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Phytochemical Analysis and Antibacterial Activity of Ocimum Gratissimum Used In Treatment of Cough

[1*]Bageya Godfrey, [2]Adaku Christopher, [3]Adia Madina Mohammad

[1*]Department of Chemistry, Faculty of Education, Busoga University P.O.Box 154, Iganga –Uganda
[2]Department of Chemistry, Faculty of Science Mbarara University of Science and Technology P.O.Box 1410, Mbarara – Uganda
[3]Department of Chemistry, College of Natural Sciences, School of Physical Sciences, Makerere University P.O.Box 7062, Kampala - Uganda.
Correspondence Author: E-mail: bageyag@yahoo.com, Tel: +256 782 022 685

Abstract: **The aim of the study was to analyse the Phytochemical constituents and antibacterial activity of the leaves of *ocimum gratissimum*. Phytochemical analysis of the extracts revealed the presence of antimicrobial principles including tannins, alkaloids, flavonoids, anthraquinone, cardiac glycoside, steroidal terpenes and phlobatanins. The antimicrobial efficacy of water, ethyl acetate and methanol extracts of dried leaf of *Ocimum gratissimum* against *pseudomonas aeruginosa* and *streptococcus pneumoniae* were determined using the Agar well diffusion method. The results revealed that the ethyl acetate extract was most potent, inhibiting all isolates with diameter zones of inhibition 13 mm, followed by methanol extract, and water extract being the least. The *S. pneumoniae* showed no resistance to the water extract. All the extracts inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs ranging between 125 - 1000 mg/ml, while MBCs gave a range of 13.5 - 250 mg/ml. The UV/Vis spectra of the crude extract revealed three major and three minor compounds. The HPLC chromatograms and IR spectra of *O.grattisimum* extract showed the presence of O=C=O, C=C=C (allenes), N=O, – N-H (amines) and -C=C- (alkenes) groups. These results suggested that probably a pyrrolizidine derivative of an alkaloid and eugenol of the type gamma eudesmol are present in the leaves of *Ocimum gratissimum*, which may provide a considerable evidence to justify *O. gratissimum* as a good candidate medicinal plant for the treatment of cough.**

***Keywords: Ocimum Gratissimum*, Phytochemical Analysis, Antibacterial activity.**

1. INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value (Kapoor et al., 1969). Traditional medicine is an important part of African cultures and local medicinal systems vary between different cultural groups and regions (Bandow et al., 2003). They are popular in developing countries on account of improved knowledge about the safety, efficacy and quality assurance of ethno-medicine.

In recent years, secondary plant metabolites (phytochemical constituents) have been extensively investigated as a source of medicinal agents. Thus, it is anticipated that phytochemical constituents with good antibacterial activity will be used for the treatment of bacterial infections like cough. This is because according to Arora et al (1998), the success of chemotherapy lies in the continuous search of new drugs to counter the challenges posed by resistant strains of micro-organisms. Studies indicate that in some plants there are many substances such as peptides, tannins, alkaloids, phenols, flavonoids and essential oils among others which could serve as sources of antimicrobial agent (Arora et al., 1998). These substances or compounds have potentially significant therapeutic applications against human pathogens (Arora et al., 1998). This investigation was designed to explore the chemical basis of the above claims and also to obtain accurate data on the biological activity of Ocimum gratissimum plant.

Cough remains one of the most serious diseases of the developing World, affecting about one to three million people annually. The devastating effects of cough are felt most profoundly in the poorest parts of the World especially in Africa. Uganda is one of the typical, highly cough-endemic, tropical African countries, where the disease is ranked one of the leading causes of morbidity and mortality (Bukenya-Ziraba *et al.,*1997). It accounts for 8 million episodes per year, 30 – 50% of outpatient visits at health facilities, 35% of hospital admissions, 9-14% of hospital deaths of which nearly half of these are in children less than 5 years of age and elderly (Kiwanuka, 2003, Yeka et al., 2012). Ugandans have been using cough syrups as one of the remedies against cough. However, such syrups can only prevent cough but not treating it. This does not fix the problem that caused the cough; it only suppresses the body's natural desire to heal (Bukenya-Ziraba *et al.,*1997).

2. MATERIALS AND METHODS

Plant source and identification

The fresh leaves of Ocimum gratissimum were collected from a garden in Igamba, Iganga Municipality Iganga District, East of Kampala, the capital city of Uganda about 118 Km. The leaves were packed in an air tight polythene bags and transported to a laboratory, chemistry Department, Makerere University. Botanical identification was done in the Department of Botany at Makerere University. The leaves were air dried under shade in the research laboratory for three weeks. The dried leaves were pounded with an electrical crushing machine into fine powder before extraction.

Sample extraction

The sample extraction procedures were carried out as described by Harbone, (1994).

The method employed was maceration (cold solvent extraction). The powdered materials were macerated in ethyl acetate, methanol and water as solvents. This method is sometimes known as cold sequential extraction.

Extraction with ethyl acetate (analytical grade; ACS,PhEur NF.)

Powdered sample (600g) was soaked in ethyl acetate (4.5 litres) in a conical flask of capacity 5 litres covered with aluminium foil (fay) with occasional shaking and left to stand for 24 hours. Extraction was repeated three more times on the same sample using (2.25, 2.00, and 1.80 litres) of ethyl acetate respectively as the conical flask was kept in a water bath at 40^{o}C. Each time the residual samples were spread for drying before the next extraction was done. The macerates obtained from each conical flask were filtered through cotton wool to obtain filtrates. The filtrates were collected and kept for concentration.

Extraction with methanol

Fresh powdered sample (600g) was soaked in methanol (2.7 litres) in another conical flask, corked and left to stay for 24 hours. The same procedure was repeated two more times using 2.0 and 1.8 litres of methanol as solvents. Each time the resulting liquid was filtered through cotton wool in a filter funnel to obtain a filtrate ready for concentration.

Extraction with water

Powdered sample (100g) was soaked in distilled water (250 ml) in a conical flask of capacity 1litre and put in a water bath kept at 35^{o}C for 8 hours. The liquid obtained was filtered through cotton wool in a filter funnel and the filtrate (120 ml) was obtained.

Concentration of the methanol, ethyl acetate and water extracts

Methanol and ethyl acetate filtrates were collected and concentrated to remove methanol and ethyl acetate in a round bottomed flask using a Buchi rotary vacuum evaporator (kept at 35 – 45^{o}C) to yield semi-solid masses whose weights were determined. The dried extracts were then transferred to small sample bottles which were placed in a desiccator containing anhydrous sodium sulphate to remove any traces of water molecules remaining in the extract. The extracts were then stored until required for bioassay.

Since butan-1-ol forms an eutectic mixture with water, it was added to the water filtrate and the content was concentrated using a Buchi rotary vacuum evaporator on a water bath at 50^{o}C running at a speed of 120 revolutions per minute.

The speed was to increase the rate at which concentration was being done. The higher the speed the less the time of concentration. The concentrated aqueous semi-solid sample (3.10 g) was kept in a fume chamber for 2 hours to remove all the butan-1-ol remnants from the extract. It was then kept for bioassay

Qualitative phytochemical analysis

Qualitative phytochemical analysis of the sample powder of the leaves was carried out for the active chemical constituents such as alkaloids, tannins, saponin, glycosides, terpenoids, steroids, flavonoids, and reducing sugar. The standard methods of analysis used were as reported by Aiyelaagbe and Osamudiamen (2009), Egwaikhide et al., (2007), Paris (1985) and Bruneton (1993).

Test for alkaloids 0.5g of each extract was dissolved in 3 drops of Dragendoffs reagent. An orange precipitate indicates the presence of alkaloid.

Test for Tannins 2g of each extract was dissolved in 10ml of distilled water in separate test tubes and 3 drops of 10% ferric chloride ($FeCl_3$) was added to 2ml of the solution. The occurrence of blackish-blue, green or blackish green coloration indicates the presence of tannins.

Test for flavonoids 0.2g of each extract was dissolved in 2ml of sodium hydroxide solution. The occurrence of a yellow solution which disappears on addition of Hcl acid indicates the presence of flavonoids.

Test for anthracenes. The presence of anthracene derivatives was detected using ammonia solution (50%, 10ml) (Bruneton.,1993). A chloroform extract was prepared from powdered sample (10 g) to detect the presence of anthracenes, O-heterosides and C-heterosides. Ammonia solution (50%, 1 ml) was added to the chloroform extract followed by stirring. Absence of a reddish colouration indicated no free anthracenes in Ocimum gratissimum .

Test for O-heterosides Distilled water (10 ml) and concentrated hydrochloric acid (1 ml) were added to chloroform extract residue in a test tube and heated for 5 minutes in a water bath. The test tube was cooled under running water. The content was filtered through cotton wool. Equal amounts of the filtrate and chloroform (5 ml) were put in a test tube, shaken and left to stand for phase separation. The organic phase was removed from the test tube and added to ammonia solution (50%, 1 ml). A red colouration of variable intensity was not observed indicating the absence of O-heterosides.

Testing for C-Heterosides. Distilled water (10 ml) and an aqueous solution of iron (III) chloride solution (10%, 1 ml) were added to the aqueous phase obtained previously in section 2.4.5 for O-heterosides. The mixture was heated for10 minutes in a water bath, and cooled under water. Chloroform (5 ml) was added, shaken and the test tube left to stand to allow separation of the organic phase. Ammonia solution (50%, 1 ml) was added to the organic phase. No red colouration of variable intensity was observed. Hence C-heterosides were absent (Bruneton., 1993).

Test for cardiac glycoside 0.5g of each extract was dissolved in 3ml of Fehling solution. A brick red precipitate indicates the presence of glycosides. This is the Keller-Killiani test.

Test for sterols and triterpenes. The presence of sterols and triterpenes were detected using concentrated sulphuric acid (Bruneton.,1993). An extract was prepared from the powdered sample (3 g) and ether (30 ml) by maceration for 1 hour. The prepared extract was used to detect carotenoids, sterols and coumarins.

Sterols and triterpenes detection was done by evaporating to dryness (60^0C) of the ether macerate (15 ml) and adding chloroform (5 ml) to the residue. The solution obtained was divided in two test tubes. Concentrated sulphuric acid (2 ml) was added to one of the test tubes, and the other acted as a control. The formation of violet ring where the phases met indicated the presence of sterols.

Carotenoids and terpene pigments were detected using antimony trichloride, SbCl3 (Bruneton.,1993). Ether extract (5 ml) was evaporated to dryness at 40oC and a saturated solution of antimony trichloride (2 drops) in chloroform (4 ml) added to residue. Partition of a blue colouration appeared, hence confirming the presence of carotenoids.

Test for Coumarins A portion of the ether extract (5 ml) (that remained after detection of sterols and triterpenes was evaporated to dryness on a water bath at 50^oC. To the residue that remained, distilled water (2 ml) at 50^oC was added followed by ammonia (25%, 1 ml) solution. Then the mixture was watched for 2 minutes under UV light at 366 nm and observation of an intense blue fluorescence indicated the presence of coumarins (Paris, 1985).

Test for saponins The presence of saponins was determined by frothing test. The sample powder (5 g) of ocimum gratissimum was vigorously shaken with distilled water (20 ml) in a test tube and allowed to stand for 10 minutes. No froth was observed indicating the absence of saponins (Kapoor et al., 1969).

Test for reducing compounds (Oses, holosides, mucilages and sugars) A decoction from the powdered plant sample (10 g) in distilled water (100 ml) was heated in a water bath at 70^{o}C for 15 minutes. Then to the cold decoction (5 ml) was added concentrated sulphuric acid (3 drops) followed by a saturated solution of thymol in ethanol (4 drops). No red colouration was observed indicating absence of oses and holosides. The absence of a flocculent precipitate on addition of pure ethanol (5 ml) to the decoction (15 ml) indicated the absence of mucilages in ocimum gratissimum (Bruneton.,1993).

Test for Reducing Sugars To the extract solution (1 ml), water (1ml) and Fehling's solution (5 drops) were added at 60^{o}C. No brick red precipitate was observed. This confirmed the absence of reducing sugars (Paris, 1985).

Testing for cyanogenic heterosides The powdered plant sample (1 g) was suspended in an equimolar solution of water and toluene (5 ml). A reagent strip impregnanted with Grignard reagent (2 g of picric acid and 20 g of sodium carbonate in 200ml of distilled water) was then added to the mixture. Absence of a red colouration on the strip indicated the absence of cyanogenic heterosides (Bruneton, 1993).

Test for cardiotonic heterosides This was determined by using potassium hydroxide in appropriate reagent which was prepared as follows. A chloroform phase was prepared from powdered sample (5 g). A mixture of ethanol (60%, 10 ml) and lead acetate solution (10%, 5 ml) were heated on a boiling water bath for 10 minutes. Chloroform (10 ml) was added to the mixture, shaken and left for phases to separate. The organic layer was removed and divided into three test tubes where each test tube had isopropanol (0.5 ml). To the first test tube, Baljet reagent (1 ml) was added. To the second test tube, Kedde reagent (1 ml) was added. (Bruneton,1993). To the third test tube, raymond-Marthoud reagent (1 ml) was added. Then potassium hydroxide (5%) in ethanol (5 drops) were added to each of the three test tubes.

After 10 minutes, no orange colouration in the first test tube, no red-violet colouration in the second tube, and a transient violet colouration in the third tube. This indicated the absence of cardiotonic heterosides in ocimum gratissimum (Bruneton, 1993).

Test for anthraquinone (Borntrger's test) To the sample extract (5 g) in a dry test tube, chloroform (20 ml) was added and shaken for 5 minutes. The mixture was filtered and the filtrate (10 ml) was shaken with an equal volume of ammonia solution (10%). A red colour was observed in the ammoniacal layer, indicating the presence of anthraquinone (Bruneton, 1993).

Test for phlobatannins 0.2g of each extract was boiled with an equal volume of 1% Hcl, the deposition of a red precipitate indicate the presence of phlobatannins.

Bioassay (Antibacterial Activity)

The crude extracts were tested against the bacteria which cause cough (pseudomonas aeruginosa and streptococcus pneumoniae). The methods used were disk diffusion agar and Agar well diffusion.

Disc diffusion assay

A preliminary screening for antibacterial activity was done by the disc diffusion method (Angeh, 2005). Disc diffusion susceptibility testing was performed using discs with a diameter of 6 mm.

The separate discs were impregnated with ethyl acetate, methanol, water crude extracts and ciprofloxacin (0.5mg/ml) as the standard drug for bacterial isolate. The discs were allowed to dry for 5 min. The plates were left at room temperature for about 10 minutes to allow the extract or the compounds to diffuse from the disc into the extract, and then incubated at 37°C for 24 hours after which the inhibition zones were measured and their diameters recorded as the zone of inhibition.

Agar well diffusion method

Bioassay was carried out on each of the three samples separately ie, the aqueous extract, methanol extract and ethyl acetate extract. Each sample had a duplicate plate. Agar well method was first used, followed by the broth dilution method to find out the minimum inhibition concentration (MIC). As described in the next section.

Then the third was to employ the minimum bactericidal concentration (MBC) to find out the minimum concentration of extracts that could kill these bacteria which cause cough i.e, pseudomonas aeruginosa and streptococcus pneumoniae bacteria.

MBC can also be determined by subculturing contents of the tubes onto antibiotic free solid medium and examining for bacterial growth.

Semi-solid nutrient agar plates were seeded with 1 ml of the standard inoculum dilution of the test bacterial isolates. The plates were swirled, allowing the inoculum to spread on the surface of the agar, and the excess discarded in a disinfectant jar. The plates were kept on the bench for about 20 minutes to set, and dried in the incubator for 30 min at 37ºC. With the aid of the sterile standard Cork borer, 6 wells were bored at equal distance around the plates. The bottoms of the wells were sealed with one drop of the sterile nutrient agar, to prevent diffusion of the extracts under the agar.

The 5th and 6th wells served as positive and negative controls. The negative control well was filled with sterile distilled water, however, for extracts, DMSO, served as the negative control. Ciprofloxacin was used as the positive control. Each prepared concentration of the extracts (0.2 ml) was aseptically introduced into Wells, 1 to 4. The plates were allowed on the bench for 40 min, for pre-diffusion and then incubated at 37ºC overnight.

The resulting zones of inhibition were measured using a ruler calibrated in millimeters. The average of the three readings was taken to be the zone of inhibition of the bacterial isolates in question at that particular concentration. Each experiment was performed in triplicates, repeated twice and were tabulated in table 4.2.

The minimal bactericidal concentrations were determined by sub culturing the contents of the tubes onto antibiotic-free solid medium and examining for bacterial growth.

MBCs were determined by first selecting tubes that showed no growth during MIC determination, a loopful from each tube was sub cultured onto extract free agar plates, incubated for further 18 hours at 37°C. The least concentration, at which no growth was observed, was noted as the MBC.

Mueller Hintron Agar (MHA) (500 millitre/microlitre) and the extract (500ul) were added to obtain one in two of the initial concentration; to this combination of standard test organism (500µl) was added to obtain a resultant concentration of one in four of the original concentration. The system was incubated at 37o C for 18 hours. The test tube with no visible growth was inoculated on Mueller Hinton Agar (MHA) for no growth. The concentration just next to the one that gave growth after 18 hours incubation was said to be the MBC. And the results are shown in table 4.3

Isolation of the compounds in ocimum gratissimum using Column Chromatography

Silica gel (240 g of 3-35 um) was mixed with hexane (750 cm3) to obtain a paste. The paste was gently poured into a column (50 cm diameter). It was then allowed to settle for about an hour. The sample (10g) was dissolved in a little ethyl acetate and then mixed with celite (11 g). The mixture was evaporated on the rotatory vapour to obtain a solid that was poured slowly onto the column. More hexane (1 litre) was poured onto the column and elution started and collection of fractions was done as the solvent system was being changed with time. The silica gel used was of analytical grade; silica gel granulation 32-63 micro m (230-400 mesh AsTM) S 0,032-0,063 mm for column chromatography.

Thin Layer Chromatography (TLC)

TLC of the collected fractions was done to allow the combination of different fractions to obtain 6 fractions. TLC of the extract was carried out on plates that were coated with a layer of silica gel of thickness (1 mm), using a solvent system of ethyl acetate: methanol: hexane (3:4:2). Fractions with similar retention factor (Rf) values were combined to give a total of six fractions labeled F1 to F6. The combined fractions from F1 to F6 were concentrated to give 0.20g, 0.15g, 0.21g, 0.23g, 0.30g, and 0.43g of powder respectively. The powdered samples were then subjected to the Ultraviolet-Visible spectroscopy for analysis.

Ultraviolet-Visible (UV/Vis) Spectroscopy procedure

Spectrophotometer cell was filled with methanol and placed in the spectrophotometer to measure the blank absorption of the solvent. The sample was filled in a different curvet and placed in the spectrophotometer. The spectrophotometer automatically measured the absorption of the compounds in the sample. The absorption range was set in the UV/Vis range of (200-700nm). The UV/Vis spectra were measured on a perkin Elmer lamba 5 spectrophotometer. This was done to characterize the chemical constituents of the most active compounds in the leaves of O. gratissimum.

High Performance Liquid Chromatography (HPLC) procedure

HPLC is an elaborate form of column chromatography, which uses packing material of small particle size and regular shape. Thus, high pressures are involved which can achieve acceptable flow rates. Ethyl acetate extract was injected (10 um) into the HPLC column of dimensions (25x4.6) cm. The wavelength UV detection was 254 nm. The gradient used water / acetonitrile, 0- 45 minutes. The sample (10 mg/ml) and the dissolving solvent was methanol and the temperature was set at 250C (Prescott, 2002).

3. RESULTS AND DISCUSSIONS

Phytochemical constituents

Phytochemical analysis of the dried leaf extract revealed the presence of the following antimicrobial principles: tannins, alkaloids, flavonoids, anthraquinoned, cardiac glycoside, steroidal terpenes and phlobatanins at different concentrations (Table 3.1). Among these phytochemicals tannins are very important with regard to cough.

Table 3.1: Phytochemical constituents from the qualitative analysis of a sample of *O.gratissimum* leaves.

Phytochemical constituents	Observations	Present(+) / Absent(-)
Alkaloids	A white-yellow precipitate	+
Flavonoids	A pink-orange colouration	+
Tannins	Red ppt insoluble in alcohol	+
O-heterosides	No red colouration of variable intensity.	_
C-heterosides	No red colouration of variable intensity	_
Cardiac glycosides	Greenish-blue colour within 3 minutes.	+
Sterols and triterpenes	Violet ring in a test tube.	+
Coumarins	Intense blue fluorescence under uv light.	+
Saponins	No froth formation.	-
Reducing sugars (oses, holosides, mucilages)	No brick-red colouration.	_
Cyanogenic heterosides	No red colouration of the strip.	_
Cardiotonic heterosides	No orange colouration in test tube 1, no red colouration in tube 2, and no transient violet colouration in test tube 3.	_
Anthraquinones	A red colour	+
Phlobatanins	A red precipitate observed.	+

Antibacterial activity of *Ocimum gratissimum* on *S.pneumoniae* and *Pseudomonas aeruginosa* .

O. gratssimum extract was screened for *S. pneumoniae* and *P. aeruginosa* using dimethyl sulfoxide as a diluent. As seen from table 3.2 below;

Table 3.2: Shows inhibition mean diameter values when different solvent extracts were subjected to *S. pneumonia* and *P. aeruginosa* with dimethyl sulfoxide (DMSO) as diluent.

	Mean diameter of inhibition (mm)		
Micro organisms	Concentration of Water extract (1000mg/ml)	Concentrationof Methanol extract (1000mg/ml)	Concentrationof Ethyl acetate extract (500mg/ml)
S.pneumoniae	00	13	13
P.auruginosa	11	10	13

Minimum inhibitory concentration (MIC) by broth dilution method for the water, methanol and ethylacetate extracts of the leaves of *Ocimum gratissimum*

The minimum inhibitory concentration assay was also employed to evaluate the effectiveness of the extracts to inhibit growth of the test microorganisms. All of them were subjected to concentrations of extracts ranging from (100 mg/ml up to 1000 mg/ml). The results obtained were recorded as in Table 3.3 below.

Table 3.3: Shows the minimum Inhibition concentration values (MICs) for *S. Pneumoniae* and *P. aeruginosa* for different extracts of *O. gratissimum* when the broth dilution method was used

Micro organisms	Water extract (mg/ml)	Methanol extract (mg/ml)	Ethyl acetate extract (mg/ml)
S.pneumoniae	250	250	125
P.auruginosa	1000	500	125

Minimum bactericidal concentration (MBC) for the water, methanol and ethylacetate extracts of the leaves of *Ocimum gratissimum*

MBCs gave 125 - 250 mg/ml for both the water & methanol extracts, whereas for the ethyl acetate extract it gave a range of 31.5 – 250 mg/ml. (Table 3.4).

Table 3.4: Shows the minimum bactericidal concentration values for *S. Pneumoniae* and *P. aeruginosa* for different extracts of *O. gratissimum*

	MBC in mg/ml.		
Micro organisms	**Water extract**	**Methanol extract**	**Ethyl acetate extract**
S.pneumoniae	250	125	250
P.auruginosa	125	250	31.5

Disc Diffusion

Sensitivity of the organisms was measured and recorded. The results in Table 3.5 shows that there is antimicrobial activity of the different concentrations of water, methanol and ethyl acetate extracts of *O.gratissimum* plant against two test pathogenic bacteria (*S. Pneumoniae* and *P. aeruginosa)* that cause cough.

Table 3.5: Shows the effect of water, methanol and ethyl acetate extracts of *O.gratissimum* on test microorganisms using disc diffusion method (inhibition zone in mm)

Micro organisms	**Water extract (50mg/ml)**	**Methanol extract (50mg/ml)**	**Ethyl acetate extract (50mg/ml)**
S.pneumoniae	4.4	5.0	10.5
P.auruginosa	4.0	7.1	12.4

The results indicated that ethyl acetate could have extracted more components from the plant that have high potency of medicinal value.

Column chromatography results

Separation of components from the ethyl acetate extract of *Ocimum gratissimum* was done using column chromatography.The gradient that consisted of different solvent system was used (Table 3.6). Altogether 48 fractions were collected using the gradient below;

Table 3.6: Solvent gradient system for ethyl acetate extract isolation

Hexane:Ethyl acetate (% of hexane)	**Volume of solvent collected /ml**
100	32
95	840
80	750
70	33
65	30
60	31
55	20
50	21
30	12

Ultraviolet-Visible (Uv/Vis) Spectrum

The absorption spectra of *Ocimum gratissimum* fractions F4 to F6 were measured in very dilute solution against a solvent blank (methanol) using an automatic recording spectrophotometer of model Pharmespac UV-1700 manufactured by SHIMADZU corporation.

Table 3.7: Results of Ultraviolet-Visible (Uv/Vis) Spectrum data

Peak	Wavelength (nm)	Absorbance
1	674.22	0.04610
2	670.31	0.59719
3	667.83	0.58811
4	660.08	0.20679
5	661.43	0.56643
6	603.57	0.51206

Basing on the visible spectral range wavelength in nm obtained, *O.gratissimum* is deemed to contain yellow flavonoids, alkaloids, and green chlorophyll at wavelengths (674.22 nm), (603.57 nm) and (661.43 nm) respectively. The findings of this study were in line with the work done by Kapoor *et al.*, (1969) and Arora *et al.*, (1998).

HPLC Spectrum for the isolated active compounds from fractions 1

Two peaks were obtained. The first peak eluted after 11 minutes and the second peak was eluted after 30 minutes. This retention time was measured from the time at which the sample was injected to the point at which the display showed maximum peak heights for the compounds present in the sample. Because different compounds have different retention times, it means that compound 'OG1' from fraction 1 had two different compounds.

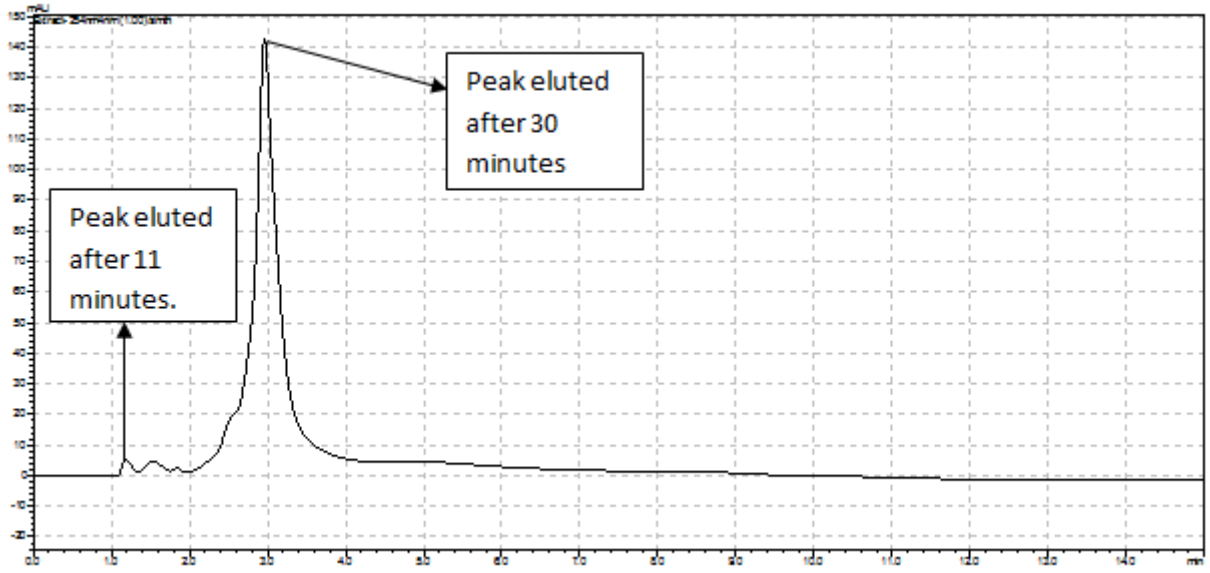


Figure 3.1: Shows the HPLC spectrum for 'Compound OG 1' from fraction 1

HPLC Spectrum for the isolated active compounds from fractions 2

One prominent peak was obtained after 32 minutes. The peak had an area of (1/2*9*150) = 675 Sq. units (calculated automatically by the computer connected to the display or screen.

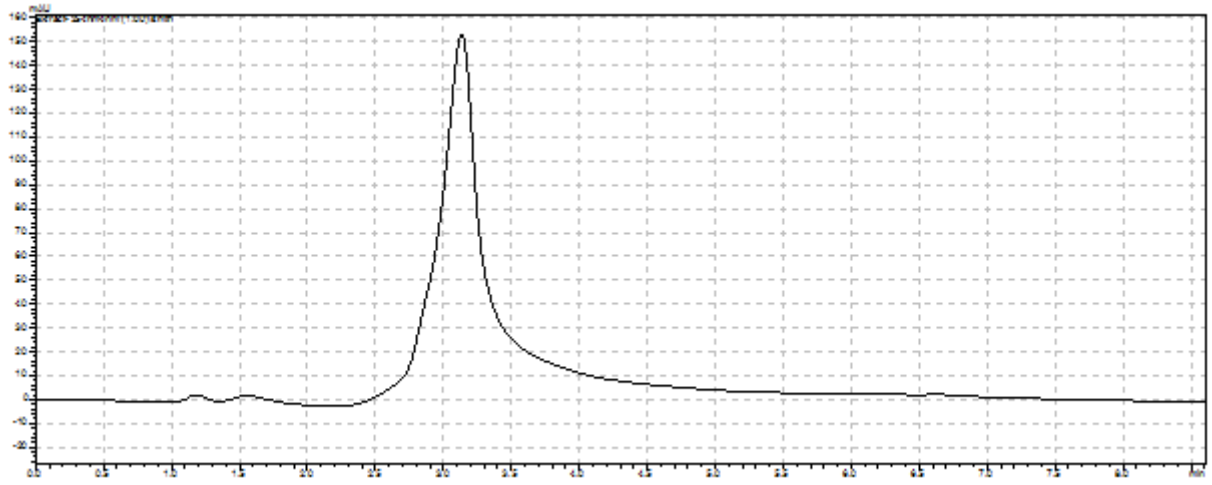


Figure 3.2: Shows the HPLC spectrum for 'Compound OG 2' from fraction 2

Infrared spectra for 'compound OG 1' from ethyl acetate extract

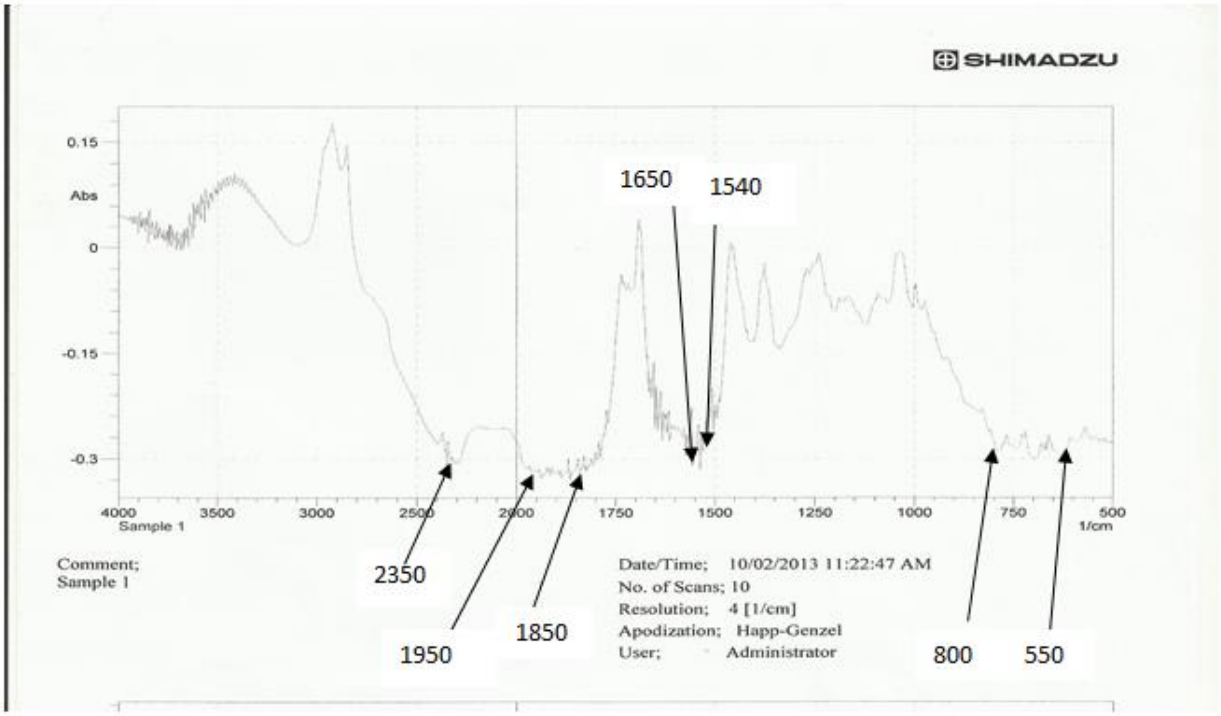
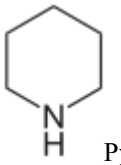


Figure 3.3: Shows Infrared spectra for 'compound OG 1' from ethyl acetate extract

The IR spectrum of *O.grattisimum* extract showed sharp bands at 3100 cm^{-1} and 3500 cm^{-1} indicating the O-H stretch in carboxylic acids. There was a strong absorption band at 2350 cm^{-1}, indicate stretching of O=C=O band of carbon dioxide groups. This has been confirmed from the earlier report of (Koushik *et al.*, 2009). The sharp peaks at 1950 cm^{-1} and 1850 cm^{-1} are due to the stretching of aliphatic carbon compounds, that is to say C=C=C in allene. The absorption band nearer to 1540 cm^{-1} indicates the presence of strong N=O strecting showing a nitro compound. The sharp peak at 1555 cm^{-1} indicates the presence of – N-H bend in amines and -C=C- stretching aliphatic at 1650 cm^{-1} in alkenes. Weak adsorption bands between 800 cm^{-1} and 550 cm^{-1} indicate the fingerprint region.

Also the IR spectrum suggested that 'compound **OG 1'** from fraction 1 obtained from ethyl acetate was not aromatic due to the absence of an aromatic C=C stretching aliphatic in the spectrum but could be cyclic. The compound also contained at least an N-H like bond.

Results of the qualitative phytochemical analsis suggested that the compound was a pyrrolizidine derivative of an alkaloid. The proposed possible structure could be;



Pyrrolizidine derivative.

Infrared spectrum for 'Compound OG 2' from ethyl acetate extract.

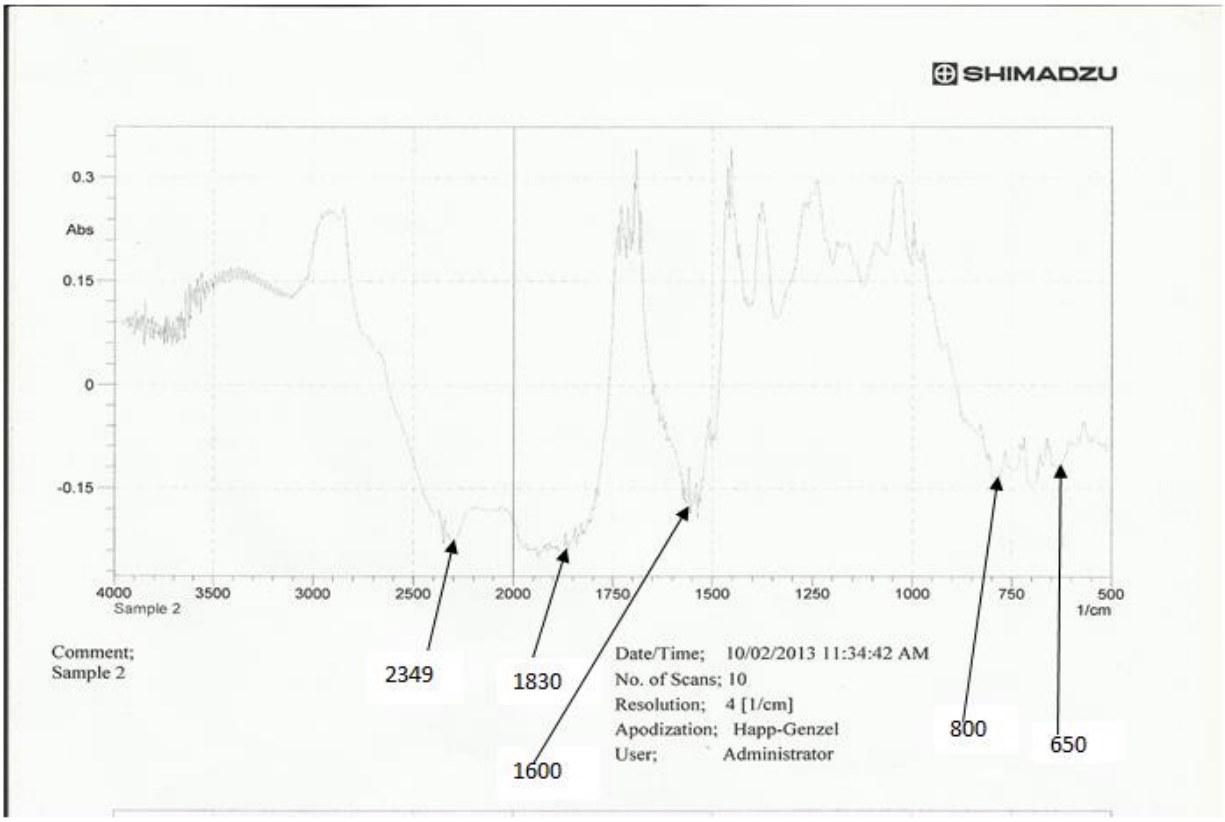
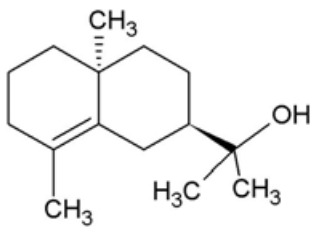


Figure 3.4: Shows Infrared spectra for 'compound OG 2' from ethyl acetate extract

The IR spectrum of *O. grattisimu* extract also showed sharp bands at 3100 cm^{-1} and 3500 cm^{-1} indicating the O-H stretch in carboxylic acids. The strong absorption band at 2349 cm^{-1},indicating stretching of O=C=O band of the carbon dioxide groups mainly from carboxylic acid groups. The broad peaks at 1820 cm^{-1} and 1830 cm^{-1} are due O=C-Cl sharp of the aliphatic acid chlorides stretching. The strong absorption bands at 1500 cm^{-1} indicate the presence of N=O strecthing from a nitro compound and the sharp band between 1600 cm^{-1} and 1585 cm^{-1} showed the C-C stretch in aromatics. The weak IR peak at 800 $cm^{-1}$and 650 cm^{-1} indicated the fingerprint region.

'Compound **OG 2'** of fraction 2 from the ethyl acetate extract was purely aromatic with O=C=O bonds suggesting the compound to be eugenol of the type Gamma eudesmol whose structure is;



Gamma eudesmol

4. CONCLUSION AND RECOMMENDATIONS

Conclusion:

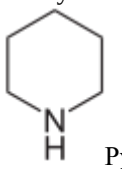
The presence of tannins also showed that the plant could be used as a purgative. It is also reportedly used in the treatment of cough, asthma and hay fever (Mekonnen, 2010). All the extracts were active against the different test bacteria but ethylacetate extract was more effective than mathanol and water extracts.

The pattern of inhibition, in general, also showed that both *S.pneumoniae* and *P. aeruginosa* were sensitive to ethyl acetate extract, and were very resistant to the water extract. The antimicrobial activity of the ethyl acetate extracts was more revealing using agar well diffusion than the disk diffusion method. This was clearly indicated by the size of the inhibition diameter, and the concentrations at which the inhibition was detected.

Thin layer chromatography of the fractions isolated from *O. gratissimum* leaves indicated the presence of fairly polar compounds as they were extracted by suitable solvent.

The presence of three prominent peaks on the UV/Vis spectrum indicated that *ocimum gratissimum* could be containing three abundant compounds. The other three minor peaks also show three minute compounds available in small concentrations.

It can also be concluded that *Ocimum grattisimum* contains the following functional groups; O=C=O, C=C=C-, N=O, –N-H, O=C-Cl indicating the presence of carbon dioxide, an allene, a nitro compound,an amine, and aliphatic acid chlorides respectively. The weak IR peak at 800 $cm^{-1}$and 650 cm^{-1} indicated the fingerprint region.

Also the IR spectrum suggested that compound 1 from fraction 1 obtained from ethyl acetate was not aromatic due to the absence of an aromatic C=C stretch on the spectrum but could be cyclic. The compound also contained at least an N-H like bond. Results of the qualitative phytochemical analysis suggested that the compound was a pyrrolizidine derivative of an alkaloid. The propossed structure was;  Pyrrolizidine derivative.

'Compound OG 2' of fraction 2 from the ethyl acetate extract was purely aromatic with O=C=O bonds suggesting the compound to be eugenol of the type gamma eudesmol. This was responsible for the anti-microbial activity.

Recommendations:

Future research should centre on the effects of different solvents and drying methods on the efficacy of the plant extracts as microbial agents. Also the sub acute toxic effects of the extracts and compunds from this medicinal plante be included as well.

More work should be done to show the synergistic effects of the compounds and the extracts, and in combination with the available drugs for treatment of cough.

The most active extracts can be subjected to isolation of the therapeutic antimicrobial principles and undergo further pharmacological evaluation, followed by nuclear magnetic resonance (NMR) for final structural elucidation of these active compounds since the clue about their spectral data is available.

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