In-vitro antioxidant activities of ocimum gratissimum, vitex doniana, carica papaya and peristrophe bicalyculata using DPPH free radical scavenging activity

Abstract

The ethanol extract 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-piricylhydrazyl (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 anti-oxidant activities 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, tannins, anthraquinones, terpenoids and carbohydrates in the medicinal plants.

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

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.1 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.2 Pharmacological functions such as anti-mutagenicity, anti-carcinogenicity, anti-aging etc are gotten from the pharmacological properties that originate from antioxidant activities.3,4 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-pircylhydrazyl radical scavenging activity assay has been widely used for antioxidant screening of fruit and vegetable juices or extracts.5 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 color 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;

\[ \text{DPPH} + (A-H) \rightarrow \text{DPPH} - H + A \]

Studies have shown that natural oxidants, especially the flavonoids, are beneficial to human health as they show a broad range of pharmacological properties such as anti-ischemic, anti-allergic, antibacterial, anti-viral, anti-inflammatory, vasodilatory and anti-proliferative activities.6 The antioxidant activities of several plant materials have been reported.4 6-11 Ocimum gratissimum popularly referred to as African basil in English, is an herbaceous perennial plant that has a woody stem, although its actual origin 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 ‘efihrin-nil’ by the Yorubas, ‘echu-anwu’ by the Igbohs, while in the northern part of Nigeria; the Hausas call it ‘daidoja’.12 The leaf has a characteristic pleasant aroma which is responsible for its name ‘Scent leaf’, and hence, its use as spice and condiments in cooking. The whole plant is used in folklore medicine and as insect repellant.13 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.14

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Received: September 24, 2018 | Published: November 09, 2018
Caricaceae is the family *Carica papaya* Linnaeus (paw paw) belongs to, and it is an herbaceous perennial plant with rapid proliferation rates. Papaya has a short life span, but has a fruitage time of 20 years. Papayas are hemaphrodites i.e. possess both male and female parts, thus, they are self-fertilizing, 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. Papayas are rich in self-defense compounds which confer a high level of immunity to attack by insects and to diseases. *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. In the Indore district of India, the locally called ‘Chotharjori’. 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. Furthermore, It is also used in the treatment of skin diseases, and serves as an antidote for metallic diseases such as diabetics, hypercholesterolemia among others.

**Materials and methods**

**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-Aldrich, U.S.A.

**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.

**Methods**

**Preparation of the extracts**

Samples of the leaf powder of each plant (50g each) were macerated with 50ml of ethanol for 72hrs 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 of the plants (Table 1). 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). The extracts were dissolved in 100µl of methanol and further diluted using methanol as standard to prepare solutions of 10, 50, and 100 µg/ml for determination of reducing potential and DPPH assay for antioxidant activity.

**Phytochemical screening**

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

**DPPH assay for antioxidant activity**

The ability of the extract to scavenge DPPH radical was determined according to Mansor et al.29 with little modification. 1.0ml of 0.3m M DPPH methanol solution was added to the solution of the extract or standard (250µg/ml, 2.5ml) and allowed to react at room temperature for 30mins. The absorbance of the resulting mixture was measured at 518nm 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 0.3m MDPPH 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.

\[
\text{AA} \% = \frac{\text{Rcontrol} - \text{Rsample}}{\text{Rcontrol}} \times 100
\]

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.

**Determination of Reducing Potential**

Reducing potential was determined according to the method of Afolabi et al. The extract or standard (100µg/ml or 250µg/ml respectively) was mixed with phosphate buffer and potassium ferricyanide. The mixture was incubated at 50°C 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.

**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).

**Results**

The phytochemical screening of the plants investigated revealed the presence of flavonoids, saponins, C. glycosides, steroids, alkaloids, tannins, anthraquinones, terpenoids and carbohydrates in the ethanol extracts of the plants (Table 1). 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 for *O. 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).
Table 1 Phytochemical Screening

| Phytochemicals   | O. gratissimum | V. doniana | C. papaya | P. bicalyculata |
|------------------|----------------|------------|-----------|-----------------|
| Flavonoids       | ++             | +++        | +++       | +++             |
| Saponins         | ++             | +          | ++        | ++              |
| C. glycosides    | ++             | +++        | +++       | +++             |
| Steroids         | ++             | ++         | ++        | ++              |
| Alkaloids        | +              | ++         | ++        | +               |
| Tanins           | +              | +          | +         | +               |
| Anthraquinone    | +              | +          | +         | +               |
| Terpenoids       | ++             | +          | ++        | +               |
| Carbohydrates    | ++             | ++         | ++        | ++              |
| Renins           | -              | -          | -         | -               |

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

Table 2 The antioxidant activities and reducing potentials of the investigated plants

| Antioxidant Activity (AOA) | IC50 (µg/ml) | Percentage Antioxidant Activity (%AA) | Reducing Potential (RP) |
|---------------------------|-------------|---------------------------------------|-------------------------|
| Control/blank (methanol)  | 0.56        | 2.5ml                                 | 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                 | 0.21        | 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).

Discussion/conclusion

Biologically active substances in plants such as flavonoids, terpenoids, 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. This study shows that the ethanol 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 potent medicinal and therapeutic effects, 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 that the 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 acid 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. 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 concur with the works of Sathiyanaranan and Arulmozhi, (2007); Edeoga et al., 2005; Miliusaska et al., 2004. O. gratissimum, C. papaya and 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 potent medicinal and therapeutic effects, and these findings are consistent with the previous works of Larson, 1988; Hudson, 1990; Hall and Cuppett, 1997.

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. These plants show potentials as likely sources for the development of new drugs, and the discoveries from this study have revealed these plants to be potent 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...
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Acknowledgments

None.

Conflict of interest

The authors declare that there is no conflict of interest.

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