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Assessment of In-Vitro Antioxidant Activities of Ocimum Gratissimum, Vitex Doniana, Carica Papaya and Peristrophe Bicalyculata Using DPPH Free Radical Scavenging Activity

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Abstract:*The ethanol extracts of the leaves of Ocimum gratissimum, Vitex doniana, Carica papaya and Peristrophe bicalyculata were assessed for antioxidant activities by the use of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. The reducing potentials of these plants were also evaluated. The phytochemical screenings of the medicinal plants were equally carried out. The percentage antioxidant activity values for the plants are: 60.0 ± 1.05%, 60.8 ± 1.28%, 62.4 ± 1.28% and 75.7 ± 2.60% for O. gratissimum, V. doniana, C. papaya and P. bicalyculata respectively. These values were dose dependent and statistically significant at P<0.05 (ANOVA). The results indicated that P. bicalyculata has the highest antioxidant activity value of 75.7 ± 2.60% while O. gratissimum has the least value 60.0 ± 1.05%. The percentage antioxidant activities of the plants were comparable to the standards used, ascorbic acid and α-tocopherol which were found to be 86.7 ± 1.08% and 97.2 ± 1.06% respectively. The reducing potentials of the plants were found to be proportionally correlated to the antioxidant activities of the plants. Phytochemical screenings revealed the presence of flavonoids, saponins, C. glycosides, steroids, alkaloids, tannins, anthraquinones, terpenoids and carbonhydrates in the medicinal plants.*

Keywords:*Antioxidant, DPPH, Ocimum gratissimum, Vitex doniana, Carica papaya, Peristrophe bicalyculata*

1. INTRODUCTION

Chemical compounds with unpaired electrons in the body act as oxidants, they are generally very reactive and are capable of causing oxidative damage to biological molecules such as proteins, lipids, and also DNA, which subsequently leads to mutation (Ellinaim et al., 2003). Free radicals play a crucial role in human health. The effect of free radical reactions in the body has been implicated in the etiology and pathogenesis of chronic diseases that are life threatening such as cancer, hypertension, cardiac infarction, arteriosclerosis, diabetes etc. Compounds that inhibit or delay the hydrolysis of other molecules by preventing the initiation or propagation of hydrolyzing chain reactions caused by oxidants are called antioxidants (Velioglu et al., 1998). Pharmacological functions such as anti-mutagenicity, anti-carcinogenicity, anti-aging etc are gotten from the pharmacological properties that originate from antioxidant activities (Cook and Samman, 1996: Huang et al., 1992).

The most important oxidants in the body are the reactive oxygen and nitrogen species, such as super oxide, hydrogen peroxide and nitric oxide radicals. Cellular and metabolic processes are responsible for generating most of the free radicals in the body; however, they can also come from external sources such as exposure to ionizing radiations, injury, oxidative drugs, pollutants, etc. Due to the constant tendency to obtain an electron from other molecules, thus, their highly reactive nature, free radicals cause damage to cells and tissues when there is excessive production and leakage from their site of origin. DPPH, diphenyl-1-picrylhydrazyl radical scavenging activity assay has been widely used for antioxidant screening of fruit and vegetable juices or extracts (Sanchez- Moreno 2002). DPPH is a stable free radical that is hydrolyzed to DPPH-H, prior to reaction with antioxidant, which as a result, decreases its absorbance and its natural purple colour changes to yellow. The degree of discoloration shows how potent the scavenging power of the antioxidant compound or extract is, in

terms of the ability to give out hydrogen. The scavenging reaction between DPPH and an antioxidant (A-H) can be represented as follows;

DPPH+ (A-H) = DPPH—H-(A)

Studies have shown that natural antioxidants, especially the flavonoids, are beneficial to human health as they show a broad range of pharmacological properties such as anti-ischemic, anti-allergic, antibacterial, antiviral, anti-inflammatory, vasodilatory and anti-proliferative activities (Cook and Samman, 1996). The antioxidant activities of several plant materials have been reported (Al-Saikhan et al., 1995; Yen and Duh, 1995: Oomah and Muzza 1996: Wang et al., 1996: Cao et al., 1996: Amarowicz et al., 1996).

Ocimum gratissimum popularly referred to asAfrican basil in English, is an herbaceous perennial plant that has a woody stem, although it's actual otigin is unknown, the plant can be found naturally in many regions. Ocimum is a genus of about six species of flowering plants in the family lamiaceae (labiate). Ocimum gratissimum is locally called 'effinrin-nla' by the Yorubas, 'nchu-anwu' by the Igbos, while in the northern part of Nigeria; the Hausas call it 'daidoya' (Effraim et al., 2003). The leaf has a characteristic pleasant aroma which is responsible for its name 'Scent leaf', and hence, it's use as spice and condiments in cooking. The whole plant is used in folklore medicine and as insect repellant (Akinmoladun et al., 2007).

Vitex doniana is a very popular plant used in traditional medicine in Nigeria. They belong to the family of verbenaceae, the local Nigerian names include; dinya (Hausa and Igala), Ucha-koro (Igbo) and Orin-ola (Yoruba). The plant has an average height of about 20-25m, and an average diameter of about 1m. They grow slowly, and have an average life-span of 60-200 years. Vitex doniana is popularly found growing in the tropical and sub tropical regions of the world and grows well in semi-arid tropical regions and humid tropical regions with seasonal rainfall ranging from 750-2000mm. they are commercially cultivated in various soil types of different origins that usually include alluvial soils and homestead gardens for its product (FAO 1983).

Caricaceae is the family Carica papaya Linnaeus (paw paw) belongs to, and it is an herbaceous perennial plant with rapid proliferation rate (Dick G., 2003). Papaya has a short life span, but has a fruitage time of 20 years. Papayas are hemaphodites i.e. possess both male and female parts (Bruce and Peter, 2008), thus, they are self- fertilizing (Jayasri, 2009), and the commercially available papayas are the hermaphrodite trees that produce fruits that are pear shaped. The plant yields natural substance (Annonaceous acetogenins) in leaf barks and tissues of twig that has potent antitumor and pesticidal activities (Mc Langhlin, 1992). Papayas are rich in self-defense compounds which confer a high level of immunity to attack by insects and to diseases (Peter, 1991).

Peristrophe bicalyculata belongs to the family Acanthaceae and the genus Peristrophe. In Nigeria, the Hausas call it 'tubanin dawaki' translated as flour of the horse. In 'Serer' and 'Wolof' languages of Senegal, it is called 'buben' and 'môto' respectively (Burkill, 1985). In the Indore district of India, the locally called 'Chotiharjori'(Dwivedi, 2002). They are found naturally in the tropical regions of Africa, in the Sahel part of the region of Mauritania, Niger and northern Nigeria as well as in India, Burma and Thailand. The herb possess anti-bacterial properties (tuberculostatic), and has proven to be effective in treating sprain, snake poison, fever, bone fracture, cold and cough and for ear and eye treatments (Rashmi, G. 2010), Furthermore, It is also used in the treatment of skin diseases, and serves as an antidote for metabolic diseases such as diabetics, hypercholesterolemia among others (Abimbola 2013).

2. MATERIALS AND METHODS

2.1. Materials

All the chemicals used for the extraction, phytochemical screening, reducing potential and DPPH assay were of analytical grade; DPPH radical was a product of Sigma-Aldriech, U.S.A.

2.2. Sample Collection and Drying

The fresh leaves of the investigated plants was harvested in July, 2017 from Faculty of Agriculture, Kogi State University Anyigba and were authenticated in the Department of Biological Sciences

Herbarium, Ahmadu Bello University, Samaru-Zaria, where the plants were assigned voucher specimen numbers: 752, 753, 754 and 756 respectively for O. gratissimum, V. doniana, C. papaya and P. bicalyculata. The leaves were cleaned of sand particles, air-dried for 14 days. They were pulverized to powder and stored in air-tight containers in the refrigerator for subsequent use. These samples were brought out and allowed to assume room temperature prior to use for analysis.

3. METHODS

3.1. Preparation of the Extracts

Samples of the leaf powder of each plant (50g each) were macerated with 50ml of ethanol for 72 hrs at room temperature. Each extract was filtered (Whatman No. 1 filter paper) and the residue re-extracted with the same solvent. The extracts were combined and concentrated in a rotary evaporator under reduced pressure to give the ethanol extract for phytochemical analysis and antioxidant- activity assay.

3.2. Phytochemical Screening

Chemical tests were carried out on the ethanolic extracts and on the leaf powder using standard procedures of Trease and Evans, 1989; Harborne, 1973 and Sofowora, (1993); Odebedy and Sofowora, (1978).

3.3. DPPH Assay for Antioxidant Activity

The ability of the extract to scavenge DPPH radical was determined according to Mensor *et al*., (2001) with little modification. 1.0ml of 0.3m M DPPH methanol solution was added to the solution of the extract or standard (250ug/ml, 2.5ml) and allowed to react at room temperature for 30 mins. The absorbance of the resulting mixture was measured at 518 nm with spectrophotometer and converted to percentage antioxidant activity (AA %). Methanol (1.0ml plus extract solution (2.5ml) was used as a blank 1.0ml of 0.3mMDPPH plus methanol (2.5ml) was used as a negative control. Solution of ascorbic acid served as positive control. Antioxidant activity (AA) was calculated as percentage inhibition relative to control using the following equation (Al-Saikhan et al., 1995).

AA% =Rcontrol – Rsample/Rcontrol X 100

Where:

Rcontrol=absorbance of control.

Rsample=absorbance with each sample.

AA%=percentage of antioxidant activity.

Each extract (sample) at a particular dose or concentration was observed in triplicate.

3.4. Determination of Reducing Potential

Reducing potential was determined according to the method of Afolabi *et al*., (2007). The extract or standard (100µg/ml or 250µmg/ml respectively) was mixed with phosphate buffer and potassium ferricyanide. The mixture was incubated at 50oC for 20mins. Trichloroacetic acid (10%, 2.5ml) was added to the mixture. A portion of the resulting mixture was mixed with ferric chloride (FeCl3; 0.1%, 0.5ml) and the absorbance measured at 700nm using a Spectrophotometer.Higher absorbance of the reaction mixture indicates higher reductive potential.

3.5 Statistical Analysis

Results were analyzed using one way analysis of variance (ANOVA). Data was expressed as Mean ± SEM, the differences between mean accepted as significant at $P < 0.05$ (ANOVA).

4. RESULTS

The phytochemical screening of the plants investigated revealed the presence of flavonoids, saponins, C. glycosides, steroids, alkaloids, tannins, anthraquinones, terpenoids and carbonhydrates in the ethanol extracts of the plants (tables 1).

Table1.*Phytochemical Screening*

Phytochemicals	*O. gratissimum*	*V. doniana*	*C. papaya*	*P. bicalyculata*
Flavonoids	++	+++	+++	+++
Saponins	++	++	++	++
C. glycosides	++	+++	+++	+++
Steriods	++	++	++	++
Alkaloids	+	++	++	+
Tanins	+	++	+	+
Anthraquinone	+	+	+	+
Terpenoids	++	+	++	+
Carbonhydrates	++	++	++	++
Renins	-	-	-	-

+++ = Abundant, ++ = Moderate, + = Present, - = Absent

From the DPPH assay carried out, the percentage antioxidant activities of the plants investigated were calculated to be 60.0 ±1.05, 60.8 ±1.20, 62.4 ± 1.26 and 75.7 ± 2.60 respectively for O. *gratissimum, V. doniana, C. papaya*and*P. bicalyculata*(table 2). While the standards used were found to have percentage antioxidant activities of 86.7± 1.08 and 97.2 ± 1.06 respectively for ascorbic acid and α-tocopherol (table 2). All these were statistically significant at $P < 0.05$ (ANOVA) (table 2).

Also, the reducing potentials of the plants investigated were found to be proportional to the antioxidant activities of these plants (table 2).These are respectively 0.8 ± 0.02, 1.0 ± 0.07, 1.2 ± 0.06 and 1.6 ± 0.03 forO. *gratissimum, V. doniana, C. papaya*and*P. bicalyculata* (table 2). Ascorbic acid and α-tocopherol as standard used showed reducing potentials of 1.8 ± 0.02 and 2.0 ± 0.02 respectively (table 2).

Table2.*The antioxidant activities and reducing potentials of the investigated plants.*

	Antioxidant Activity (AOA)	IC_{50} (µg/ml)	Percentage Antioxidant Activity (% AA)	Reducing Potential (RP)
Control/blank (methanol)	0.560	2.5ml	0.0	0.0
O. gratissimum	0.221	100	60.0 ±1.05*	0.8 ± 0.02
V. doniana	0.218	120	60.8 ± 1.20*	1.0 ± 0.07
C. papaya	0.210	100	62.4 ± 1.26**	1.2 ± 0.06
P. bicalyculata	0.122	180	75.7 ± 2.60**	1.6 ± 0.06
Ascorbic acid	0.103	120	86.7 ± 1.08**	1.8 ± 0.02
α-tocopherol	0.017	50	97.2 ± 1.06**	2.0 ± 0.02

*** Represent significant at P < 0. 001; * significant at P< 0.01 (ANOVA); AOA=Antioxidant activity, AA= Percentage antioxidant activity, RP= reducing potentials, IC50 = 50% inhibitory concentration. Experiments were carried out in triplicate and expressed as mean ± standard error of mean (SEM).*

5. DISCUSSION/CONCLUSSION

Biologically active substances in plants such as flavonoids, terpernoids, saponins, tannins, anthraquinone, carbohydrate, glycosides, steroids, and alkaloids are responsible for the medicinal effects of plants in the management of diseases. These bioactive substances are popular for their anti-inflammatory, anti-diabetic, anti-microbial, anti-atherosclerotic and anti-carcinogenic properties (Chukwuka *et. al.*, 2011).

This study shows that the ethanolic extracts of the leaves of O. *gratissimum, V. doniana, C. papaya* and *P. bicalyculata*tested positive for flavonoids, ,alkaloids, Cardiac glycosides, carbohydrates,anthraquinones, tannins, saponins,terpenoids and steroids. These compounds detected have been documented to have potentmedicinal and therapeutic effects (Afolabi *et al.*, 2007; Edeoga *et al.*, 2005; Okwu and Josiah, 2006; Liu, 1991) and these findings are consistent with the previous works of Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997.

Futhermore, the result of the DPPH scavenging assay showed thatthe percentage antioxidant activity of *P. bicalyculata* was found to be the highest at 75.7 ± 2.60 % and this can be very much compared to the antioxidant activities of α-tocopherol and ascorbic acids which were used as standards and obtained as 97.2 ± 1.06 % and 86.7 ± 1.08 % respectively. The percentage antioxidant activity of *P. bicalyculata* was found to be statistically significant at P = 0.001 (ANOVA). The high percentage antioxidant activity value obtained could be attributed to its abundance of flavonoids, Phenols and ascorbic acid which have been evaluated to be 1.72, 1.86 and 44.03mg/100g dry weight respectively (Okwu and Josiah 2006). This is equally concurs with previous works of Nieto *et al*., (1993); Das and Pereira, (1990); and Foti *et al*., (1993).

In addition, the percentage antioxidant activities of *C. papaya*, *V. doniana* and *O. gratissimum* were 62.4 ± 1.26 %, 60.8 ± 1.20 % and 60.8 ± 1.20 % respectively. These were significant at P < 0.01 (ANOVA). The values were comparable to the standards used. These concurs with the works of Sathiyanaranan and Arulmozhi, (2007); Edeoga *et al*., 2005; Miliuaska *et al*., 2004. *O. gratissimum*,however, had the least percentage antioxidant activity of 60.0 ±1.05%. This can also be compared to the percentage antioxidant activities of the α-tocopherol and ascorbic acid used as standards (97.2 ± 1.06 and 86.7 ± 1.08 respectively). This is statistically significant at P < 0.05 (ANOVA).

The reducing potential of these plants were noted to have a direct linear relationship with the percentage antioxidant activity, and this concurs with the work of Duan *et al*., 2007. These plants show potentials as likely sources for the development of new drugs, and the discoveries from this study have revealed these plants to bepotent antioxidants. This property could be utilized in drug development, in the search of powerful antioxidants which are urgently needed to challenge free radicals in biological systems. It will consequently help to prevent ailments originating from free radicals. However, further study needs to be done to isolate and characterize the active principles in these plants.

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