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Full Length Research Article

ANTIOXIDANT POTENTIAL OF HABISCUS CANNABINUS METHANOLIC LEAF EXTRACT

James SA[1], MJ Ladan and Goje DJ

Department of Biochemistry, Kaduna State University, Kaduna.

[1]Corresponding author email: sjgamong@yahoo.com

ABSTRACT
The antioxidant properties of Methanolic leaf extract of Hibiscus cannabinus (Malvaceae family), was investigated using 1,1-diphenyl-2-picryhydrazyl (DPPH) assay method, reducing power and hydrogen peroxide scavenging effect assays. The Methanolic leaf extract, exhibited significant scavenging effect on DPPH free radical and hydrogen peroxide production at a lower concentration of the extract when compared with ascorbic acids and α- tocopherol standard antioxidants. The highest free-radical scavenging and percentage scavenging effect of hydrogen peroxide by the Methanolic leaf extract was observed at concentrations 31.25 and 250µg/ml respectively; with 76.03% and 62.26% inhibition/scavenging effect respectively. However, reducing power showed low activity for the leaf extract.

Keywords: DPPH, Ascorbic acid, α-tocopherol, Hibiscus cannabinus, flavonoid, hydrogen peroxide, free-radical.

INTRODUCTION

Reactive oxygen species (ROS) are produced during the interaction of metabolism with oxygen in the mitochondrial respiratory chain, liver mixed function oxidasess, by bacterial leucocytes, through xanthine oxidase activity, atmospheric pollutants, and from transitional metal catalysts, drugs and xenobiotics (Atawodi, 2005 and Smirnoff, 2005) . These oxygen species produced have the potential to cause oxidative damage by reacting with biomolecules (Smirnoff, 2005) thereby precipitating a disease condition. Antioxidants suppress the formation, dispose the species, scavenge and oppose the action of pro-oxidants (Murry et al; 2003). In a normal cell, there is an appropriate balance between the pro-oxidants and antioxidants, these antioxidants may include NADPH, reduced glutathione (GSH), ascorbic acids, α-tocopherol, etc. However, this balance can be shifted towards the pro-oxidant when production of oxidants increases or when levels of antioxidants are diminished. This state is known as "oxidative stress", and can result to disease state such as diabetes mellitus, arterial and cardiac diseases, arthritis, cataracts and also premature ageing along with several chronic diseases and other serious cell damage when the stress is massive or prolonged (Murry et al; 2003; Mishra et al; 2007).

The recent quest of natural plant product in altering human abnormal biochemical changes as a result of disease condition, apart of its dietary importance plants have shown to be either therapeutic or and chemopreventive. It is also known that plant accumulates antioxidant chemicals as secondary metabolite through evolution as a natural means of surviving environmental stress (Atawodi, 2005); to avoid such stress that make the plant vulnerable to active oxygen, it thus develop numerous potent antioxidant such as terpenoids, flavonoids, essential oils and other aromatic compounds that are non-nutritive and once thought to be unimportant (Mishra et al; 2007).

Since most medicinal plants occur naturally in a large number of countries, a plant of potential importance in one country may well have been studied by scientists elsewhere. Yet pools of information is especially critical when it comes to drugs as a value judgement on safety or efficacy of a particular drug can rarely be based on the results of a single study. Therefore, a combination of information can ascertain its uses (Farnsworth, et al; 1985).

Hibiscus cannabinus L. (Malvaceae) has long been used as folk medicine in India and Africa for the treatment of various disease conditions (Duke, 1983). The plant Hibiscus cannabinus is also known as Kenaf and commonly called Rama in northern Nigeria, where it is used as vegetables in the preparation of local dish called 'pate' (i.e. maize portage) and the preparation of a local salad. Literatures have indicated not much work have been done in respect to antioxidant activity of Hibiscus cannabinus in Nigeria. In fact, work done by Atawodi (2005) did not include Hibiscus cannabinus among the plants used as antioxidant in Nigeria or in West Africa. But work done by Agbor et al (2005) and Ibrahim et al (2005), implicated the plant Hibiscus cannabinus to have antioxidant activity. Therefore this study is to ascertain the antioxidant potential of Hibiscus cannabinus in comparison with the known natural and synthetic antioxidant.

MATERIALS AND METHODS

Reagents

All chemicals and reagents used for the study were of analytical grade.

Plant Material

The leaves of *Hibiscus cannabinus* were collected from four different communities around Kaduna State, Nigeria between the months of July and August, 2009 and authenticated by the Department of Biological Sciences, Kaduna State University, Kaduna, Nigeria.

Fresh leaves were collected and dried for four days, crushed to powder and stored in an airtight container at 25^{o}C.

Extraction

The powdered plant material (50g) was soaked in methanol and stirred (with the aid of a magnetic stirrer) for one hour, then stored in the dark for 48 hours. After 48 hours, the mixture was filtered first with muslin then filtered again with cotton wool in funnel. The filtrate was then evaporated with the aid of rotary evaporator.

Phytochemistry

Standard methods were used to determine the presence of alkaloids and glycosides in Methanolic leaf extract of *Hibiscus cannabinus* as described by El-Olemy *et al* (1994).

Antioxidant Assay

1,1-diphenyl-2-picrylhydrazyl DPPH free radical scavenging activity:

Free radical activity of *Hibiscus cannabinus* leaves extract was measured by following the decrease in the absorbance of methanolic solution of DPPH (33mgL^{-1}), it was prepared in methanol which gave an initial absorbance of 2.450. Five ml of this stock solution was then added to 1 ml of the methanolic

leaf extract at different concentrations (31.25 – 1000 µg/ml). This was repeated in triplicate. After 30min, absorbance was measured at 517nm, the mean values noted and compared with standards. Scavenging activity was expressed as percentage inhibition calculated using the following (Kumar *et al.*, 2007):

$$\% \text{ Anti-radical activity} = \frac{\text{Control Abs} - \text{Sample Abs}}{\text{Control Abs}} \times 100$$

Reducing power assay:
In triplicate, different concentration of the methanolic leaves extract (31.25 – 1000 µg/ml) in 1 ml of methanol were mixed with 2.5 ml of 0.2Mphosphate buffer (at pH 6.6) and 2.5 ml of 1% potassium ferrocyanide. The mixtures were incubated at 50°C for 20 min. To the mixture, 2.5 ml of 10% tricholoroacetic acid was added and was then centrifuged at 3000 rpm for 10 min. 2.5ml of the supernatant was transferred to a test tube and mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride ($FeCl_3$) and the absorbance was measured at 700 nm, the mean values noted and compared with standards. Increase in absorbance of the reaction mixture indicated increased reducing power (Kumar *et al.*, 2007).

Scavenging of hydrogen peroxide:
A solution of 40mM hydrogen peroxide was prepared in phosphate buffer of pH 7.4. Different concentrations (31.25 – 1000 µg/ml) of the methanolic leaves extract were added to 0.6 ml of 40mM hydrogen peroxide solution. This was repeated in triplicate. Absorbance of hydrogen peroxide at 300 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of scavenging of hydrogen peroxide by *Hibiscus cannabinus* and the standard compounds was calculated using the following formula and the mean values noted (Kumar *et al.*, 2007):

$$\%\text{Scavenging } [H_2O_2] = [A_o - A_I] \times 100$$

where A_o was the absorbance of the control, and A_I was the absorbance in the presence of the sample and standard.

RESULTS
The Methanolic leaf extract of *Hibiscus cannabinus* gave a weight of 6.2g on extraction. Phytochemical screening of the Methanolic leaf extract indicates the presence of flavonoids and other polyphenols such as tannins and anthroquinone as shown in table 1.

The evaluation of the free radical scavenging activity of *Hibiscus cannabinus* was compared with ascorbic acid and α-tocopherol *in vitro* using DPPH radical with garlic acid as standard. In the reaction between DPPH and the antioxidants results indicates that at lower concentration of 31.25µg/ml and 62.5µg.ml inhibition (76.03 ± 0.003 and 67.0 ± 0.005 % respectively) of free radical activity of *Hibiscus cannabinus* is significantly high as compared to ascorbic acid or α-tocopherol (see figure 1). As the concentration increases, only ascorbic acid shows a significant increase in the % inhibition of the free radical scavenging activity.

Also, the reductive capacities of the extract were compared with Ascorbic acid and α-tocopherol. The reducing power of the *Hibiscus cannabinus* extract was found to be concentration dependent and significantly low when compared with Ascorbic acid and α-tocopherol (Figure 2). Figure 3 illustrate the percentage scavenging effect of hydrogen peroxide generation in *Hibiscus cannabinus* methanolic leaf extract as compared with Ascorbic acid and α-tocopherol. At lower concentration of 31.25 - 250µg/ml, *Hibiscus cannabinus* showed higher percentage hydrogen peroxide scavenging activity over α-tocopherol with a scavenging effect of 62.3% at 250µg/ml; but at higher concentration of 500 and 1000µg/ml, α-tocopherol showed high scavenging effect of 62.3 and 98.7% respectively. Ascorbic acid showed a significant scavenging effect across the concentration except at 250µg/ml where the effect is significantly low.

Table 1: Phytochemical analysis on methanolic leaf extract of *Hibiscus cannabinus*

Phytochemical test	Test	Inference
Alkaloids		
General test	Mayer's test	+
Ergonovine	Ferric chloride test	-
Codine	Potassium ferrocyanide test	+
Quinine	Erythroquin test	+
Morphine	Potassium ferrocynide test	+
Theophylline	Ferrous sulphate test	+
Theobromine	Silver nitrate test	-
Glycosides		
General test	Fehlings test	+
Saponins	Frothing test	+
,,	Emulsifying test	+
Tannins	Ferric chloride test	+
Flavonoids	Sodium hydroxide test	+
Cardiac glycoside	Keller-Killiani test	+
Anthroquinone	Bromine test	+

Key: + = Present - = Absent

Table 2: Antioxidant profile of *Hibiscus cannabinus* leaf extract

Sample tested	Sample conc. (µg/ml)	DPPH radical scavenging activity (% inhibition)	Reducing power activity[a] (absorbance)	Percentage scavenging of hydrogen peroxide
H. cannabinus	31.25	76.03 ± 0.003	0.506 ± 0.001	62.26 ± 0.680
Ascorbic acid	1000	82.90 ± 0.116	1.278 ± 0.006	90.50 ± 0.064
α-tocopherol	1000	48.10 ± 0.081	0.714 ± 0.002	98.70± 0.175

[a]Increase absorbance indicates increased reducing power

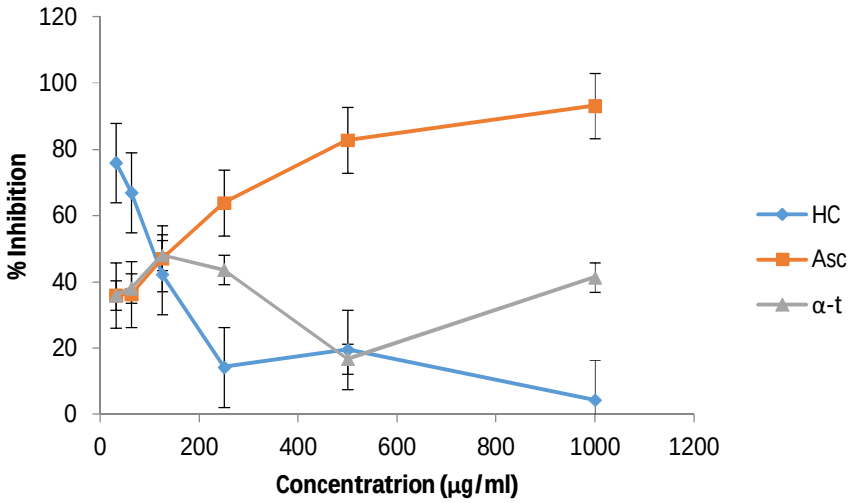

Figure 1: Free radical (1,1-diphenyl-2-picrylhydrazyl) scavenging by *H. cannabinus*, ascorbic acid and α- tocopherol. Results are means ± SD of three parallel measurements. (*H. cannabinus*, ascorbic acid and α- tocopherol are represented by ◊, Δ and □, respectively).

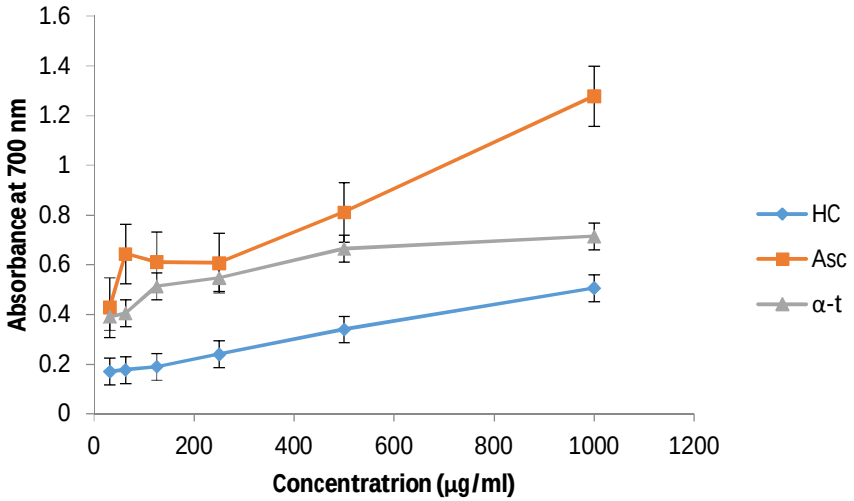

Figure 2: Reducing power of *Hibiscus cannabinus* Methanolic leaf extract, Ascorbic acid and α-tocopherol. Results are means ± SD of three parallel measurements.

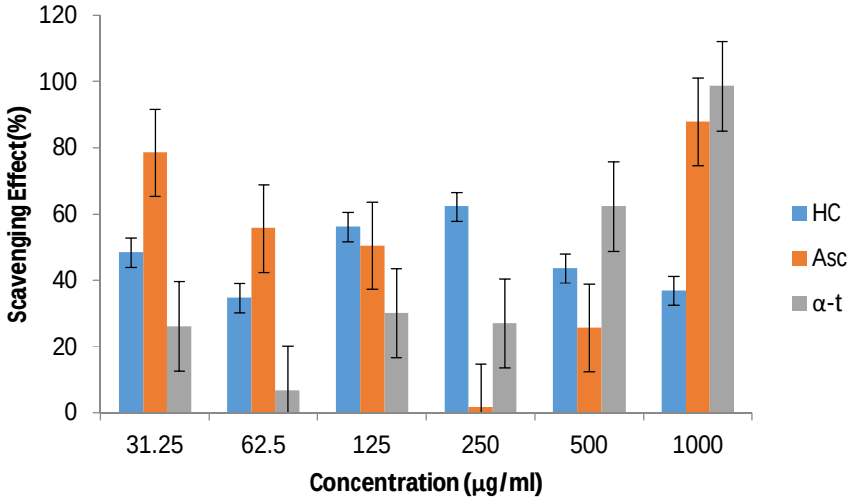

Figure 3: Comparison of the percentage scavenging effect of hydrogen peroxide generation in *Hibiscus cannabinus* Methanolic leaf extract, Ascorbic acid and α-tocopherol. Results are means ± SD of three parallel measurements.

DISCUSSION

The presence of flavonoid and other polyphenols in the Methanolic leaf extract of *Hibiscus cannabinus* suggest its antioxidant potentials. Flavonoids and polyphenols scavenge free radical and prevent oxidative damage of tissues hence combating pathological disorder generated by cellular metabolic processes (Mishra *et al;* 2007). A variety of flavonoids, lignans, an alkaloid, a bisbenzyl coumarins and terpenes exhibit the ability to inhibit lipid peroxidation in the brain, kidney and erythrocyte hemolysis; these compounds have at least one free aromatic hydroxyl group in structure, which is obviously a very potent antioxidant compound (Ng *et al*. 2000) which protect the body from various disastrous and chronic diseases, like cancer, arthritis, common cold cough, cataracts etc. which weaken the immune system of the body (Mishra *et al;* 2007).

Also, results of the evaluation of the free radical scavenging activity by *Hibiscus cannabinus* indicates that the Methanolic leaf extract has a noticeable effect on scavenging the free radical at a lower concentration. For the determination of reducing ability of a compound (transformation of Fe^{3+} to Fe^{2+}), serves as a significant indicator of its potential antioxidant activity (Kumar *et al.* 2007), hence its determination in *Hibiscus cannabinus* Methanolic leaf extract which shows a reductive capacities of the extract.

Work done by Agbor *et al* (2005) showed that aqueous leaf extract of *Hibiscus cannabinus* extract has the ability to inhibit the accumulated lipid peroxidation product in the plasma as well as maintaining the antioxidant activity of the enzymes such as superoxide dismutase and catalase. Also Ibrahim *et al* (2005) showed that the Methanolic leaf extract yield 66.93% at 500µg/ml when he assayed 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity thus supporting the findings that *Hibiscus cannabinus* has natural plant product that can initiate and maintain antioxidant activity.

CONCLUSION

The antioxidant potential of *Hibiscus cannabinus* Methanolic leaf extract and standards were compared using various *in vitro* methods. The extract shows the presence of flavonoids and other polyphenols. The antioxidant study indicates that the plant *Hibiscus cannabinus* has antioxidant activity at lower concentration when compared with standard antioxidant compounds *in vitro*. However, the active substance responsible for the antioxidant activity of *Hibiscus cannabinus* is not yet clear. Hence, the need for further work to isolate and identify the antioxidant factor(s) in *Hibiscus cannabinus.* Also further work on the safety of the extract is suggested.

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