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Phytochemical Analysis and Antibacterial Activities of Spinach Leaf

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Abstract

The nutritional and medicinal benefits of vegetables provide a better support for human wellbeing. There are several edible vegetables which are used in day to day kitchen in different forms. The usages of green leafy vegetables are limited to a specific geographical location. The study screened for phytochemicals and determined antibacterial activities of *Spinacia oleracea* which is one of the most important vegetable used in Nigeria. The presence of phytochemicals including phitobatamin, saponins, phenol, tannins, glycosides, flavonoids, steroids, terpenes and cardenolides were determined in the *Spinacia oleracea*. The extract of *Spinacia oleracea* exhibits the presence of phitobatamin in aqueous extract but absent in ethanol and ethyl acetate crude extracts. The aqueous extract and ethyl acetate crude extract s exhibits presence of saponin but absent in ethanol crude extract. Phenol present in ethanol crude extract but absent in aqueous extract and ethyl acetate crude extract, also flavonoids present in aqueous extract and ethyl acetate crude extract but absent in ethanol crude extract, tannin present in ethanol and ethyl acetate crude extracts but absent in aqueous extract, terpenes present in aqueous extract but absent in ethanol and ethyl acetate crude extracts, steroids present in ethanol crude extract but absent in aqueous extract and ethyl acetate crude extract, glycoside present in ethanol crude extract but absent in aqueous extract and ethyl acetate crude extract and the extract exhibits the presence of cardenolides in ethyl acetate crude and aqueous extract. The antibacterial activities of aqueous extract, ethanol and ethyl acetate crude extracts of the leaves (*Spinach oleracea*) were evaluated in the present study by analysing the antibacterial activity of this extract. The presence of these bioactive constituents is associated with the antibacterial activity of the plant. The study revealed the absent of antibacterial activity in both ethanol crude extract and aqueous extract (100%, 50% and 25%) against selected bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) but found effective in ethyl acetate crude extract against tested bacteria which revealed the high effective of the extract at 100% concentration but was not able to inhibit the growth of the tested bacteria at 50% and 25% concentration. It was concluded that the extracts of *Spinacia oleracea* consists of important constituents for pharmacological activities.

Keywords: *Spinacia oleracea*; Phytochemical steroids; Antibacterial activity

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Introduction

Spinach (*Spinacia oleracea*) is an edible flowering plant in the family *Amaranthaceae*. This plant grows to a height of up to 30 cm. Spinach may survive over winter in temperate regions. The leaves are alternate, simple, and ovateto triangular - based, varying in size from about 2-30 cm long and 1-15 cm broad. The larger leaves are at the base of the plant and small leaves higher on the flowering stem. The flowers are inconspicuous, yellow - green, 3-4 mm in diameter, maturing into a small, hard, dry, lumpy fruit cluster about 5-10 mm across containing several seeds. Spinach has a high nutritional value. It is a rich source of

vitamin A, vitamin C, vitamin K, magnesium, manganese, folate and iron. The biochemical components present in green leafy vegetables are of great pharmacological or medicinal importance. The phytonutrients present in green leafy vegetable gives many common health benefits such as protection from eye disorders, oxidative stress, iron deficiency etc., Consumption of green leafy food is good for human health as it improves nutritional status and reducing risks of specific diseases like diabetes, cancer and hepatotoxicity. The present study determines availability of phytochemicals in vegetable and their pharmacological benefits. *Spinacia oleracea* is used since ancient periods as food source. It contains many nutrients and minerals which are helpful in maintaining human health. Especially in developing countries the nutrition and health of increasing world population is a major upcoming challenge. Plant food acts as sources of energy, supplying nutrients and micronutrients essential for health. This also includes added health benefits like antioxidant activity [1]. Several studies on the chemical composition of leafy vegetables have shown that, they contain enormous amount of micronutrients, several agronomic advantages and economic value. They also contain some chemical compounds that are having important medicinal uses, human well-being and healthy lifestyle. Some of the vegetables are also reported to cure more than one health problem. The medicinal values of vegetables and fruits are believed to be dictated by their phytochemical and other chemical constituents [2]. Fruits and vegetables are important sources of phytochemicals and it is studied that some antinutritional content of these vegetables have exhibited potential for reducing the risk of certain diseases in human beings [3]. These diseases include high blood pressure, heart attack, stroke, and other cardiovascular diseases [4]. These antinutrients or phytochemicals carry out their healing activities by combining with vitamins or with other nutrients [5]. Information is however scanty on the nutritional and phytochemical contents of the leafy vegetables [6]. Phytochemicals are naturally occurring components in fruits, vegetables, legumes and grains. Plants are getting specific colour, flavour, smell and are part of plant's natural defense system i.e., disease resistance. Phytochemicals are bioactive, non-nutrient plant compounds in fruits, vegetables, grains and other plant foods that have been linked with reducing the risk of major degenerative diseases [7]. Thus there is a need to evaluate the potential of local vegetables in relation to the provision of basic nutrients and phytochemicals, which will help in providing vital data for food processors, nutritionist, dieticians, as well as the consumers for the selection of proper green leafy vegetables. Medicinal plants are used locally in the treatment of infections caused by fungi, bacteria, viruses and parasites [8,9]. Many people in Nigeria rural areas depend on the traditional medicine for the treatment of their ailments and since prehistoric times, various parts of plants has been used in the treatment and prevention of various diseases [10]. Different plants have been used as a source of inspiration in the development of novel drugs either in a pure compound form or their extract form and it provides unlimited opportunities to develop a variety of new drugs. Plant-derived medicines are widely used because they are relatively safer than the synthetic alternatives. Antibiotic resistance has become a global concern and is being threatened by emergence of multidrug resistance-pathogens [11,12]. Therefore, increase in failure due to chemotherapeutics and antibiotic resistance leads to screening of several medicinal plants for their antimicrobial effect [13]. Relevant experimental work on the phytochemical and antimicrobial activity of the plant has not yet been explored. Therefore, the study is to evaluate the phytochemical and antibacterial activity of the plant extract of *Spinacia oleracea*.

Methodology

Collection of plant

The plants (*Spinacia oleracea*) were collected from Faculty of Agricultural, LAUTECH Ogbomoso, Oyo State, Nigeria. The plants were collected with plastic zip lock bags and brought to the laboratory which thereafter washed thrice with tap water to remove any debris and then rinsed with distilled water as well shed dried and used for the extraction.

Preparation of plant extracts

The collected plant samples were dried and crushed to powder form. Five (5 g) of powdered plant sample was soaked with 50 ml of water, ethanol, and ethyl acetate separately. The entire mixture was incubated at 4°C for 48 hr. After the incubation period was over, the mixture was filtered and centrifuged at 10,000 rpm at 4°C. The extracts were concentrated to dryness in rotary evaporator (IKA-RV 10 Control) and were stored at 4°C until further used.

Phytochemicals analysis

Phytochemical analysis of the test sample was carried out according to standard methods [14].

Test for saponin: About 5 ml of the extract was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filterate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion which confirms a positive presence of Saponin.

Test for taninns: 1 ml of extract was boiled in 20 ml of water in a test and then filtered. A few drops of 0.1% ferric chloride was added and observed green or a blue-black coloration which confirms the presence of tannin.

Phenol 5 ml of the extract was pipetted into a 30 ml test tube, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added and left to react for 30 min. Development of bluish green colour was taken as a positive presence of phenol.

Test of flavonoids: 3 ml of 1% Aluminium chloride solution were added to 5 ml of each extract. A yellow coloration was observed indicating the presence of flavonoids. 5 ml of dilute ammonia solution were added to the above mixture followed by addition of concentrated H_2SO_4. A yellow coloration disappeared on standing. The yellow coloration which disappeared on standing indicates a positive test for flavonoids.

Test for cardiac glycosides and cardenolides (Keller-killani test): 5 ml of each extracts was treated with 2 ml of glacial acetic

acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates a deoxysugar characteristics of cardenolides which confirms a positive presence of cardenolides. A violet-green ring appearing below the brown ring, in the acetic acid layer, indicates the positive presence of glycoside.

Test for steroids: 2 ml of acetic anhydride was added to 2 ml extract of each sample followed by careful addition of 2 ml H_2SO_4. The colour changed from violet to blue or green indicate the presence of steroids.

Test for terpenoids (Salkowski test): 5 ml of each extract was mixed with 2 ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface was formed to show positive results for the presence of terpenoids.

Test for phlobatannins: Deposition of a red precipitate when 2 ml of extract of each plant samples was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

Preparation of bacteria innoculum

A 24 h old culture of bacterial isolate was emulsified in sterile normal saline and adjusted to 0.5 McFarland standard (by compared the desired inoculum with a 0.5 McFarland standard).

Bacterial sensitivity testing: Well Diffusion Method: For each aqueous extract and crude extract, a solidified muller Hinton agar plate was assigned to it and aseptically, each plate was seeded by flooding with specific bacteria solutions (*Staphylococcus aureus and Pseudomonas aeruginosa*) and then allowed to settle for 10 min. Three holes were bored in the agar with the aid of sterile cork borer and filled with extract based on the prepared concentrations (100%, 50%, and 25%) using sterile syringe. Incubated at 37°C for 24 hr and monitored.

Discussion

The results of the phytochemical test carried out on the extracts were recorded as shown in **Table 1.** Preliminary phytochemical screening revealed the presence of Saponins, flavonoids, terpenes, cardenolides and phitobatamin in aqueous extract but absent in ethanol crude extract, Tannin, phenol, glycoside and steroids were also present in ethanol crude extract but absent in ethyl acetate crude extract, phenol was less abundant in ethyl acetate crude extract. Cardenolides was much abundant in ethyl acetate crude extract, found minute in aqueous extract but absent in ethanol crude extract. Phytochemical constituents in the various part of the plant vary significantly. Several medicinal plants are used in traditional medicines for curing many diseases. The spinach leaf extract with their phytoconstituents are reported for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities [15]. In the present investigation, the aqueous extract of *Spinacia oleracea* shows the abundant presence of Terpenes, and steroids. Steroids are of immense importance in pharmacy because of their relationship with compounds like sex hormones and can be used for drug production [16]. The study revealed that Cardenolides, glycosides, flavonoids, phenol and saponin were found to be present in *Spinacia oleracea* extract with different versions of the extract. Several studies reported that, glycosides play an important role in lowering the blood pressure and they are also used in treatment of congestive heart failure and cardiac arrhythmia [17]. Flavonoids show anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity. Flavonoids are generally distributed throughout the plant kingdom and are 15 carbon compounds. Tannin and phenol are necessary for the animal body for repair and maintenance. In the present work some plants exhibited the presence of proteinsand carbohydrates, thus reflecting their nutritional importance as protein and carbohydrate supplement which cannot be ignored [18]. Natural or synthetic phitobatamin show a biological or pharmacological activity, and some of them show antitumor activity as well. They embody some claims in herbal medicines. These applications include purgative (sennosdes), antibacterial (rhein and saprorthoquinone), anti-tumor (emodin and jugone), inhibition of PGE2 biosynthesis (arnebinone and arnebifuranone) and anti-cardiovascular disease (tanshinone). It has been reported that terpenesare used in the treatment of cough, asthma and hay fever. In the present study, the ethyl acetate crude extracts of the *Spinach oleracea* was found to exert the antibacterial activity against the two test organisms at 100% (*Staphylococcus aureus* and *P. aeruginosa)* but not effective at 50% and 25%, why aqueous extract and ethanol crude extract were not effective in all tests carried out at difference concentrations (100%, 50% and 25%). The ethyl acetate crude extract showed maximum antibacterial effect among the three solvents **(Table 2).** This study confirms the potential antibacterial activity of ethyl acetate crude extract of

Table 1 Phytochemical Screening of Spinach Leaf (*Amunun tutu*).

Parameter	Water	Ethanol	Ethyl acetate
Saponins	++	--	+++
Tannins	--	++	+
Phenol	--	+	++
Flavonoids	+++	--	+
Glycosides	--	++	--
Steroids	--	+	--
Terpenes	+++	--	--
Cardenolides	+	--	+++
Phlobatamin	+	--	--

Legend: +++ (Much abundant); ++ (Less abundant); + (Minute); - (Absent)

Table 2 Antibacterial Activity of Spinach Leaf (*Amunun tutu*).

Extract (Conc)	*Staphylococcus aureus*	*Pseudominas aeruginosa*
Extract+H_2O (100%) Extract+H_2O (50%) Extract+H_2O (25%)	Resistant Resistant Resistant	Resistant Resistant Resistant
Extract+ethanol (100%) Extract+ethanol (50%) Extract+ethanol (25%)	Resistant Resistant Resistant	Resistant Resistant Resistant
Extract+ethyl acetate (100%) Extract+ethyl acetate (50%) Extract+ethyl acetate (25%)	Sensitivity Resistant Resistant	Sensitivity Resistant Resistant

Spinach oleracea. This credit to maximum activity of ethyl acetate crude extract being an organic solvent and will dissolve organic compounds better, hence liberate the active component required for antimicrobial activity [19].

Conclusion

The commonly consumed green leafy vegetable in Nigeria selected for the present study contain substantial amount of phytochemicals, which are helpful in the prevention of some deadly diseases [20-26]. The phytochemicals were not affected by cooking except for flavonoids and alkaloids. This implies that the fear of losing these plant chemicals as a result of cooking need not arise [27-31]. Vitamins and minerals can be lost (leached out) during cooking but not the phytochemicals. This work also showed that the *Spinacia oleracea* was one of the most cherished vegetables in Nigeria most especially in south western zone which is very rich in most of these phytochemicals. Further studies are required to isolate the active compound from ethyl acetate version of the extract of *Spinacia oleracea,* responsible for this antibacterial effect which might be a lead compound in antibacterial arena.

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