Comparative Study of the phytochemical Composition of Aqueous Extracts of *Ocimum gratissimum* (Scent Leaves) and *Murraya koenigii* (Curry Leaves)

Ike Chiamaka J., Eze-Steven Peter E. and Ani Anita Chikaodinaka

Department of Applied Biochemistry, Faculty of Applied Natural Sciences Enugu State University of Science and Technology (ESUT) Nigeria.

**ABSTRACT**

There are significant evidences supporting the process of bioactive moieties in fruits and vegetables, helpful to attenuate lifestyle related disorders. These phytomolecules exert potential health benefits by mitigating generative risk factors. The comparative study of the phytochemical composition of aqueous extracts of scent leaves *Ocimum gratissimum* and curry leaves *Murraya koenigii*. Phytochemical extracts is a process of tracing the medicinal value of plants constituents in some chemical substance that produce a definite physiologic action on the human body. The aim of the study was to have the knowledge of the quantity of the phytochemical compounds present in the extracts of scent leaf and curry leaf and the study also aims at accessing the potential medicinal effects of the phytochemicals present in the leaves. The quantitative phytochemical analysis of *Ocimum gratissimum* and *Murraya koenigii* was carried out in order to ascertain the presence of some plant secondary metabolites. The determinations were done by utilizing standard conventional protocols as illustrated by Harborne, Trease and Evans. The result phytochemical screening of *Ocimum gratissimum* showed that the plant leaves contain tannins (flavonoids 0.570±0.030, alkaloids 0.161±0.021, glycoside 0.035±0.015 sterol 0.002±0.000 and saponins 0.143±0.032 tannins 0.152±0.007 and *Murraya koenigii* showed that the plant contains (flavonoids 1.717±0.764, alkaloids 0.086±0.007, glycoside 0.030±0.002, sterol 0.006±0.001 and saponins 0.769±0.123. The phytochemical and elemental component of the leaves of *Ocimum gratissimum* was analysed. The result indicated that the leaf of the plant contains some major bioactive compounds that can inhibit the growth of microorganism, thereby proving it as an effective potentials source of antibiotic. However, the result revealed that the plant leaf contains saponins, tannins and alkaloids which help to inhibits bacterial growth. The study showed that *Ocimum gratissimum* and *Murraya koenigii* have similar phytochemical compounds but Murraya koenigii contains more of the phytochemical compounds.

Keywords: Comparative, phytochemical, composition, aqueous, Scent Leave, curry leaves.

**INTRODUCTION**

The main purpose of eating food is our appetite, but it also gives nutrients which are required by the human body. It also helps to prevent the human body from different dietary diseases and keeps the health in good condition. Consumption of junk food is a major trend leading towards a number of diseases due to improper nutrition. A good quality food has a good impact on the consumer health as there is a direct relationship between food and health.

In this aspect, functional and nutraceuticals have opportunities for food industries, not only to develop novel food products but also reduce care cost and improve source of revenue for rural populations. There are significant evidences supporting the process of bioactive moieties in fruits and vegetables, helpful to attenuate lifestyle related disorders. These phytomolecules exert potential health benefits by mitigating generative risk.
factors. Phytochemical extracts is a process of tracing the medicinal value of plants constituents that produce a definite physiologic action on the human body [1]. The most important of these bioactive compounds of *Murraya koenigii* and *Ocimum gratissimum* are alkaloids, flavonoids, phenolic, tannins, saponins, glycosides etc. Knowledge of the chemical constituents of these plants is desirable, not only for the discovery of the therapeutic agents but also because, such information may be of value in disclosing new sources of such economic materials such as tannins, oils, gum, precursors for the synthesis of complex chemical substances. *Ocimum gratissimum*, is an aromatic perennial herb with erect stem, much branched, glabrous and woody at the base often with epidemic peeling in strips. *Ocimum gratissimum*, is grown for the essential oil in its leaves and stems while engenol and to a lesser extent, thymol extracted from the oil are substitutes from clove oil and thyme oil. The essential oil possesses antibacterial properties and it is also an important insect repellants, so also are the leaves when left dry and burnt. They are primarily used as vegetables, as spice due to its aromatic nature to spice various kinds of soups (e.g. pepper soup) and other delicious meals like porridge [2]. The whole plant has many applications in traditional medicine, especially in Africa and India. The applications include; in the treatment of ringworms, gout and fungal infections, malaria, catarrh, aches, colon pain etc. The juice gotten from squeezing its leaves can be used to cure several stomach related illnesses like; cholera, diarrhea, dysentery, vomiting and convulsion. [3]. *Murraya koenigii* commonly known as curry leaves belongs to family *Rutaceae*. It is an aromatic, pubescent, deciduous shrub or small tree. It is widely distributed in south-east Asia, Australia and the Pacific Islands. In India, it occurs in wild and cultivated forms. In India, it is a very popular and medicinal plant. It has strongly aromatic leaves so they can retain their colour and flavour even after drying. Curry leaves has a slightly pungent, bitter, and feebly acidic taste [4]. The fresh leaves of *Murraya koenigii* are a good source of B-Carotene. The extracts of the leaves of *Murraya koenigii* is thought to be an anti-diabetic agent, as a spice and condiment in India. In tropical countries like Malaysia, it is found to be very effective in the treatment of kidney illness. These leaves also prevent the renal failure. *Murraya koenigii* a rich source of minor constituents such as; calcium, potassium, magnesium, phosphorus, along with iron, manganese, and zinc in trace amounts [5]. Bioactive compound of curry leaves have many functional properties. It contains alkaloids like; muconicine, koenimbine, koenine, mahanimbidine etc. which have bioactive functions like anti-cancer, anti-diabetic and anti-ulcer. Extracts of spices, aromatic herbs and medicinal plants are most beneficial natural products that are widely utilized as ingredients in food products, pharmaceuticals and cosmetics because of their intense flavor, medicinal and antiseptic properties. Curry leaves contains significant amount of phenolic and flavonoids compound which are phytost_pduents responsible for lipid lowering anti-obesity activities because of strong antioxidant potential. *Ocimum gratissimum* and *Murraya koenigii* are known to have common phytochemical compounds which are significant in traditional medicine for the treatment of several ailments.

**Statement of the Problem**

The increased use of food additives has encouraged the scientific investigations into their content. The aim is to understand the chemical compositions which could be vital in the supply of nutrients required for a balanced health. Lack of these nutrients are indicated in several ill health and biochemical abnormalities.

**Aims and Objectives**

The aim of this project work is to justify the comparative study of the phytochemical composition of the
aqueous extracts of Murraya koenigii and Ocimum gratissimum.

MATERIALS AND METHODS

Sample Collection
Ocimum gratissimum and Murraya koenigii used for this project work was gotten from Eke market, Agbani in Nkanu West local government, Enugu State on July 2018. Sample collection, preparation and analysis were done in the laboratory of the department of Applied Biochemistry, Enugu State University of Science and Technology, Nigeria.

Sample Preparation
Ocimum gratissimum and Murraya koenigii were washed and air dried less than two weeks and was later pulverized to fine powder, using a mechanical grinder.

Phytochemical Analysis
The quantitative phytochemical analysis of Ocimum gratissimum and Murraya koenigii was carried out in order to ascertain the presence of some plant secondary metabolites. The determinations were done by utilizing standard conventional protocols as illustrated by [6]; [7]. The preliminary analysis involved testing for the presence of flavonoids. terpenoids, steroids, saponins, alkaloids, tannins, glycosides and phenols.

Qualitative Phytochemical Analysis of the Extracts.

Test for Tannins
Extract (0.1 g) was stirred with 10ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2ml of each filtrate. The presence of a blue-black or blue green precipitate indicated the presence of Tannins [8].

Test for Alkaloids
A quantity of the extracts (0.1g) was dissolved individually in dilute Hydrochloric acid and filtered.

a) Dragendroff’s Test: Filtrates were treated with Dragendroff’s reagent (solution of potassium Bismuth Iodine). Formation of red precipitate indicates the presence of alkaloids [9].

b) Hager’s Test: Filtrates were treated with Hager’s reagent (saturated picric acid solution). Presence of alkaloids was confirmed by the formation of yellow colored precipitate [10].

Test for Saponins
A quantity of each extract (0.1 g) was boiled with 5 ml of distilled water and filtered. To each filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as evidence for the presence of saponins [11].

Test for Glycosides
Each extracts (0.1 g) was mixed with 10 ml of distilled water and heated in a water bath for 5 minutes. To 5 ml of each filtrate, 0.3 ml of Fehling’s solution A and B until it turns alkaline. The solutions were heated in a water bath for 7 minutes. A brick-red precipitate indicated the presence of glycoside [12].

Test for Terpenoid
Each extract (0.1 g) was dissolved in ethanol. Acetic anhydride (1 ml) was added followed by the addition of concentrated H2SO4. A change in color from pink to violet showed the presence of terpenoids [13].

Test for Flavonoids
A quantity (0.1 g) of each of the extract was dissolved in water and filtered. 5 ml of each of the filtrates, 3 ml of lead ethanoate solution was added. Appearance of a buff-colored (pale yellow-brown) precipitate indicated the presence of flavonoids [14].

Liebermann-Buchard Test for Steroids
To 0.1 g of each extract, 2 ml of acetic acid was added. The solution was cooled well in ice followed by the addition of concentrated H2SO4 carefully. Color development from violet to blue or bluish-green indicated the presence of a steroidal rind [15].
Ferric Chloride Test for Phenols
About 0.1 g of each extracts was boiled with distilled water and then filtered. To 2 ml of each filtrate, few drops of 10% ferric chloride solution were added. A green-blue or violet coloration indicated the presence of a phenolic hydroxyl group [16].

Quantitative Phytochemical Analysis of the Extracts
Determination of Alkaloids (was done using Harborne, 1973).
The samples were weighed (0.5 g) using electric weighing balance into a 250 ml beaker; 50 ml of 10% acetic acid in ethanol was added to the sample and covered. The mixtures were allowed to stand for four hours for proper extraction to take place. The sample was filtered with filter paper and the extracts were concentrated on a water bath to form precipitate of the alkaloid in the filtrate. The filtrate was weighed with NH4OH and filtered. The filter paper was weighed before using it to filter. After filtering, the filter papers and the precipitate were dried in an oven at 40°C and weighed.

Determination of Saponins
The extracts were weighed (1.0 g) using an electric weighing balance into 250 ml conical flask and soaked with 25 ml of 20% ethanol for three minutes and heated for three hours at 55°C proper extraction and then filtered. The residue was re-extracted with another 25 ml of 20% ethanol. The two extracts were combined and heated to 20 ml at 90°C on a water bath. The concentrate was transferred into a 500 ml separating funnel and 10 ml of petroleum ether was added and shaken vigorously, the upper layer was discarded and the lower was collected in a weighed beaker and heated to dryness. The beaker is allowed to cool in a desiccator and re-weighed.

Determination of Tannins (was done using Robinson method, 1981).
1.0 g of the extracts was placed into a plastic bottle and 50 ml of distilled water was added and shaken for 3 hours in a vibrator. The sample was filtered into a 50 ml volumetric flask and make up to mark. 5 ml of the filtrate was dispensed into a test tube and mixed with 1ml of 0.1 m FeCl2 in 0.1 NHCL and 1ml potassium ferrocyanide, the absorbance was measured at 720 nm for 10 minutes.

Determination of Flavonoids (was done using Bohn and Kocipal-Abyassan method, 1994).
The samples were repeatedly extracted with 25 ml of 80% aqueous methanol at room temperature; the solution was shaken for 30 minutes and filtrate was transferred into a weighed beaker and evaporated to dryness over a water bath and weighed again. The time for the first extraction was 1 hour, 45 minutes for the second extraction and 30 minutes for the third extraction.

Determination of Steroids (was done using Okeke and Eloku method).
The extracts (1.0 g) were dispensed in 100 ml of distilled water into a conical flask; the mixtures were shaken for 3 hours and allowed to stand overnight. Then it was filtered, the filtrate was eluted with 10 ml normal ammonium hydroxide solution, 2 ml of the elutes were put into test tubes and mixed with 2 ml of Chloroform and also 3 ml of acetichydride was added to the mixtures, followed by 2 ml of concentrated H2SO4, dropped wisely. The absorbance was measured in a spectrophotometer at 420 run.

Quantification of Terpenoid content
The determination of the terpenoid was carried out according to the method of [17]. 0.1 g of the extracts were weighed out separately, macerated with 20 ml of ethanol and filtered through Whatmann no.1 filter paper. The filtrates (1 ml) were pipetted out and 1 ml of 5% phosphomolybdic acid solution was added and shaken. Gradually 1 ml of concentrated H2SO4 was added to each. The mixtures were left to stand for 30 minutes. Ethanol (2 ml) was added and absorbance was measured at 700 nm.

Quantification of Glycoside Content
The determination of glycoside was carried out according to the method of [18]. The extracts (0.1 g) were weighed out separately, macerated with 20 ml of distilled water and 2.5 ml of 15% lead acetate was added and filtered. Chloroform (2.5 ml) was added to the filtrates, shaken vigorously and the lower layer collected and evaporated to dryness. Glacial acetic acid (3 ml) was also added together with 1 ml of 1% ferric chloride and 1 ml of concentrated H2SO4. The mixture was shaken and put in the dark for 2 hours. Absorbance was measured at 530 nm.

RESULTS

Table 1: Result of the Quantitative Phytochemical Content of Murraya koenigii

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>ABSORBANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>+++</td>
</tr>
<tr>
<td>Saponin</td>
<td>+++</td>
</tr>
<tr>
<td>Steroid</td>
<td>++</td>
</tr>
<tr>
<td>Tannin</td>
<td>+++</td>
</tr>
<tr>
<td>Glycoside</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>++</td>
</tr>
</tbody>
</table>

Key:
+++ = Highly present
++  = Moderately present
-   = Not present

Table 2: Result of the Qualitative Phytochemical Analysis of Ocimum gratissimum

<table>
<thead>
<tr>
<th>Phytochemicals</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+++</td>
</tr>
<tr>
<td>Saponin</td>
<td>+++</td>
</tr>
<tr>
<td>Steroid</td>
<td>+++</td>
</tr>
<tr>
<td>Tannin</td>
<td>++</td>
</tr>
<tr>
<td>Glycoside</td>
<td>+++</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
</tr>
<tr>
<td>Phenol</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 3: Result of the Quantitative Phytochemical Analysis of Murraya koenigii

<table>
<thead>
<tr>
<th>PHYTOCHEMICAL</th>
<th>CONCENTRATION (Mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>1.717±0.7638</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>0.863±0.006</td>
</tr>
<tr>
<td>Steroid</td>
<td>0.006±0.007</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.769±0.123</td>
</tr>
<tr>
<td>Glycoside</td>
<td>0.295±0.002</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.183±0.009</td>
</tr>
</tbody>
</table>
Table 4: Result of the Quantitative Phytochemical Analysis of *Ocimum gratissimum*

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONCENTRATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>0.570±0.030</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>0.161±0.021</td>
</tr>
<tr>
<td>Steroid</td>
<td>0.002±0.000</td>
</tr>
<tr>
<td>Saponin</td>
<td>0.143±0.032</td>
</tr>
<tr>
<td>Glycoside</td>
<td>0.035±0.015</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.152±0.007</td>
</tr>
</tbody>
</table>

DISCUSSION

The result of phytochemical screening of *Ocimum gratissimum* and *Murraya koenigii* showed that the plant leaves contains tannins, flavonoids, alkaloids, glycoside, sterol and saponins. The phytochemical and elemental component of the leaves of *Ocimum gratissimum* was analysed. The result indicated that the leaf of the plant contains some major bioactive compounds that can inhibit the growth of microorganism, thereby proving it as an effective potentials source of antibiotic. The plant extract might also be a potential source for drugs formulation as the plant leaves are used traditionally for curing of many infectious diseases. These metabolites are known to have varied pharmacological actions in man and animals, the presence of these metabolites suggest great potentials of the plants as a source of useful phytomedicines. The phytochemicals are naturally occurring chemicals in plants which serve as medicinal for the protection of human disease; the phytochemical are non nutritive plants chemical that have protection or disease preventive properties. *Murraya koenigii* is very rich source of organic compounds with different chemical composition such as alkaloids, flavonoids carbohydrates, and sterol is present in the plant extract prepared in solvents such as petroleum ether, ethyl acetate, chloroform, ethanol and water. The major chemical constituents are explained for the confirmation of the phyto-constituents in the plant extracts, various numbers of tests were performed on both plants: The presence of alkaloids was confirmed by using Hager’s reagent, which shows formation of yellow colored precipitates in the extract of *Murraya koenigii*. Phenolic compounds were confirmed by formation of white precipitate by the addition of few drops of 10% ferric chloride solution to the aqeous extracts of *Murraya koenigii*. The presence of flavonoids is detected by a pale-yellow brown precipitate. Presence of Saponins is considered when the extract showed frothing formation which persisted on warming. Presence of sterol is indicated by the addition of acetic acid which was shaken with few drops of concentrated sulphuric acid from to form the bluish-green coloration. The result of the quantitative phytochemical analysis of *Murraya koenigii* and *Ocimum gratissimum* indicated that the extracts of *Murraya koenigii* contains more quantity of the phytochemical compounds than in *Ocimum gratissimum*.

CONCLUSION

The results from this work has shown that extracts of the plants under study contains many phytochemical compounds which include alkaloids, steroids, flavonoids, glycosides, saponins and tannins which are responsible for their various activities and also may account for medicinal benefits. The selected plants contain...
substantial amount of phytochemical which are helpful in the prevention of some deadly diseases. Scent leaf (*Ocimum gratissimum*) and curry leaf (*Murraya koenigii*) leaves could help fulfill the growing demands of plant based foods for human nutrition. I recommend that further studies should still be carried out on the phytochemical constituents of curry leaf and scent leaf.

REFERENCES


