac{100}{1}$$

Where:

AO= Absorbance of Positive Control

AS= Absorbance of Sample

Result:-

Table 1. Antioxidant parameters of Mucana flagellipes

Biomolecules	Contractions (%)
Phenol	1.13
Flavanoid	11
Anthocyanin	0.0024mg/g
Ascorbic acid	46.30

Table 2. Reducing power of the Mucana flagellipes and ascorbic acid (standard)

Concentraction (mg/ml)	Samples(%)	Acsorbic acid (std) (%)
30	94.70	98.60
50	110.10	115.00
70	120.80	129.40

Table 3. Hydrogen peroxide scavenging of the sample and ascorbic acid standard

Concentraction (mg/ml)	Samples (%)	Acsorbic acid (std) (%)
30	13.90	9.40
50	15.60	15.10
70	19.70	17.80

Discussion:-

The antioxidative properties of the leaves of *mucuna* f*lagellipes* were properly investigated. The biomolecules determination was represented in table 1. The phenol content of *Mucuna flagellipes* leave was found to be 1.13%. The flavonoid content of the leave was 11%. The anthocyanin content of the leave was 0.00243mg/g. The ascorbic acid content of the leave was 46.3%. It is well known that plant phenolics are highly effective free radical terminators (scavengers) and antioxidants. Antioxidative activity of vegetables and fruits are derived from phenol, flavonoid, ascorbic acid, anthocyanin and beta carotene compound (Bravo, 1998), and the content of the biomolecules in this leave extract was appreciable.

Scavenging assays:-

Reducing power:-

Reducing power is used as the absolute measure of antioxidant activity. The reducing power potential of the sample and the ascorbic acid (standard) increases as the concentrations increases. The antioxidants present in the extract of *Mucuna flagellipes* caused the reduction of Fe^{3+} ferricyanide complex to the ferrous form Fe^{2+} with a change in color from yellow to bluish green (Lou *et al.*, 2006), and thus proved the reducing power ability. The reducing power potential of the leave was lower than the reducing power potential of the ascorbic acid (standard) at the concentrations of 30mg/ml, 50mg/ml and 70mg/ml.

Hydrogen peroxide scavenging activity:-

Hydrogen peroxide is highly important because of its ability to penetrate into biological membranes. The scavenging of the hydrogen peroxide by extracts may be attributed to their phenolics, which can donate electrons to hydrogen peroxide, thus neutralizing it to water (Ebrahimzadeh *et al.*,2009*).* The result showed that the hydrogen peroxide scavenging potential of the leaves of *Mucuna flagcllipes* was higher than the hydrogen peroxide potential of the ascorbic acid standard at the concentrations of 30mg/ml and 70mg/ml. However, at concentration of 50mg/ml, the hydrogen peroxide potential of the leave was not different from the hydrogen peroxide potential of the ascorbic acid standard. Therefore, *mucuna flagellipes* plant has a strong hydrogen peroxide scavenging potential than the ascorbic acid used as the standard.

The presence of these biomolecules (flavonoid, anthocyanin, phenol and ascorbic acid) that were determined in *Mucuna flagellipes* showed its ability to inhibit carcmogenous molecules which causes death or aging in living tissues and the scavenging of hydrogen peroxide and reducing power at different concentrations of the extract makes the leave an important source of antioxidant.

Conclusion:-

The leaves of *mucuna flagellipes* exhibited higher potency of free radical scavenging activity. The presence of these biomolecules such as flavoid, anthocyanin, phenol and ascorbic acid creates an anti-oxidative properties and activities. The result of this research work revealed that the extract of the *mucuna flagellipes* leave can be utilized as an effective and safe antioxidant source.

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