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Phytochemical analysis and hepatoprotective potential of aqueous leaf extract of *Ocimum gratissimum* (Scent leaf)

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Abstract
The aim of this study was to evaluate the phytochemical composition and hepatoprotective potential of aqueous leaf extract of *Ocimum gratissimum* (Scent leaf). Freshly harvested leaves of *O. gratissinum* were thoroughly washed with tap water before being dried at room temperature after which there were ground to fine powder. 50g of the powdered plant sample was soaked in 100 ml of distilled water for 30 minutes. The resulting extract was filtered and the filtrate concentrated by gentle evaporation. Evaluation of the phytochemical composition and hepatoprotective potential of the extract were determined by standard methods. Phytochemical analysis on the extract revealed the presence of tannins (49.19±2.86mg/100g), saponins (42.28±2.85mg/100g), flavonoids (24.05±2.02mg/100g), steroids (38.89±1.06mg/100g), phenols (25.55±2.36mg/100g) and alkaloids (21.02±1.03mg/100g). However, serum hepatomarkers of animals in group 2 orally administered with only CCl_4 was elevated thus: AST (110.1±2.34 IU/L), ALT (45.4±2.9 IU/L), ALP (63.4±4.86 IU/L) and TB (2.6±0.87 IU/L) compared to those of group 1 reported thus: AST (90.3±2.12 IU/L), ALT (35.3±4.34 IU/L), ALP (40.2±3.91 IU/L) and TB (0.2-1.31 IU/L) that was administered with only 2ml/kg of distilled water. However, administration of 200mg/kg, 400mg/kg and 600mg/kg b.w of extract significantly shielded the liver evident by the dose dependent reduction in the levels of the serum hepatomarkers of animals in groups 3-5 respectively. In conclusion, *Ocimum gratissimum* habours compound (s) with hepatoprotective potentials, and thus should be explored to enhance benefits.

Keywords: *Ocimum gratissimum*, serum hepatomarkers, liver, phytochemical

Introduction
Liver is known as the most massive organ of the human body weighing about 1500g. It is positioned in the upper right corner of the abdomen [1]. The organ which is closely associated with the small intestine is saddled with the responsibility of processing the nutrient rich venous blood that vacates the gastrointestinal tract [2] prior to delivery of substances to systemic circulation [3]. It is a vital organ that plays major roles in the elimination of xenobiotics from the body in addition to many critical functions. With this, it is obvious that a total loss of liver functions could result to death [4].

Liver damage has been identified as a major health problems in developing countries due to dietary habits, excessive alcohol consumption, poor hygiene, unsupervised drug use and smoking etc. [5], and a challenge of immense concern to health care experts and pharmaceutical industries as conventional drugs available for the treatment of liver diseases are associated with a wide range of adverse effects, thus paving way for scientific trials on botanicals in an effort to develop an efficient and effective therapies in the prevention and treatment of liver diseases.

Ocimum gratissimum L. which is commonly known as clove basil or lemon basil is a polymorphic branched aromatic shrub measuring about 0.5-3m tall. It belongs to the family *Lamiaceae*. In Nigeria, this plant is known as nchuanwu or ahuji in Igbo, efirin in Yoruba and daidoya among the Hausas.

The usefulness of this plant is justified by its numerous medical applications some of which are hepato-oriented and include improvement of cardiac functions, digestive processes, and regulation of blood sugar levels, anti-mutagenic and wound healing properties among others. Phytochemicals are biologically active chemical compounds in plants which are known for their characteristic medicinal potential evident by the fact that they have been implicated in the therapeutic abilities of plants according to research outcomes [6]. Owing to the fact that not much is either known or has been done on hepatoprotective drugs, it is imperative to explore the hepatoprotective potential of plants that have demonstrated hepato-oriented therapeutic potentials such as *Ocimum gratissimum* (scent leaf) in an effort to develop a capacity therapy to shield the liver against attacks by hepato-destructive agents.

Materials and Methods

Collection and processing of plant

Fresh leaves of *Ocimum gratissimum* was harvested from a home garden, the leaves were thoroughly washed with tap water, after which there were dried at room temperature. Dried leaves of *O. gratissimum* were subsequently ground to fine powder with the aid of an electric blender. In order to obtain the aqueous extract of the powdered plant sample, 50g of the sample was soaked in 100 ml of sterile distilled water in conical flask and was allowed to stand for 30 minutes, after which the extract was filtered using Whatman filter paper No. 42 (125mm). The filtrate was concentrated by gentle evaporation on a heating mantle [7].

Qualitative phytochemical analysis

Test for tannins

Precisely 0.5 g of extract was dissolved and stirred in 10 ml of distilled water, after which it was filtered. Few drops of 1% ferric chloride solution were introduced into a test tube containing 2 ml of the filtrate. Appearance of a blue-black, green or blue green precipitate indicated the presence of tannins [8].

Test for saponins

Exactly 1 g of extract was boiled with 5 ml of distilled water, filtered. To the filtrate, 3 ml of distilled water was added and shaken vigorously for about 5 minutes. Frothing which persisted on warming indicated the presence of saponins[9].

Test for alkaloids

Exactly 50 mg of extract was stirred in 2 ml of dilute hydrochloric acid (HCl) and filtered, after which few drops of Mayer's reagent were added by the side of the test tube. Formation of white or creamy precipitate indicated the presence of alkaloids [10].

Test for flavonoids

Exactly 0.5 g of extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were then added to the filtrate. This was followed by the addition of few drops of conc. HCl. Appearance of pink, orange, or red to purple colour showed that flavonoids were present [7].

Test for phenols

Few drops of neutral 5% ferric chloride solution was introduced into a test tube holding 50 mg extract dissolved in 5 ml of distilled water. The appearance of dark green colour showed that phenolic compounds were present [11].

Test for steroids

Exactly 5 ml of distilled water was added to a test tube holding 0.5 g of extract and the mixture shaken vigorously and observed for a stably persistent froth. The resulting froth was mixed with 3 drops of olive oil and shaken vigorously. The formation of emulsion showed that steroids were present [12].

Quantitative phytochemical analysis

Determination of tannins

The Follins Dennis spectrophotometric method as in Pearson [13], was employed. 5 g of the extract was dispersed in 50 ml of distilled water and shaken. The mixture was allowed to stand for 30 min at room temperature, and shaken every 10 min. At the end of the 30 min, the mixture was filtered with the aid of the Whatman filter paper and the filtrate was used to determine the quantity of tannins present. 2 ml of the extract was introduced into a 50 ml volumetric flask. Similarly, 5 ml of standard tannic acid solution and 5 ml of distilled water were introduced into separate flasks to serve as standard and blank respectively. They were further diluted with 35 ml distilled water separately and 1ml of follin-Dennis reagent was added to each of the flasks, followed by 2.5 ml of saturated sodium carbonate solution (Na_2CO_3). The content of each flask was then made up to 50 ml (with distilled water) and incubated for 90 min at room temperature. The absorbance of the developed colour was measured at 620 nm wavelength in a spectrophotometer. Readings were taken with the reagent blank at zero. The tannin content was then calculated using the formular below

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1000} \times \frac{Vf}{Va} \times D$$

Where: W = Weight of sample analyzed. Au = Absorbance of the test sample. As = Absorbance of the standard solution in mg/ml. C = Concentration of standard solution in mg/ml. Vf = Total volume of extract. Va = Volume of extract analyzed D = Dilution factor where applicable.

Determination of phenols

Total phenol was determined by weighing 5 g of extract into a 250 ml titration flask, after which 100 ml n-hexane was introduced twice at 4 h interval. The mixture was filtered and the filtrate discarded. Subsequently, 50 ml diethyl ether was added twice, heated for 15 min after each drop and was cooled up to room temperature and was filtered into a separating funnel. This was followed by the addition of about 50 ml of the 10% NaOH solution twice and shaken vigorously. The mixture was washed thrice with 25 ml de-ionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 50 ml dichloro methane (DCM) twice to acidify the aqueous layer in the separating flask. Subsequently, the organic layer was collected, dried and then weighed [14].

Determination of flavonoids

In the determination of flavonoids, 5 g of extract was placed in 250 ml titration flask, prior to the addition of 100 ml of the 80% aqueous methanol at room temperature and shaken for 4 h with the aid of an electric shaker. The resulting solution was filtered through Whatman filter paper no. 42 (125 mm). This process was repeated and the filtrate transferred into a crucible and evaporated to dryness over a water bath and the quantity of flavonoids determined [15].

Determination of saponins

Saponins were determined by dissolving 5 g of extract in 100 ml of 20% ethanol. The suspension generated was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The filtrate and the residue were re-extracted using another 100 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a separating funnel and 20 ml of diethyl ether was introduced and shaken vigorously. While the aqueous layer was recovered, the ether layer was discarded. The process of purification was repeated and about 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath.

After evaporation, the samples were oven dried a constant weight. The saponin content was calculated in percentage [16].

Determination of alkaloids

Alkaloids was determined by placing 5 g of extract into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was subsequently introduced, covered and allowed to stand for 4 h. This was filtered and the filterate concentrated with the aid of a water bath in order to evaporate one-quarter of the original volume. Concentrated ammonium solution was added drop-wise to the resulting substance until precipitation was completed. The entire solution was allowed to settle and the precipitate was collected by filtration, after which it was weighed [16].

Determination of steroids

Exactly 1ml of test extract of steroid solution was transferred into 10 ml volumetric flasks. Sulphuric acid (4N, 2ml) and iron (III) chloride (0.5% w/v, 2 ml), were introduced, prior to the addition of potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The resulting mixture was subjected to heating in a water-bath at 70±2 ^{0}C for 30 minutes with occasional shaking and diluted to the mark with distilled water. The absorbance was read at 780 nm against the reagent blank to determine the quantity of steroids in the sample [17].

Hepatoprotectiveactivity

Experimental animal

Thirty five (35) adult male albino rats (150–180 g) were used in the study. Rats were housed in plastic cages, and were fed standard diet, and allowed free access to water. The animals were kept for one week to acclimatize prior to commencement of experiment which was performed in six groups of five rats per group as shown below:

Group 1: Normal control group, and was administered with (2ml distilled water) p.o for six consecutive days.

Group 2: was administered with (Carbon tetrachloride) CCl_4 without treatment.

Groups 3-5: were administered with 200mg/kg, 400mg/kg and 600mg/kg of extract respectively for six consecutive days p.o.

Group 6: received the standard drug silymarin (100 mg/kg per day in 0.05% CMC, p.o.) for six consecutive days.

Acute toxicity study

This was performed using the method described of Lorke [18]. In the first phase, rats were divided into 3 groups of 3 rats per group and were orally administered with 10mg, 100mg and 1000mg of the extract per kg body weight orally. The animals were observed for 24 hours for signs of toxicity, including death. In the absence of observable signs of toxicity, the second phase was initiated and constituted of 4 rats which were split into 4 groups of 1 rat per group. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

Induction of liver damage

Groups 2–6 were administered with CCl_4 in olive oil (1:1 v/v, 1.5 ml/kg, i.p.) as a single dose on the 7th day to induce liver damage. On day 8, all rats were sacrificed by cervical decapitation. Blood sample was collected and analysed accordingly.

Determination of liver functions

The collected blood sample was centrifuged at 3000 rpm for 10 min. The clear serum obtained was used to determine the levels of ALT, AST, ALP, and TB with the aid of commercial kits as described by Somasundaram *et al*. [19].

Statistical analysis

Data were expressed as Mean ±SD. The data were analysed using analysis of variance (ANOVA). The differences in mean were compared using Duncan Multiple Range Test. $P<0.05$ was considered significant.

Result

Table 1: Effect of the aqueous extract of *Ocimum gratissimum* (scent leaf) on serum hepatomarkers

	Serum Hepatomarkers				
Groups	**Treatment**	**AST(IU/L)**	**ALT(IU/L)**	**ALP(IU/L)**	**Bilirubin (mg/dL)**
Group 1	2ml distilled water	90.3±2.12^d	35.3±4.34^d	40.2±3.91^d	0.2-1.31^d
Group 2	CCl_4 only	110.1±2.34^a	45.4±2.9^a	63.4±4.86^a	2.6±0.87^a
Group 3	Extract$_{200mg/kg}$+ CCl_4	103.4±3.23^b	43.4±2.7^b	50.8±5.67^b	2.3±0.56^b
Group 4	Extract$_{400mg/kg}$+ CCl_4	98.6±2.86^c	40.2±3.2^c	47.8±6.56^c	1.8±4.92^c
Group 5	Extract$_{600mg/kg}$+ CCl_4	91.6±1.02^d	36.1±2.30^d	41.2±4.76^d	1.2±3.98^d
Group 6	silymarin$_{100mg/kg}$+ CCl_4	90.1±2.36^d	34.8±3.76^d	40.5±3.40^d	0.9±5.23^d
Values are means ± SD of five determinations. Values with different superscript in a column are significantly different ($P<0.05$)					

Table 2: Qualitative and Quantitative phytochemical analysis of *Occimum gratissimum* (scent leaf)

Phytochemicals	Qualitative	Quantitative (mg/100g)
Tannins	++	49.19±2.86
Saponins	++	42.28±2.85
Flavonoids	+	24.05±2.02
Steroids	++	38.89±1.06
Phenols	+	25.55±2.36
Alkaloids	+	21.02±1.03

Discussion

The liver is indispensably saddled with the task of metabolizing and detoxification of xenobiotics [20]. Its exposure to several endogenous and xenobiotic agents and consequent generation of intermediate and end products culminate to cellular death and constitute the principal causes of liver disease [21]. Phytochemicals are known for their therapeutic significance and have been implicated as key factor that determines the therapeutic strength of medicinal plants. Table 1.0 shows the effect of the aqueous extract of *Ocimum gratissimium* (scent leaf) on serum hepatomarkers. Administration of CCl_4 resulted in elevated levels of serum

hepatomarkers, an indication that the liver was damaged by the hepatotoxic agent. However, administration of 200mg/kg, 400mg/kg and 600mg/kg of aqueous leaf extract of *Ocimum gratissimum* gave rise to a dose dependent decrease in the levels of serum hepatomarkers. This may be as a result of the phytochemicals present in the plant [23] this result is consistent with the finding of Handa *et al*. [24] who reported the hepatoprotective potential of *Ocimum santum* against CCl_4 induced liver damage. Table 2.0 shows the qualitative and quantitative phytochemical analysis of aqueous leaf extract of *Ocimum gratissimium.* Phytochemicals reportedly present include tannins, saponins, flavonoids, steroids, phenols and alkaloids. While the amount of tannins in the extract was the highest among the reported phytochemicals, followed by saponnins, the amount of alkaloids was the least.

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