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The Phytochemical and Proximate Analysis of the Ethanol Extract of Ocimum Gratissimum (Scent Leaf)

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ABSTRACT: The present investigation was centered on the phytochemical and proximate analysis of ethanol extract of *Ocimum gratissimum*. The phytochemical and proximate constituents of the ethanol extract of the leaves were determined using standard reference methods. The qualitative phytochemical constituents indicated the moderate presence of alkaloid, saponin, tannin, flavonoid and `a trace amount of cardiac glycoside and absence of steroid and terpenoid. The quantitative phytochemical constituents indicated the alkaloid content 8.00 ± 0.09%, flavonoid 9.20 ± 0.06%, tannin 8.40 ± 0.06%, saponin 9.90 ± 0.20%. The proximate composition indicated the moisture content 12.30 ± 0.01%, Ash 10.50 ± 0.02, Crude fibre 13.00 ± 0.02%, crude protein 18.23 ± 0.02%, Fat 12.50 ± 0.02% and carbohydrate 33.47 ± 2.20%. The result indicates that the bioactive compounds in the vegetable are good and available nutrients which justify the use of scent leaf as spice in food preparation and as medicinal plant in ethnomedicine.

KEYWORDS: bioactive, ethnomedicine.

I. INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities at large[5]. The medicinal values of some plant lies in the chemical substances known as phytochemicals which produce definite physiological actions in the human body. The most important of these bioactive compounds are alkaloids, tannins, flavonoids and phenolic compounds. Phytochemicals are naturally occuring, biologically active compounds in plants. The prefix "Phyto" is from a greek word meaning plant. The presence of certain types of phytochemicals in some plants can act as a natural defence system providing protection against such things as attack by insects and grazing animals[1]. they act as synergistic agents, allowing nutrients to be used more efficiently by the body. in wide ranging diets, phytochemicals are found in fruits , vegetables, legumes, wholegrain, nuts, seeds and herbs. Many phytochemicals particularly the figment molecules are often concentrated in the outer layer of the various plant tissues[2]. levels vary from plant to plant depending on the variety, growing condition, processing and cooking method. In general, the plant chemical protects the plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack. Recently, it is clearly known that they play a role in the protection of human health[6]. In contrast, other plants produce phytochemicals that provide colour, aroma and flavour. These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelets aggregation and modulation of hormone metabolism and anticancer property[4]. Phytochemicals are not essential nutrients and are not required by human body for sustaining life but have important properties to prevent or fight some common diseases.

II. LITERATURE SURVEY

A typical scent leaf has a curvy peak with serrated/wavy edge. Both the front and back of the leaf is not smooth and are textured. They are cousins with mint leaf. It is found in the tropics of africa and Asia , having the most variant of the species. In west africa, ocimum gratissimum is found around village huts and gardens, cultivated for medicinal and culinary purposes. The leaf have strong and are popularly used to flavour soup and spice meat. Antifungal activity: Antifungal study of ocimum gratissimum on three major dermatophytes-trichophyton, microsporium, epidermophyton together with malassezia furfur isolated from scalp, skin , toes and feets showed that scent leaf has great potential of use as an antidermatophyte agent. The volatile oil of scent leaf produce inhibitory effect against the oral microbial flora in the study. Antibacterial activity: Ethanol extract of ocimum gratissimum demonstrated antimicrobial activity against

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N.gonorrhea, S. typhi and other bacteria. This justifies the traditional medicinal uses of ocimum gratissimum for treating diarrhea, respiratory infections and fever. (Adewole,2014). The principal constituent of essential oil of scent leaf is eugenol which is responsible for the biological activities of this plant . The volatile oil contains phenols particularly thymol which is responsible for the antimicrobial actions.

III. MATERIALS AND METHODS

Leaves of *Ocimum gratissimum*, Thomas-willey milling machine, water bath, weighing balance, whatman No.1 filter paper, measuring cylinder, beakers, pipette, kjeldahl apparatus, conical flask, seperatory funnel, dessicator, spectrophotometer, sohxlet extractor.The leaves of ocimum gratissimum was procured, identified and authenticated by a taxonomist and prepared for analysis. The leaves was air-dried at room temperature for 7 days and ground into uniform powder using a Thomas-Willey milling machine. The ethanolic extract of the sample was prepared by soaking 500g of dried powdered samples in one litre of absolute ethanol and shaken intermittently. it was sieved with a cloth and further sieved with Whatmann no.1 filter paper and was placed in a water bath at 50^0C to remove the ethanol by evaporation. The phytochemical and proximate analysis was carried out using standard analytical procedures.

A. TEST FOR ALKALOIDS

About 2g of the ethanolic extract was stirred with 5ml of 1% aqueous HCL or a steam bath 1ml of the filterate was treated with a few drops of wagner's reagent (solution of iodine and pottassium iodide) . The presence of alkaloid was indicated by the formation of a reddish brown precipitate.

B. TEST FOR TANNINS

About 2g of the dried powdered sample was boiled in 20ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and oberved for brownish green or a blue black colouration which indicates the presence of tannin.

C. TEST FOR SAPONIN

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. 10ml of filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil, shaken vigourously and then observed for the formation of emulsion.

D. TEST FOR FLAVONOIDS

5ml of 10% dilute ammonia solution was added to a portion of aqueous filterate of the plant extract, followed by addition of concentrated H_2SO_4. A yellow colouration was observed that indicates the presence of flavonoid. The yellow colouration disappeared on standing.

E. TEST FOR STEROIDS

2ml of acetic anhydride was added to 0.5g ethanol extract of the sample with 2ml H_2SO_4. The colour change from violet to blue or green indicates the presence of steroids.

F. TEST FOR TERPENOIDS (SALKOWSKI TEST)

5ml of the extract was mixed in 2ml of chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface indicates positive result for the presence of terpenoids.

G. TEST FOR CARDIAC GLYCOSIDES (KELLER-KILLANI TEST)

5ml of the extract was treated with 2ml of glacial acetic acid containing 1drop of ferric chloride solution (0.1%). This was underplayed with 1ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxysugar characteristics of cardenolides . A violet ring may appear below the brown ring while in the acetic layer, a greenish ring may form just gradually throughout thin layer. The quantitative analysis of the phytochemicals was determined using standard procedures as described by Harborne (1973).

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H. PROXIMATE ANALYSIS OF THE SAMPLE USING AOAC (2002) METHOD

Determination of moisture content

The method used is the drying method. 2g of the sample was placed into a pre-heated ,cooled and weighed silica dish and dried in the oven for 24 hours at regulated temperature of 80^{o}C to a constant weight. The dish and the content was allowed to cool in a desiccators and weighed. The moisture was determined as percentage moisture.

Determination of Fat content

The method to be used was soxhlet extraction method. 250ml boiling flask was dried in an oven at 105-110^{o}C for 30minutes and transferred into desiccators and then allowed to cool. 2g of the sample were was weighed into the thimble. labeled boiling flask was weighed and filled with 300ml of petroleum ether (boiling point 40-60^{o}C). The extraction thimble was plugged lightly with cotton wool. The soxhlet apparatus was assembled and allowed to reflux for some hours. The thimble was carefully removed and petroleum ether in the top corner of the set-up was collected and drained into a container for re-use. when the flask is almost free from petroleum ether , the sample was removed and dried in an oven at 105^{o}C -110^{o}C for one hour and then was transferred into a desiccator and allowed to cool and then weighed.

Determination of Ash content

An empty crucible was washed thoroughly and dried in an air circulation oven for 2 hours and allowed to cool in the desiccators. The empty dry crucible was transferred into a muffle furnace to burn off all organic matter and the weight of the crucible was stabilized and noted. 2g of deffated sample was dispersed into the crucibles and placed in a muffle furnace and incinerated at 600oc for 3 hours. each crucible was labeled appropriately. The ash samples was removed from the furnace and cooled to room temperature and then weighed. The experiment was carried out in triplicates and the percentage was determined.

Determination of Crude fibre

2g of the sample was defatted with petroleum ether and boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml of solution. the solution was filtered through linen on a fluted funnel and was washed with boiling water. the residue was transferred to a beaker and boiled for 30minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but close pad of washed and ignited asbestors in a goosh crucible and dried in an electric oven and weighed. it was incinerated, cooled and weighed. The percentage crude fibre was determined.

IV. RESULTS

TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF THE *OCIMUM GRATISSIMUM*

Phytochemicals	Occurance
Alkaloid	++
Saponin	++
Tannin	++
Flavonoid	++
Steroid	–
Terpenoid	–
Cardiac glycoside	–

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Key
+ = Trace/mildly present
++ = moderately present
+++ = Abundantly present
- = Absent

TABLE 2: QUANTITATIVE ANALYSIS OF THE PHYTOCHEMICAL CONSTITUENT OF *OCIMUM GRATISSIMUM*

Parameters	Phytochemical composition (%)
Alkaloid	8.00 ± 0.09
Flavonoid	9.20 ± 0.06
Saponin	9.90 ± 0.20
Tannin	8.40 ± 0.06

TABLE 3: RESULT OF THE PROXIMATE ANALYSIS OF THE *OCIMUM GRATISSIMUM* LEAF

Parameters	Proximate composition (%)
Moisture content	12.30±0.01
Ash content	10.50±0.01
Fat	12.50±0.02
Protein	18.23±0.20
Crude Fiber	13.00±0.02
Carbohydrate	33.47±2.20

V. DISCUSSION

The qualitative analysis of the phytochemical constituents of *ocimum gratissimum* showed the presence of a moderate amount of alkaloid, saponin, tannin, flavonoid and a trace amount of cardiac glycoside but showed the absence of steroid and terpenoid. The study revealed that the plant is rich in secondary metabolites and is said to be responsible for its physiological actions in the body. Flavonoid have exerted biological properties which include anti inlammatory, anti-tumor properties and are potent water soluble anti-oxidants and are free radical scavengers which prevents oxidative cell damage and have strong anti-cancer activity.

VI. CONCLUSION

Ocimum gratissimum have very high nutritional value due to its rich content in bioactive compounds which are extremely valuable for the body and creates need for recovery, good health and active life. The chemical constituents of the plant encourage the consumption of the vegetable as both food for nutrients and as medicine. Generally, vegetables are the cheapest and most valuable source of important nutrients. they are ideal for obese people and vegetarians who can satisfy their appetite without consuming much carbohydrate for utilizable energy.

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