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Chemical and phytochemical constituents of *Elettaria cardamomum* seed extract

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Abstract
Preliminary phytochemical investigation of aqeous extract of *Elettaria cardamomum* revealed the presence of flavanoids, tannins, terpenoids, alkaloids, saponins and glycosides. Chemical test was carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents. The aqeous extract gave a clear zone of inhibition against the growth of the test bacteria (*Staphylococcus aureus*, *Escherichia colia*, *Pseudomona aeruginosa*, *Salmonellae typhi*, *Klebsiellae pneumonae*) at moderate to high concentrations, as well as a test on fungi (*Candida albicans*, *Aspergillus niger*, *Penicillium notatum* and *Rhizophus stolonifera*) at high concentration. The plant showed presence of 9 compounds in total which was identified using Gas Chromatographic-Mass Spectrometric (GC-MS) analysis of the aqeous fraction of *Elettaria cardamomum* with about six major constituents; Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid (44.04%), Acetic acid, methoxy-(.27.82%), Butane, 2-ethoxy-(5.64%), 2,3-Butanediol (14.13%), 7-Hexadecenoic acid, methyl ester, (Z)-(4.36%), Ethyl 9-hexadecenoate (4.00%). Some phytochemical constituents present in the extract in small amount include ethyl ester, 9-Octadecenoic acid, trans-2, 4-Decadienol, Methyl myristoleate, (Z)-9-Tetradecenoic acid. The GC-MS of the extract revealed various bioactive compounds in which their biological activity can be attributed to ethno medicinal uses of plant which was found Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid to be the principal compound in extract of the seeds and was responsible for antibacterial and antifungal infections. The presence of these phytochemicals is an indicator that the seed can be a potential source of precursors in the development of pharmaceutics. It is recommended that further study should carry on the characteristic of the isolated chemical constituents using nuclear magnetic resonance (^{1}H and ^{13}C), and HPLC.

Keywords: Phytochemical, GC-MS, *Elettaria cardamomum*, plant seed extract

1. Introductions
Ever since ancient times, in search for rescue for their disease, the people looked for drugs in nature. The beginnings of the medicinal plants' use were instinctive, as is the case with animals [1]. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Plants are enriched with various secondary metabolites with unique structural diversity and extraordinary biological properties which serves as the potential source of many important drugs. [2].

There is a vast variety of medicinal plants and *Elettaria cardamomum Maton* is one of a kind [3], it is commonly called as Green Cardamom in English and Choti Elaichi in Hindi and belongs to the family Zingiberaceae which is comprised 53 genera and almost 1300 different species which are evenly distributed in South and South-East Asia. The plants of this family have an effective role in the food, cosmetics, perfumery and pharmaceutical industries due to their color, taste, odor and extraordinary chemical profile that consists of alkaloids, carbohydrates, proteins, phenolic acids, flavonoids, and diarylheptanoids and essential oils. [4]. *Elettaria cardamomum* is an important spice and also entitled as Queen of Spices. It is categorized under the world's most expensive and commercially important spices and considered as 3rd most expensive spice after saffron and vanilla. It is believed that the botanical name of the plant has been originated from the popular South Indian language,

2. Materials and Methods

2.1 Sample collection and preparation

Fresh sample of *E. cardamomum* was collected from Maiduguri, Monday market, Borno state, Nigeria.

2.2 Sample Identification

Botanical identification and authentification of the plant seed was carried out in plant Biology Department, University of Ilorin, Kwara State, Nigeria.

2.3 Preparation of the plant seed extract

The samples were dried under control conditions to avoid many chemical change occurring. The dried seeds were pulverized and the resulting powdered samples were used for analysis. The dried seeds were grinded into smaller pieces using blinder machine. The powdered sample was weighed and extracted with distilled water.

2.4 Reagents and Solvent Used

The solvents were purchased from local chemical suppliers and purified by distillation before usage. Distilled water, methanol, chloroform, ammonia, acetic anhydride, ferric chloride, ammonium hydroxide, 1% dilute hydro chloric acid, sodium chloride, silica gel, ascorbic acid were used.

2.5 Apparatus

Separating funnel, cotton wool, aluminum foil, funnel, spatula, rotary evaporator, paper tapes, weighing balance, water bath, filter paper and beaker bottles were used. Test tubes and racks, beaker of different sizes, water bath, filter papers, cotton wool, aluminum foil and detergent were also used for phytochemical test.

2.6 Extraction procedure of *Elettaria cardamomum* seed extract

Successful Maceration method was used in the extraction of the whole seeds. The solvent used was aqeous which was firstly distilled to remove any form of impurities. The pulverized plant seed sample was soaked for seven days after which it was decanted filtered with the help of cotton wool to separate the plant extract. The extract was then fixed dried for some days to expel the remaining solvent. The crude extract becomes solidified and the extract is then weighed and sends for phytochemical analysis.

3. Phytochemical Screening of *E. cardamomum* seed extract

Phytochemical test was conducted at University of Ilorin chemistry Laboratory and was done on the aqueous extract of the powdered specimens using standard procedures to identify the constituents as described by [5].

3.1 Qualitative Phytochemical Screening of *E. cardamomum* seed extract

3.2Test for Alkaloids

Wagner Test**:** 2ml filtrate of sample with 1% HCL + steam. Then 1ml of solution with 6 drops of wagner's reagent. Brownish red precipitate indicated the presence of alkaloids. [6].

3.3 Test for Flavonoids

NaOH Test: The extract was treated with dilute NaOH, followed by addition of dilute HCL. A Yellow solution with NaOH, turns to colorless with dilute HCL indicate the presence of flavonoids [7].

3.4 Tst for Saponin

Frothing test**:** 0.5ml of sample filtrate with 5ml of distilled water and then shakes well. Persistence of frothing indicates the presence of saponin [8].

3.5 Test for Terpenoids

Liebermann-Burchardt test: 1ml of methanolic sample extract, add 1ml of chloroform, 2 to 3ml of acetic anhydride, 1 to 2 drops of concentrated sulphuric acid. A pink or red coloration indicates the presence of terpenoid [9].

3.6 Tests for Tannin

Braemer's test: 10% alcoholic ferric chloride will be added to 2-3ml of methanolic sample extract (1:1). Dark blue or greenish grey coloration of the solution indicates the presence of Tannin. [9]

3.7 Test for Cardiac glycosides

Keller-Kiliani test: 2ml sample filtrate with 1ml of glacial acetic acid, 1ml of ferric chloride and 1ml of concentrated sulphuric acid. A green-blue coloration of solution indicates the presence of cardiac glycosides [8].

4. Quantification of Phytochemical Screening of *E. cardamomum* seed extract

4.1 Determination of Alkaloids

1g of powdered *Elettaria cardamomum* seeds were extracted with 10 ml of methanol: (1:1v/v) mixture and solvent evaporated. The resultant residue was mixed with 20 ml of H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous then extracted with excess chloroform to obtain the alkaloid fraction. The chloroform extraction was repeated several times and the bulk of extract was concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to the initial weight of the sample powder [10].

4.2 Determination of Saponin

20 g of powdered extract was placed in a 500 ml flask containing 300 ml of 50% alcohol. The mixture was boiled under reflux for (30 minutes) and was immediately filtered while hot through a coarse filter paper. 2g of charcoal was added, to the content, boiled and filtered while hot. The extract was cooled (some saponin was separated) and an equal volume of acetone was added to complete the precipitation of saponins. The separated saponins were collected by decantation and dissolve in the least amount of boiling 95% alcohol and filtered while hot to remove any insoluble matter [10]. The filtrate was allowed to cool to room temperature there by resulting in precipitation of saponin. The separated saponin were collected by decantation and suspended in about 20 ml of alcohol and filtered. The filter paper was immediately transferred to a desiccator containing anhydrous calcium chloride and the saponins were left to dry. They were weighed with reference to the weight of extract used.

4.3 Determination of Tannin

0.1 g Powdered samples was put into a 100 ml conical flask and 50 ml of distilled water was added. The flask was gently heated to boiling for 1 hour, and the filtrate was collected in a 50 ml volumetric flask. The residue was washed several

times and the combined solution made to the volume with distilled water. To 10 ml of sample solution in a 50 ml volumetric flask, 2.5 ml of Folin-Denis reagent and 10 ml of $NaCO_3$ solution were added and made to volume with distilled water. The same treatment was made to the all the samples and the flasks were allowed to stand for 20 minutes after which optical density was measured at 760 nm using spectrophotometer [10].

4.4 Determination of Cardiac glycosides

1g of the sample was extracted with 10 ml 70% alcohol and the mixture filtered. From the filtrate, 8ml was transferred to a 100 ml volumetric flask and the volume was completed to the mark with distilled water. 8ml of the mixture was added to 8ml of 12.5% lead acetate (to precipitate resins, tannins and pigments). The mixture was shaken well, completed to the volume (100 ml) with distilled water and filtered. 50 ml of the filtrate was pipette into another 100 ml volumetric flask and 8ml of 4.7% disodium hydrogen phosphate (Na_2HPO_4) solution was added to precipitate excess lead. The mixture was made up to the volume with distilled water and mixed. This was filtered twice through filter paper. Baljets reagent (10 ml) was added to 10 ml of the purified filtrate. A blank sample of 10 ml of distilled water was also added to 10 ml baljets reagent. The two solutions were allowed to stand for one hour (time necessary for maximum colour development) a blank of 20 ml distilled water was used. The intensity of the colour was read at 495nm using spectrophotometer. The colour was stable for several hours [10].

4.5 Determination of Flavonoids

To determine flavonoids, 5 g of each plant sample was weighed in a 250 ml titration flask, and 100 ml of the 80% aqueous methanol was added at room temperature and shaken for 4 h in an electric shaker. The entire solution was filtered through Whatman filter paper no. 42 (125 mm) and again, this process was repeated. The filtrate as a whole was later transferred into a crucible and evaporated to dryness over a water bath and weighed [11].

4.6 Determination of Terpenoids

Dried plant extract 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hours. The extract after filtration, was extracted with 10 mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula [12].

(wi-wf/wi × 100)

5. GC-MS analysis

Chemical constituent was conducted at Federal department of Fisheries Laboratory. GC-MS analysis was carried out by injection (0.1 µL) of the seed oil on a QP-2010 instrument with a mass selective HP 597A detector fitted with Ulbon HR-1 capillary column (30 m x 0.25 mm, film thickness 0.25 µm). GC-MS operation condition split mode: carrier gas helium at a flow rate of 1.5 ml/min; temperature program 60-300 °C (100 C/min), injector temperature 300 °C and detector temperature 280 °C. The mass spectrometry conditioned was as follows: ionization voltage, 70 eV; emission current, 40 mA; mass range 0-400 Da, ion source temperature, 200 °C.

6. Results and Discussion

6.1 Phytochemical Screening of *E. cardamomum* seed extract

In the presence study, the preliminary phytochemical screening of the extract of *Elettaria cardamomum* seed revealed that they are good source of natural products. The results of phytochemical screening indicate that the plant seed extract contained alkaloids, saponin, tannin, glycoside, flavonoids and terpenoids. Terpenoids have medicinal properties such as anti-carcinogenic, anti-ulcer, anti-microbial, anti-malaria [13]. Flavonoids are hydroxylated phenolics substances, they are potent water soluble antioxidant which prevent oxidative cell damage and have strong anticancer activity and free radical scavenger also responsible for their therapeutic potency against wide array of microorganisms [14]. Glycoside is used in the treatment of heart diseases and as antibiotics. Saponins are group of naturally occurring plant which exhibit microorganism activities, anti-cancer properties. Tannins provide several advantages like having astrigents, antimicrobial, antioxidant, anticancer, virucide and anti-inflammatory properties. [15].

The role of alkaloids is quite important in plants as they have anti-bacterial, anti-fungal, antiviral, antiherbivory and herbicidal activities [16].

7. Gas Chromatograph-Mass Spectroscopy analysis of aqueous extracts of *Elettaria cardamomum* seed

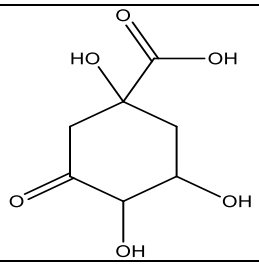
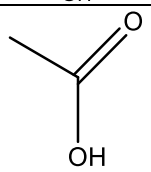
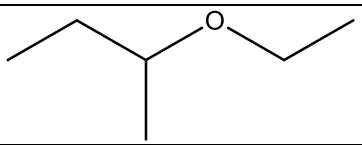
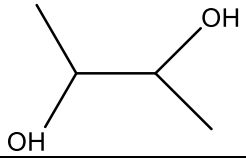
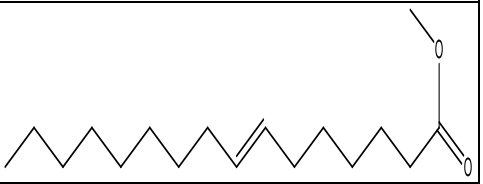
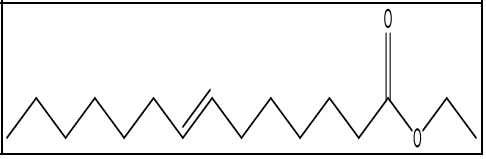
The presence of 9 compounds in total was identified using Gas Chromatograph-Mass Spectroscopic (GC-MS) analysis of the aqeous fraction of *Elettaria cardamomum* with about six major constituents; Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid (44.04%), Acetic acid, methoxy-(27.82%), Butane, 2-ethoxy-(5.64%), 2,3-Butanediol (14.13%), 7-Hexadecenoic acid, methyl ester, (Z)-(4.36%), Ethyl 9-hexadecenoate (4.00%). Some phytochemical constituents present in the extract in small amount include ethyl ester, 9-Octadecenoic acid, trans-2,4-Decadienol, Methyl myristoleate, (Z)-9-Tetradecenoic acid.

Table 1: Phytochemical screening of aqueous Extracts of *Elettaria cardamomum* seed

Constituents	Qualitative screening	Quantitative screening (%)
Flavonoids	+	16
Terpenoids	+	46
Saponin	+	1.5
Alkaloids	+	38
Tannins	+	
Steroids	-	
Glycoside	+	
Anthraquinones	-	

Keywords: + Present-Absence

Table 2: GC-MS Analysis of aqeous extracts of *Elettaria cardamomum* seed

S/N	Compound Name	Formula	Molecular Weight (g/mol)	% of abundance	Retention time (min)	Structure
1	Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	$C_7H_{10}O_6$	190	44.04	4.293	
2	Acetic acid	$C_2H_4O_2$	60	27.82	4.494	
3	Butane, 2-ethoxy-	$C_6H_{14}O$	102	5.64	4.736	
4	2,3-Butanediol	$C_4H_{10}O_2$	90	14.13	5.144	
5	7-Hexadecenoic acid, methyl ester, (Z)	$C_{17}H_{32}O_2$	268	4.36	17.076	
6	Ethyl 9-hexadecenoate	$C_{18}H_{34}O_2$	282	4.00	17.683	

8. Conclusion and Recommendation

The result of phytochemical screening of the crude extracts revealed the presence of alkaloids, saponins, flavonoids, terpenoids, cardiac glycoside and tannin. Therefore, it's an indicator that the seed can be a potential source of precursors in the development of pharmaceutics. The GC-MS of the extract revealed various bioactive compounds in which their biological activity can be attributed to ethno medicinal uses of plant was found Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid to be the principal compound in extract of the seeds, which is responsible for antibacterial and antifungal infections. The study is recommended further research should be conducted on the characterize the isolates using Nuclear Magnetic Resonance (^1H and ^{13}C), and HPLC.

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