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RESEARCH ARTICLE

QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND TOTAL FLAVONOID CONTENTS OF ETHYLACETATE FRACTION OF CHIKADOMA AS A BACTERICIDAL AGENT

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ABSTRACT

Background: Total Phenolic and total flavonoid contents are among or perhaps, the most important indices for the evaluation of antioxidant activity of herbal remedies. Chikadoma is commonly employed in traditional Nigerian medicine. **Objectives:** To estimate the total phenolic and total flavonoid contents (TPC and TFC) of Chikadoma as a bactericidal agent. **Materials and Methods**: The leaves of Chikadoma were screened for the estimation of the total phenolics and flavonoids content using Folin-Ciocalteu and Aluminiumchloride colorimetric assays respectively. Gallic acid was employed as internal standard for total phenolics. Quercetin was used as internal standard for total flavonoids. **Results**: The total phenolic content for ethyl acetate fraction of Chikadoma showed significant value of 48 ± *1.5* mg gallic acid equivalent (GAE) /g of extract; while the total flavonoid content for ethyl acetate fraction of Chikadoma showed significant value of 140 ± *2.0* mg quercetin equivalent per gram (QE/g) of extract. **Conclusion:** correlation between TPC and TFC of Chikadoma had strong positive significance as a bactericidal agent.

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INTRODUCTION

The preponderance of antioxidants such as phenolics, flavonoids, proanthocyanidins and tannins in plants may provide protection against a number of health disorders. Therefore, the demand for natural antioxidants and food preservatives is increasing such that medicinal plants are being investigated for their antioxidant properties (Peschel, 2006). Natural antioxidants have the ability to scavenge free radical (Saeed, 2012). The development of a number of disorders have implicated free radicals. These disorders include neurodegeneration (Halliwell, 2006), cancer (Halliwell, 2007), and inflammation (Ferguson, 2010) giving rise to studies of antioxidants for the prevention and treatment or diseases. Chikadoma (Ohadoma, 2019) is the common Nigerian name for *Lupinusarboreus;* a bushy shrub with bright yellow flowers blended with white and purple colours. It belongs to the family of Fabaceae.

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Other common names of Chikadoma are "yellow bush" (U.K) and "coastal bush" (Northern California, USA) (Pliny, 2009; Rickart, 1998). Despite the fact that this plant is widely used in Nigerian traditional medicine, not substantial scientific studies have been conducted save the extensively pioneer novelty investigations of the pharmacological utility by a lead researcher Dr. Chika Ohadoma from whom it derived its name[(9)]. Phenolic and flavonoid compounds remain widespread in plant kingdom where they serve as antioxidants and free radical scavengers (Geetha, 2016). In our previous studies we reported the antimicrobial activity (Ohadoma, 2014), and free radical scavenger effects of this plant (Ohadoma *et al*., 2018), and molecular potentials of Chikadoma as a bactericidal agent (Ohadoma, 2019) in which the ethylacetate fraction was implicated for this activity. The objective of this present study was to determine the total phenolic and flavonoid contents of the ethylacetate fraction of Chikadoma as a bactericidal agent.

Materials and methods

Plant material and preparation extract and fractions: The plant materials, extract preparation, preliminary phytochemical constituents and determination of the acute toxicity are in

accordance with earlier documented report (Ohadoma, 2010). Briefly, fresh leaves of Chikadoma were collected from Owerri, Nigeria and authenticated in the Department of Pharmacognosy, Madonna University Elele, Nigeria by Dr. Osuala, F. N. and voucher specimen was deposited at the Herbarium. At room temperature, the leaves were air-dried for 28 days and pulverized.

The powdered leaf (2kg) using absolute methanol (sigma Aldrich, Germany) was extracted by cold maceration for 18h. The methanol extract was obtained by filtering the mixture and subsequently evaporated using a rotary evaporator (RV 05 Basic IB, IKA, Staufen, Germany). The concentrated methanol extract was stored in a refrigerator. The ethyl acetate fraction was obtained using column chromatography (Ohadoma, 2014).

Total phenolic content: The determination of the total phenolic content of ethyl acetate fraction of Chikadoma was carried out using Folin-ciocalteu method (Baba, 2015). In brief, 200 µL of the fraction (1 mg/ml) were made up to 3 mL with distilled water. For 3 min, it was mixed thoroughly with Folin-ciocalteu (0.5 mL), followed by the addition of Sodium carbonate (2 mL of 20% w/v). The mixture was further allowed to stand for 60 min in the dark before absorbance was measured at 650 nm. The total phenolic content was calculated from the calibration. The results were expressed as mg of gallic acid equivalent per g weight of extract.

Total flavonoid content: The total flavonoid content of ethyl acetate fraction of Chikadoma was done using Aluminiumchloride colorimetric method [16]. Briefly, an aliquot (1ml) of quercetin solution was added to 10 ml volumetric flask containing 4 ml of 5% $NaNO_2$. After 5 min, 0.3 ml of 10% $AlCl_3$ was introduced, followed by 2 ml of 1 MNaOH and the total volume was made up to 10 ml with distilled water.

For the test solution the same dilutions were also prepared, using methanol, blank determination was done in place of test or standard solutions. The absorbance at 358 nm was taken against the blank. The total flavonoid content was calculated from calibration curve of quercetin. The result was expressed as mg of quercetin equivalent per g weight extract.

Statistical analysis: With GraphPadprism 6 software (GraphPad Software, Inc. U.S.A), the statistical analysis was carried out. The results are expressed as means ± standard deviation.

RESULTS AND DISCUSSION

The total phenolic content of the ethyl acetate fraction of Chikadoma, calculated from the calibration curve ($R^2 = 0.998$), was 48 ± 1.5 mg gallic acid equivalent/g; while the total flavonoid content ($R^2 = 0.999$) of the ethyl acetate fraction was 140 ± 2.0 mg of quercetin equivalent/g (Table 1). The quantities of phenolic and flavonoid contents found in the ethyl acetate fraction of Chikadoma are stupendous.Phenolics act as antioxidants because they have redox properties [17]. The total phenolic concentration may be employed as a basis for rapid determination of antioxidant activity as their free radical scavenging ability is facilitated by their hydroxyl groups. This is facilitated by their hydroxyl groups.

Table 1. Total phenolic and flavonoid contents of ethyl acetate fraction of Chikadoma leaves

Total Phenolics content [x]	48 ± 1.5
Total Flavonoids content [y]	140 ± 2.0

Where x = mg gallic acid equivalent (GAE)/g of fraction
y = mg quercetin equivalent (QE)/g of fraction
The values are means of three biological replications.

This is in agreement with an earlier report. The antimicrobial activity of Chikadoma as a bactericidal agent is not unconnected with the total phenolic and flavonoid contents therefore, justifying a documented study that the antimicrobial and antioxidant activities of extract are positively associated with the total phenolics and flavonoid contents of the extracts (Baba, 2015). Enzyme inhibition through reaction with sulfhydryl groups by oxidized compounds and/or via more non-specific interactions with the protein are the mechanisms of actions thought to be implicated for phenolic toxicity to micro-organisms [13]. Plant flavonoids are known to have antioxidant activity both in-vitro and in-vivo [18, 19]. In plants, the presence of antioxidants (phenolics, flavonoids, proanthocyanidins and tannins) may avail protection against a variety of diseases. To buttress this, ingestion of natural antioxidants have been inversely related to morbidity and mortality from degenerative disorders (Gulcin, 2012). The antioxidant activity of flavonoids (flavones, flavanols and condensed tannins) which are plant secondary metabolites depends on the presence of free OH groups, especially 3-OH (Shimoi, 1996). As hydroxylatedphenolics, flavonoids occur as C_6-C_3 unit linked to an aromatic ring; and are extremely effective scavengers of most oxidizing molecules such as singlet oxygen implicated in several disease conditions (Bravo, 1998). Hence, it should not be absurd that they have been found to be effective antimicrobial agents against a large group of microorganisms. The mechanism of action of flavonoids may be due to their ability to complex with bacterial cell walls where most bactericidal agents such as β-lactams belong and to complex with extra cellular and soluble proteins (Ohadoma, 2019); suppression of reactive oxygen formation, scavenge reactive species, chelate trace elements involved in free-radical production, up regulate and protect antioxidant defenses (Agati *et al*., 2012).

Conclusion

Our findings suggest that Chikadoma is a potential source of antioxidants and agree with the reported molecular potential as a bactericidal agent hence may serve as a lead in the search of natural bactericidals.

Conflicts of interest: No conflict of interests have been declared by the authors.

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Contributions

OSC Conceived and designed the experiments
AGC and ALU performed the experiments.
OSC and MHU analyzed the data
OSC, AGC and ALU wrote the paper
OSC supervised the project.

All authors read and approved the paper.

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