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Quantification of the Total Phenolic and Flavonoid Contents, and Antibacterial Activity of *Dioclea Reflexa*

Edewor TI*[1], Akpor OB[2], Owa SO[2]

[1]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria.
[2]Department of Biological Sciences, Landmark University, Omu aran, Nigeria.

*Corres.author: ibitheresa@yahoo.com, Phone: +234-8037272625

Abstract: Phytochemical screening of the extracts revealed presence of terpenes only in the n-hexane extract while flavonoids, steroids and terpenoids were present in the methanolic extract. Alkaloids, saponins and anthraquinone glycosides were absent in all the extracts. The methanolic extract was active against *K. pneumoniae* and *P aeruginosa*, and inactive against *E. coli, P. vulgaris* and *S. aureus.* The total phenolic content obtained was 41.58 ± 0.15 mg Gallic acid equivalent/ g extract while the total flavonoid content was 29.82 ± 0.31 quercetin equivalent/ g extract.
Key words: *Dioclea reflexa*, phytochemical, antibacterial, phenolic, flavonoid

Introduction

For years some plants have been used traditionally for the treatment of sicknesses and diseases. These plants known as medicinal plants have been shown to possess chemical components that exhibit therapeutic properties. There is a growing interest in the study of medicinal plants that possess antioxidant property. These natural antioxidants have been shown to be effective in the inhibition of oxidation of food, reduction of age related diseases[1, 2] and have the ability to increase the antioxidant capacity of the plasma thereby reducing the risk of contracting cancer and heart related diseases[3]. These antioxidants act as free radical scavengers that can remove free radicals and reactive oxygen species that are formed during normal physiological processes thereby preventing cell injury[4, 5].

Dioclea reflexa is a woody climber. It is found in Nigeria and other West African countries such as Senegal. The plant seed is known for its flavor and sweet aroma. Traditionally, it is used for the treatment of pains and respiratory tract infections. Most research work has been on the plant seed[6-8]. Ogundare and Olorunfemi (2007) reported the antimicrobial efficacy of the leaves[9]. The aim of this study was to determine the phytochemicals present, evaluate the total phenolic, flavonoid contents and to examine the antibacterial activity of the methanolic leaf extract of *Dioclea reflexa*.

Materials and Methods

Gallic acid and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethanol, methanol, hexane, sodium hydroxide, sodium carbonate, aluminum hydroxide, Folin-Ciocalteau reagent and sodium nitrite were obtained from E. Merck (Darmstadt, Germany).

Genesys 10S vl. 200 2l7H311008 spectrophotometer was used for absorbance measurements.

Plant material and extraction

Fresh plant leaves were collected from Kaba, Kogi State, Nigeria. These were taken to the Department of Biological Sciences Landmark University for identification. The leaves were pulverized into fine powder and stored in a container. 500 g of the pulverized leaves was extracted using a soxhlet extractor using solvents of different polarities (n-hexane and methanol). The extracts were concentrated under reduced pressure to give the crude extract.

Phytochemical screening

The n-hexane and methanolic extracts were examined for presence or absence of phytochemical components using the method described by Tona *et al*., 1998[10]. Precoated Merck silica gel TLC plates were spotted with each extract and developed using n-butanol-acetic acid-water (4:1:5) solvent mixture. The spots were then visualized with a solution of 1% $AlCl_3$ in methanol and viewed under the UV-lamp at 366 nm. For the detection of steroids and terpenes in the extracts, the solvent mixture n-hexane:dichloromethane in the ratio 1:9 was used to develop the chromatogram while the spots were identified using Libermann-Burchard reagent after heating the plates for 10 mins. at 100^{o}C. The method described by Harborne, 1981[11] was used for detection of tannins using $FeCl_3$ while Dragendorf"s reagent was used for the identification of alkaloids. Frothing was used to test for presence of saponins while Molisch"s reagent was used to identify presence of glycosides.

Antibacterial assay

Test organisms

Pure cultures of *E. coli, K. pneumoniae, S. aureus, P. vulgaris* and *P. aeruginosa* were used for the antibacterial assay.

Preparation of the medium

Nutrient agar medium was prepared by dissolving 28 g of nutrient agar in 1000ml of distilled water. The medium was sterilized in an autoclave at 121^{o}C for 15 minutes. It was cooled to 45^{o}C and poured into sterile petri dishes to solidify. 5 mm sized cork borer was used to bore the solidified plates which were used for the antibacterial assay.

Preparation of test samples

10 mg each of the extracts were dissolved in 10 ml of each redistilled solvent (n-hexane, methanol). The activity of Streptomycin, a standard antibiotic was also determined used as the positive control; 10.0 mg of it was dissolved in 10 ml of distill water. Distill water and redistilled solvents were used as negative control.

Method

Disc diffusion method of Bauer-Kirby *et al.*, (1966) was employed[12]. This involved the use of filter paper disc as carrier for the antibacterial agents. Sterilized discs cut from Whatman no. 1 filter paper were impregnated with solutions of the antibacterial agents at different concentrations (1.0 mg/ml, 2.0 mg/ml). The solvent was evaporated and the disc dried properly. The nutrient agar medium was inoculated with the test organism and the impregnated disc placed on the surface of the nutrient agar. The antibacterial agent upon contact with the agar diffused into all directions. The ability of the test organism to grow or not in the presence of the test sample was then determined within 24 hours by measuring the zones of inhibition. The plates were incubated upside down at 37^{O}C. Redistilled solvents were used as control. All tests were done in quadruplicate and the antibacterial activity was expressed as a mean of inhibition diameters (mm) produced by the leaf extract and the streptomycin used as the standard.

Total flavonoid content

Aluminium colorimetric method was used to determine the total flavonoid content of *Dioclea reflexa*. 5 mg of the extract was weighed and dissolved with 5ml of 50% methanol and to 1 ml of this solution was added 0.7 ml of 5% (w/w) $NaNO_2$ and 10 ml of 30% (v/v) ethanol. This mixture was stirred for 5 mins and 0.7 ml of 10% $AlCl_3$ (w/w) added to it and stirred for 6 mins. 5 ml of 1mol/l NaOH was added to the mixture and diluted to 25ml with 30% (v/v) ethanol. The mixture was allowed to stand for 10 mins and the absorbance measured at 500 nm using a uv spectrophotometer. Quercetin was used as the standard and the total flavonoid content was expressed as quercetin equivalent in mg/g extract[13].

Total phenolic content

The total phenolic content of the methanolic extract was determined using the Folin-Ciocalteau method. 5 mg of the extract was weighed and dissolved with 5ml of 50% methanol using a vortex mixer. 0.5 ml of this solution was pipette into a test tube and 3.5 ml of distilled water, 0.25 ml Folin-Ciocalteau reagent added to it. It was left to incubate for 8 mins at room temperature. Then 1 ml of 20% Na_2CO_3 was added and left to incubate for 2 hrs. The absorbance was measured at a wavelength of 765 nm against a reagent blank using a UV spectrophotometer. Gallic acid was used as the standard and the total phenolic content of the extract expressed in mg Gallic acid equivalents/g extract[14].

Results

Table 1: Phytochemical screening of crude extracts of *Dioclea reflexa* leaves

extracts	Anthraquinone glycosides	tannins	flavonoids	saponins	steroids	alkaloids	Terpenoids
n-hexane	-	-	-	-	-	-	-
methanol	-	-	+	-	+	-	+

Table 2: Antibacterial analysis of the crude extracts of *Dioclea reflexa* leaves

Extracts	Zones of inhibition (mm)				
	E. coli	K. pneumoniae	aureus	P. vulgaris	P aeruginosa
n-hexane	-	-	-	-	-
methanol	-	12	-	-	20

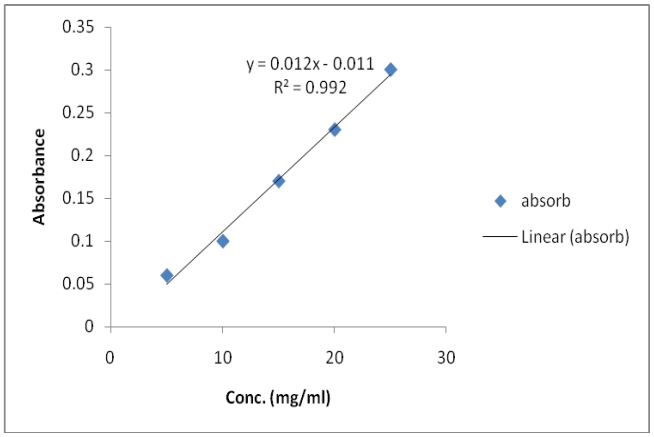

Fig. 1: standard curve for quercetin

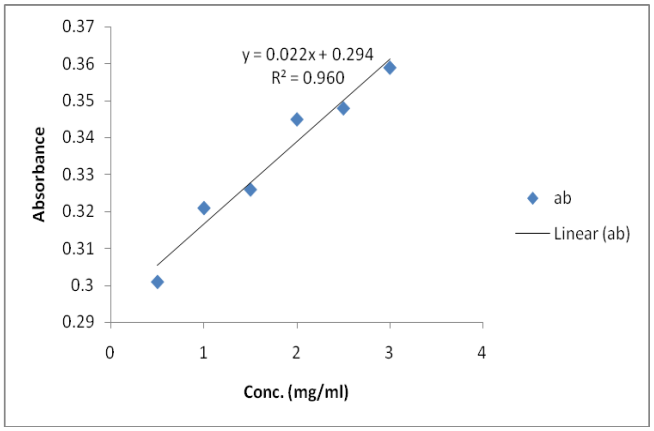

Fig. 2: Standard curve for Gallic acid

Table 3: Total phenolic and flavonoid contents of the methanolic leaf extract of *Dioclea reflexa*

Plant part	Total Phenolic content (mg Gallic acid equivalent/g extract)	Total Flavonoid content (mg quercetin equivalent/g extract)
leaves	41.58 ± 0.15	29.82 ± 0.31

Phytochemical screening

The result of the phytochemical screening revealed presence of flavonoids, steroids and terpenoids and absence of alkaloid, saponins, tannins and anthraquinone glycosides in the methanolic extract while none was found in the n-hexane extract (Table 1).

Antibacterial assay

The n-hexane extract did not exhibit any antibacterial activity while the methanolic extract was active against *K. pneumoniae* and *P. aeruginosa* (Table 2).

Total phenolic and flavonoid contents

The spectroscopic determination of total phenolic content from the methanolic leaf extract was carried out using Folin-Ciocalteau reagent and linear Gallic acid standard curve ($y = 0.023x + 0.294$, $R^2 = 0.960$. The flavonoid content was determined using the aluminium colorimetric assay. Quercetin was used as the standard with a standard curve ($y = 0.012x - 0.011$, $R^2 = 0.992$). The total phenolic content obtained was 41. 58 ± 0.15 mg GAE/g extract while the total flavonoid content obtained in this study was 29.82 ± 0.23 mg QRT/ g extract (Table 3).

Discussion

Researchers have discovered that plants are reservoirs of chemical compounds some of which have shown chemotherapeutic properties. These properties have aided their increased demand in the pharmaceutical and cosmetic industries. The phytochemicals identified in the methanolic leaf extract of *Dioclea reflexa* were flavonoids, steroids and terpenoids. These phytochemicals have been repeatedly shown to possess antimicrobial, antiviral, antifungal and other pharmacological properties[16]. This result is in contrast to the findings of Ogundare and Olorunfemi (2007)[17] who showed that the methanolic extract of the plant leaves contain alkaloids, tannins, glycosides and phenols. This might be as a result of both climatic, environmental and soil factors; since their plant material was collected from the western part of Nigeria while ours was collect from the north central part of Nigeria. This also resulted in the discrepancy in the antibacterial activity of the methanolic leaf extract. While ours was active against *Kliebseilla pneumoniae* and *Pseudomonas aeruginosa* and inactive against *E. coli, Staphylococcus aureus* and *Proteus vulgaris*; theirs was active against *Staphylococcus aureus, Streptococcus pneumoniae, Kliebsella pneumoniae, Samonella typii, Proteus mirabilis* and *E. coli*, and inactive against *Pseudomonas aeruginosa*. Comparing the antibacterial activity of the methanoilc leaf extract to that of streptomycin, the leaf extract was observed to be more active against *Kliebseilla pneumoniae* and *Pseudomonas aeruginosa* than the standard antibiotic used (streptomycin). The plant methanolic leaf extract was shown a have a reasonable quantity of phenolics and flavonoids. The Phenolics and flavonoids are very important plant phytochemicals that has several hydroxyl groups. These hydroxyl groups have been shown to be responsible for these chemicals radical scavenging ability. This ability makes them to act as antioxidants. They have been found to show inhibitory effects on inflammation, allergies, mutagenesis and carcinogenesis in humans[18-25], prevent the decomposition of hydroperoxides into free radicals and slow down the rate of conjugated diene formation, retard oxidative degradation of lipids thereby improve the nutritional value and quality of food[26-28]. However, it was noted that synthetic antioxidants possess higher activity than the natural antioxidants[27]. In the Folin- Ciocalteau assay, the molybdotungstate present in the reagent oxidizes the phenolics to yield a coloured product which absorbs around 745-750 nm. This method is not specific to polyphenols alone but other hydroxyl compounds present in the extract may cause interference by reacting with the reagent thereby giving a false total phenolic content of the extract. Therefore, the antioxidant activity of the extract may be a combination of effects of polyphenolics and other antioxidant compounds present in the extract[29].

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