

RESEARCH ARTICLE

Phytochemical and Antioxidant Potentials of Some Indigenous Vegetables Cultivated in Malete, Kwara State, Nigeria

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Abstract: Indigenous vegetables are important sources of natural antioxidant which play important role in prevention of degenerative diseases. In this study, five indigenous vegetables (*Ocimum basilicum*, *Celosia argentea*, *Amaranthus virides*, *Talinum triangulare* and *Corchorus olitorius*) cultivated in Malete, Nigeria were extracted in 95 % methanol and investigated for their phytochemical compositions and antioxidant activities using 2, 2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS^{•+}) and hydrogen peroxide radical scavenging assays. The comparative thin layer chromatography (TLC) of the extracts was carried out on silica gel coated plate developed in a mixture of organic solvents and visualized with the aid of ultraviolet visible light (uv) at 254 nm, ferric chloride solution and vanillin-H₂SO₄ spray reagent. The result of TLC analysis showed many visible components under uv light at 254 nm and after treated with spray reagents, indicating the presence of many important phytochemicals in the extracts of vegetables. The results of the phytochemical screening revealed the presence of various medicinally active compounds such as flavonoids, phenols, alkaloids, terpenoids, saponins etc. in various extracts of indigenous vegetables. Among the five indigenous vegetables tested, the *C.olitorius* and *O.basilicum* extracts exhibited the highest antioxidant potentials against DPPH, ABTS and Hydrogen peroxide. This finding provides insight into the bioactive components and antioxidant potentials of indigenous vegetables cultivated in Malete.

Keyword: phytochemicals, Antioxidant, Indigenous vegetables.

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Introduction

Vegetables are the fresh and edible parts of herbaceous plants. They are an essential foodstuff and extremely valuable for health maintenance and disease prevention. They comprise valuable foodstuff that can be effectively used for the development and repair of different parts of the body [1]. Plants based foods like fruits, vegetables and whole grains contain many components beneficial to human health [2].

Research supports that some of these foods, as part of an overall healthful diet, have the potential to delay the onset of many age-related diseases and these have further led to continued research aimed at identifying dietary bioactive components including antioxidants, which may be responsible for improving and maintaining health [3].

The awareness of consumption of indigenous vegetables is becoming increasingly popular for their health beneficial effects because they are sources of natural antioxidant that can protect human against oxidative stress and thus play an important role in the chemoprevention of diseases that have their aetiology and pathophysiology in reactive oxygen species [4].

Epidemiological studies have shown that a considerably higher amount of natural antioxidants in the human diet may protect us against various life threatening diseases, like hepatitis and aging, cancer [5]. The development of these disorders is mainly due to the presence of reactive oxygen species (ROS) in the human body [6]. Vegetables as medicinal plants contain no or less toxic

effects [7,8], and have the ability to synthesize several secondary metabolites of relatively complex structures with wild pharmacological activities like antimicrobial [9-11], antidiabetic [12], anti-histaminic[13], anti-carcinogenic[14] and hypolipidemic [15] and possess preventive or curative properties against cardiovascular disease, ageing, obesity, hypertension, insomnia, ageing[16-18], coronary heart disease, stroke, and cancers [19].

Leafy vegetables are natural source of antioxidants and rich in phytochemicals [20, 21] like phenolic acids and flavonoids [22] and micronutrients [23] which can be helpful in management of oxidative stress and age related human ailments [24]. The present work was therefore designed to investigate the phytochemical constituents and antioxidant potentials of five leafy vegetables namely *Ocimum basilicum*, *Celosia argentea*, *Amaranthus virides*, *Talinum triangulare* and *Corchorus olitorius* cultivated in Malete, Moro local government area, Kwara State, Nigeria.

Materials and Method

Materials

TLC was performed on aluminum baked plates, pre-coated with silica gel. Spots were detected at UV-254nm. Visualization was attained by spraying with ferric chloride (FeCl₃), Iodine vapour, (a mixture of silica gel with few iodine crystals) and 1% vanillin solution (prepared with 5% H₂SO₄ in Ethanol) followed by heating. The indigenous vegetables were sourced from Malete, Moro Local Government area, Kwara State, Nigeria.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS (2,2-azinobis(3-ethylbenzothiazoline-6-acid)), hydrogen peroxide, ascorbic acid Acetic acid, sodium hydroxide, n-Hexane, ethylacetate, tetraoxosulphate(VI) acid (H₂SO₄), methanol (CH₃OH), ferric chloride. All chemicals and reagents were of analytical reagent grade.

Method

Collection and Identification of Samples

The vegetables were collected from Malete environ, Kwara State, Nigeria and taxonomically identified and authenticated at the department of plant and environmental

biology, Kwara State University, Malete, Nigeria.

Preparation of Samples

The collected samples were washed under running tap water and cut into smaller pieces and dry at ambient temperature for seven (7) days. The dried samples were pulverized and then kept in a polyethylene bag until further uses.

Extraction of Samples

The air-dried pulverized leaves of *Corchorus olitorius*, *Ocimum basilicum*, *Celosia argentea*, *Amaranthus virides* and *Talinum triangulare* were separately extracted in 95 % methanol at ambient temperature for 72 hours by maceration method. The extracts were filtered and the filtrates were concentrated over a reduced pressure by rotary evaporator at 40 °C. The extracts were separately weighed and refrigerated at 4°C in dark sample bottle for consequent experiments. The percentage yields of the extracts were determined using the equation.

$$\text{percentage yield} = \frac{\text{weight of product}}{\text{weight of starting material}} \times 100\%$$

Qualitative Phytochemical Screening of the Extracts

Phytochemical analysis of methanolic leaves extracts of *C. olitorius*, *O. basilicum*, *C. argentea*, *A. virides* and *T. triangulare* was carried out by employing the standard procedures of [25], to reveal the presence of chemical constituents such as alkaloids, flavonoids, tannis, terpenes, saponins, phenols, steroids, carbohydrates and glycosides.

Test for Saponins

A 5.0 ml aliquot of the extract was diluted with 20 ml of deionized water, shaken vigorously and observed. Persistent foaming indicated the presence of saponins.

Test for Alkaloids

A known quantity of the extract, 0.1 mg was added to 6ml of dilute hydrochloric acid and boiled, after boiling; it was cooled and filtered. The filtrate was divided into three portions and subjected to the following tests. To the first portion, 2 drops of Dragendorff's reagent were added. The formation of reddish brown precipitate indicated the presence of

alkaloids. To the second portion, 2 drops of Meyer's reagent were added. A creamy white precipitate indicated the presence of alkaloids. To the third portion, 2 drops of Wagner's reagent were added. A reddish-brown precipitate indicated the presence of alkaloids.

Test for Tannins

The extract, 1 ml was added to 10 ml of deionized water and then treated with 3 drops of ferric chloride. A green or blue colouration indicates the presence of tannins.

Test for Flavonoids

Acid Test (Dilute H_2SO_4)

A 1.0 mL of aqueous extract was treated with few drops of dilute H_2SO_4 solution. Formation of intense yellow-orange colour was used as indication of the presence of flavonoids.

Shinodal Test (Mg-chips and conc. HCl)

To 1 ml aqueous extract, a few magnesium-chip was added followed by addition of few drops of conc. HCl, pink scarlet, crimson red or occasionally green to blue colour development indicated the appearance of flavonoid.

Test for Steroids and Triterpenoids

Liebermann Buchard Test

To 1 mL of aqueous acetone extract in a test tube, a few drops of acetic anhydride was added, boil and cool after which the concentrated H_2SO_4 was added from the side of the test tube, there was a formation of brown ring at the junction of the two layers and its upper layer which turned green and this can be taken as indication of presence of Steroids while the formation of deep red colour indicated the presence of triterpenoids.

Salkowski's Test

0.5g of aqueous extract was treated with 2 mL chloroform with few drops of concentrated H_2SO_4 . This was shaken and allows standing for 30 min.

The appearance of red colour in the lower layer indicated the presence of Steroids while the formation of yellow colour indicated the presence of triterpenoids.

Test for Phenols

Ferric Chloride Test: A 1.0 mL aqueous extract was treated with 3-4 drops of ferric chloride. Formation of bluish black colour indicated the presence of phenols.

Test for Glycosides:

Keller Killani Test (cardiac glycosides):

To 0.5g of aqueous extract in a test tube, 0.4ml glacial acetic acid containing a trace amount of ferric chloride was added followed by addition of 0.5 mL of concentrated H_2SO_4 along the sides of the test tube. The appearance of blue colouration in the acetic layer indicates the presence of cardiac glycosides.

Test for Reducing Sugar

Five milliliters (5ml) of a mixture of equal parts of Fehling's solution A and B was added to 5ml of extract and then heated in a water bath for 5 min. Brick red precipitate showed the presence of reducing sugar.

Thin Layer Chromatography (TLC) of the Extracts

Thin layer chromatography of the crude extracts was carried out to identify the unique distinguishing chemical constituents present in the extracts. The extracts were comparatively analyzed by applied a microgram quantity of each extract on TLC plate through a thin capillary glass tube and developed in n-hexane/ethyl acetate (8:2). Visualization of the chromatogram was attained under the UV lamp at 254nm, by stained in iodine vapour, spraying with ferric chloride ($FeCl_2$) solution and 1% vanillin- H_2SO_4 followed by heating.

Antioxidant Assays of the Methanol Crude Extracts

The antioxidant activities of the crude extracts were determined using three different methods: DPPH and ABTS and Hydrogen peroxide scavenging activity assays [26].

DPPH Radical Scavenging Activity

Free radical scavenging activity of the crude extracts was evaluated using DPPH assay according to the method of [26] with slight modifications. 0.004 g DPPH reagent was dissolved in 100 mL methanol.

Various concentration of Sample (ranging from 10-150µg/mL was prepared). In 96-well plates, 50 µL various concentrations of sample and 150 µL DPPH solution were added. The plate was incubated for 30 min at room temperature in the dark, and finally, absorbance was recorded at 595 nm wavelength.

Ascorbic acid at different concentrations ranging from 10 to 150 µg/mL was used as Standard. Methanol was used as Negative Control. Samples, Standards and Control were run in Triplicates. The degree of scavenging was calculated using the following equation:

$$\% \text{ DPPH Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

ABTS assay was carried out according to [26] with modifications. ABTS radicals were prepared freshly according to the original method. After 12-16 hr, ABTS•+ solution was diluted to an absorbance of 0.9 ± 0.02 at 745 nm. Various concentration of Sample (ranging from 10-150µg/mL was prepared). In 96-well plates, 50 µL of various concentration of sample was added followed by addition of 150 µL of ABTS radical solution. The plate was shaken for 10 seconds at medium speed,

and absorbance was measured at 745 nm following incubation in the dark for 5 min. Ascorbic acid at different concentrations ranging from 10 to 150 µg/mL was used as Standard. Methanol was used as Negative Control. Samples, Standards and Control were run in Triplicates.

The degree of scavenging was calculated by the following equation:

$$\% \text{ ABTS scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

Determination of Hydrogen Peroxide Scavenging Activity

The assay was performed according to the method of [27] with modifications. 100 µL of Hydrogen peroxide (40 mM) prepared in phosphate buffer (pH 7.4) was added to 100 µL of extract and its fractions at a concentration of 100 µg/mL in a Quartz microplate and mixed. The initial absorbance

was recorded at 230 nm and the mixture was incubated for 10 mins.

The absorbance was read again at 230 nm after 10 mins. Ascorbic acid at concentration 100 µg/mL was used as Standard. Phosphate Buffer was used as Negative Control. Samples, Standards and Control were run in Triplicates. The degree of scavenging was calculated by the following equation:

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Absorbance of control

Results and Discussion

Results

Results of Extraction and Percentage Yield of Methanolic Vegetable Extracts

Table 1: showed the results of extraction and percentage yield of the extracts

s/n	Name	Code	Weight of Pulverized Sample (g)	Weight of Crude Extract (g)	Colour	Percentage Yield (%)
1	<i>Corchorus olitorius</i>	MECO	37.11	7.4	Green	19.9
2	<i>Talinum triangulare</i>	METT	20.66	6.0	Green	29
3	<i>Amaranthus viride</i>	MEAV	51.39	5.8	Green	11.2
4	<i>Celosia argente</i>	MECA	24.47	6.7	Green	27.3
5	<i>Ocimum basilicum</i>	MEOB	47.84	19.2	Green	40.1

Key: MECO = methanol extract of *Corchorus olitorius*, METT = Methanol extract of *Talinum triangulare*, MEAV = methanol extract of *Amaranthus viride*, MECA = methanol extract of *Celosia argente*, and MEOB = methanol extract of *Ocimum basilicum*

Results of Phytochemical Analysis of the Crude Extracts of Indigenous Vegetables

Table 2: showed the results of phytochemical screening for the crude extracts of various indigenous vegetables.

s/n	Phytochemicals	CRUDE EXTRACTS				
		MECO	METT	MEAV	MECA	MEOB
1.	Flavonoids	+	+	+	+	+
2.	Alkaloids	+	+	+	-	+
3.	Terpenoids	+	+	+	-	+
4.	Tannins	+	+	+	+	+
5.	Saponins	+	+	-	+	+
6.	Carbohydrate	+	+	+	+	+
7.	Phenols	+	+	+	+	+
8.	Steroids	+	+	+	+	+
9.	Glycosides	+	-	+	+	+

Key: + = present, - = absent

Result of Thin Layer Chromatography (TLC) Analysis of Crude Extracts



Figure 1: Comparative TLC of extracts (a) after treated with iodine vapour, (b) after treatment with vanillin-H₂SO₄ at 70 °C, (c) after treatment with ferric chloride solution

Results of Antioxidant Potentials of the Methanol extracts of Different Indigenous Vegetables

Table 3: Results of antioxidant activity of the crude extracts of indigenous vegetables

S/N	SAMPLE	DPPH (IC ₅₀ µg/mL)	ABTS (IC ₅₀ µg/mL)	Hydrogen peroxide (IC ₅₀ µg/mL)
1	MECO	2.23 ± 0.002	2.50 ± 0.022	0.07 ± 0.001
2	METT	3.85 ± 0.021	5.1 ± 0.01	1.3 ± 0.011
3	MEAV	3.85 ± 0.021	4.21 ± 0.031	0.82 ± 0.21
4	MECA	4.8 ± 0.001	6.5 ± 0.011	0.57 ± 0.013
5	MEOB	2.35 ± 0.021	1.52 ± 0.001	0.13 ± 0.012
	VITAMIN C	2.16 ± 0.001	3.50 ± 0.13	0.12 ± 0.012

Discussion

The results of phytochemical screening (Table 1) revealed the presence of medicinally active constituents in the leaves extracts of indigenous vegetables. The presence of flavonoids, terpenoids, steroids, alkaloids, tannins, carbohydrates, phenols, saponins and glycosides were confirmed in the extracts of *Corchorus olitorius* and *Ocimum basilicum*. The absence of glycosides was determined in *Talinum triangulare* extract (METT); this is coincided with the findings of

[28]. In methanol extract of *Amaranthus viride* (MEAV), saponins were absent, this in agreement with the finding of [29]. More so, the presence of alkaloids and terpenoids were absent in the extract of *Celosia argente* extract (MECA). The comparative thin layer chromatographic analyses of the extracts (figure 1) revealed that *C. olitorius* (MECO) showed the presence of 8 components when treated with vanillin-H₂SO₄ reagent at 70 °C and some of these components gave positive reaction with ferric chloride solution and iodine vapour indicating the presence of

phenolic compounds in this extract. The chromatogram of *Ocimum basilicum* revealed the presence of 7 prominent components when treated with vanillin-H₂SO₄ reagent at 70 °C and also showed positive reaction with iodine vapour and ferric chloride test. This is an indication that, *O. basilicum* extract is rich in phenolic compounds and this corroborates the result of phytochemical screening in Table 1. Some of the components observed in *O.basilicum* are similar to those of *C.olitorius* extract (MECO). The chromatogram obtained from vanillin sprayed of *Amaranthus viride* (MEAV), *Talinum triangulare* and *Celosia argente* showed few components in which some of these components disappeared when treated with iodine vapour and ferric chloride solution, although their origins showed positive reaction to these reagents indicating that the phenolic components in these extracts are needed to be separated using polar solvent systems as mobile phase with reverse phase silica or sephadex as stationary phase. The result of antioxidant assay (Table 3) of the crude methanol extracts of indigenous vegetables demonstrated excellent antioxidant activities against DPPH, ABTS and Hydrogen peroxide. *C.olitorius* extract (MECO) demonstrated the highest antioxidant activity in which its IC₅₀ value statistically comparable to *O. basilicum* extract and standard drug, vitamin C. Therefore, *C.olitorius* and *O.basilicum* exhibited excellent antioxidant activities against DPPH, ABTS and Hydrogen peroxide than other extracts. Other crude extracts (METT, MEAV and MECA) showed good DPPH, ABTS and hydrogen peroxide scavenging activities as shown in Table 3 which are statistically similar to those of vitamin C. Therefore, the strong antioxidant activity of methanol extract of these leafy indigenous vegetables could be traced to the presence of different phytochemicals such as flavonoids, phenols, terpenoids glycosides, alkaloids, saponnins and this suggests that, the vegetables can be considered as viable sources of natural antioxidant and might be exploited for nutraceuticals uses.

Conclusion

The present study validates the antioxidant potentials of some leafy indigenous vegetables cultivated in Malete area and their phytochemical constituents such as

(flavonoids, steroids, terpenoids, saponnins, alkaloids, phenols and glycosides) were also determined. The presence of these phytochemicals in some indigenous leafy vegetables establishes their potential sources of natural therapeutic agents which can be used against different diseases. From the results of this finding, it is therefore recommended that the vegetables cultivated in Malete area should research further for the isolation and identification of bioactive compounds responsible for their antioxidant activities.

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