Original Research Article

Evaluation of Phytochemical, Antimicrobial Activities and Toxicological Analysis of Scent Leaf (*Ocimum gratissimum* L.) Leaf Extracts

ABSTRACT

Aim: The phytochemical screening, antibacterial activities and in vivo toxicity of extracts of the leaves of scent leaf (*Ocimum gratissimum*) were investigated.
Methods: All the analyses were carried out using standard scientific procedures.
Results: The phytochemical analysis according to standard screening tests using conventional protocols revealed the presence anthraquinone, saponins, tannins, terpenoids and alkaloids, which were detected in methanol extracts analyzed. But, flavonoids, glycosides, phlobatannins and steroids were not detected in the methanol extracts analyzed. While, only flavonoids was detected in chloroform extract. All other phytochemicals were absent. The extract fractions generally exhibited slight antibacterial activities on *Staphylococcus aureus, Bacillus subtilis, Salmonella Typhi and Escherichia coli*. But, the extracts showed no effect against *Candida albicans*. The minimum inhibitory concentration of *Ocimum gratissimum* was determined with *Staphylococcus aureus* and *Bacillus cereus* recording MICs at lowest concentration (12.5mg/ml) of the methanol and chloroform extracts used. While, methanol and chloroform extracts were found to have recorded moderate activity *Salmonella Typhi* and *Escherichia coli* at the MIC of 50mg/ml. The methanol and chloroform extract recorded MBC of 50mg/ml on *Bacillus subtilis* and *Salmonella Typhi*. However, *Salmonella Typhi* was inhibited at the concentration of 100mg/ml of chloroform scent leaf extract. The MICs of *Candida albicans* were not determined in the methanol and chloroform scent leaf extract analyzed. The in vivo toxicity of *Ocimum gratissimum* extracts against albino rats revealed that the plant extracts were found to exhibit mild toxicity at higher doses, but the overall remark showed that the plant extract was safe at various concentrations.
Conclusions: The plant can be used in the treatment of various diseases caused by the test microbes.

Keywords: Ocimum gratissimum, Phytochemistry, Toxicology, Antimicrobial, Minimum Inhibitory Concentration

1. INTRODUCTION

The use of synthetic and chemically based drugs in the treatment of various bacterial diseases leads to a long-term complication to the recipients, since most of the chemically synthetic drugs possess serious side effects that might make their disadvantages to outweigh their advantages, because some chemical constituents can be carcinogenic, cytolytic or cytotoxic when administered in large doses. Therefore, the use ethnomedicinal or natural plants as substitutes of chemically synthetic drugs is imperative in order to prevent negative side effects and toxicity of the orthodox drugs with the natural means of treatment.

Research on herbs, spices and medicinal plants originated with our ancestors thousands of years ago it's now a popular subject that appeals to life scientist due to the problems of drugs resistance and cost of drugs. Scientist in Africa and other developing countries are conducting researches into local plants which are used in traditional medicine [1].

Majority of chemically synthesized drugs have serious adverse effects to the recipients, which may lead to temporary or permanent disability and incapacitations. Also, gastrointestinal disorders, dysentery, diarrhea and candidiasis are very serious infections that can lead to frequent morbidity and mortality in tropical countries like Nigeria. These disorders are serious diseases that can affect many people at various stages of their lives causing distress and discomfort. Sometimes, the disorder can even lead to hospitalization. Majority of the etiologic agents of gastroenteritis were found to be resistant to variety orthodox drugs, as such complementary and alternative therapy is the only future to the success of pharmacology [2].

Treatment of diseases has always been associated with administration of drugs gotten from plants, animals and mineral sources. The use of plants or herbs extract in the treatment of human ailments is a very ancient art. Investigation of African medicinal plants for antimicrobial activities ranks highest among biological test carried in many plants and their extracts [3]. Herbal medicines tend to look primitive and unscientific when compared to synthetic (conventional) drugs, which are thought to be more reliable than those made from plants. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries for primary health care [4].

The perennial plant *Ocimum gratissimum* (scent leaf) is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae and it is the most abundant of the genus Oscimum. In the southern part of Nigeria, the plant is called "*effinrin-nia*" by the Yoruba speaking tribe, "*Nchonwu*" in Igbo, while in the northern part of Nigeria, the Hausas call it "*Daidoya*" [5]. These perennial plants are woody at the base. The plants are found to grow somewhere around 1-3 metres in height. The stems of these plants are dark brown in color bearing leaves from top to bottom. The leaves are narrow and oval in shape growing 5-13 cm in length and have 3-9 cm width, but sometimes the leaves are green in color. The flowers are pale yellow in color and the plants give out a sweet scent of camphor [6].

A lot of research has been carried out on the herb, *Ocimum gratissimum*. Though, literature search has not revealed any study on the effect of *Ocimum gratissimum* on the histology of the lung of albino rat, some works closely related to it has been documented. This plant is used by herbalists to treat a variety of maladies, from bacterial infections and diabetes to pain and liver damage. Several studies have been performed that lend credence to herbalist use of this plant for treating diarrhoea and other gastrointestinal infections [7].

In folk medicine, *Ocimum gratissimum* is extensively used throughout West Africa as a febrifuge, anti- malarial and anti-convulsant. The crushed leaf juice is used in the treatment of convulsion, stomach pain and catarrh. Oil from the leaves have been found to possess antiseptics, antibacterial and antifungal activities [7]. In the coastal area of Nigeria, the plant is used in the treatment of epilepsy, high fever and diarrhea. While in the savannah areas decoctions of the leaves are used to treat mental illness [8].

Ocimum gratissimum is used by the Igbos of southern Nigeria in the management of the baby's cord. It is believed to keep the baby's cord and wound surface sterile. It is used in the treatment of fungal infections, fever, cold and catarrh. Clinical trials in creams formulated against dermatological disease have yielded favorable results. Several tonics are produced from these herbs and used in treating as skin infections, bronchitis and conjunctivitis.

Antiseptics are produced from the herb that treats well in dressing wounds. Crushed leaves are extracted to form remedies for cough. The plant roots act as sedatives for children [9].

Ocimum tea is an infused form of the herb used in treating fever and diaphoresis. The volatile oil acts as a good antimicrobial agent, Nutritional importance of this plant centers on its usefulness as a seasoning because of its aromatic flavor. It is used as a flavour in spicing meat products [7].

In Congo, scent leaf decoction is used for diarrhoea, gonorrhoea infection, vaginal douches for vaginitis and used in treatment of mental illness. Extracts of scent leaves has also been reported to have lowered blood pressure, strong insect repellent effects and kill many micro-organisms that cause diseases, including candida. Several studies have confirmed the efficacy of *Ocimum gratissimum* in treating various conditions after it is condensed into an essential oil. This is largely credited to the plant's high concentrations of a phenylpropene compound called eugenol [7]. Studies suggest that *Ocimum gratissimum* effectively combats several types of invasive bacteria. These range from shigella and salmonella to Escherichia and Proteus strains. The oils of the plant also were effective in fighting strains of *E. coli*, dysentery and typhoid. Some research also confirms that *Ocimum gratissimum* is effective in treating various veterinary problems, from killing worms in goats to increasing libido in laboratory mice. The plant extracts can be used in relaxing intestinal muscles. The herbaceous plant has anti-nociceptive effects. It is effective in reducing blood glucose, and it is helpful in preventing convulsions and seizures [7].

Use of natural means of treating infectious diseases will be the future of pharmacology in the development of effective drugs with low or no toxicity to the recipient. Scent leaf has been used traditionally for the treatment of gastrointestinal disorders, dysentery, diarrhea and candidiasis caused by various gastrointestinal inhabiting microorganisms. Direct oral administration of raw scent leaf juice has been used for long in many tribes in order to treat gastrointestinal disorders, dysentery, diarrhea and candidiasis of varying degrees. Accessibility; traditional medicine is more accessible to most population in the world than orthodox medicine. In fact it is reported that 60-80% of the population of every country of the developing countries has to rely on traditional or indigenous forms of medicine [10]. Therefore this research was designed to determine the antimicrobial effect of scent leaf (*Ocimum gratissimum*) and to identify the common phytochemicals constituents of scent leaf that may be inhibitory to gastrointestinal pathogens as well as the in vivo toxicity level of the extract. The research findings can further be used by the clinics, pharmaceutical industries and other medical sectors in tackling the menace of the aforementioned disorders.

2. MATERIAL AND METHODS

2.1 Plant Materials

Fresh plant leaves of scent leaf (*Ocimum gratissimum*) was collected from Samaru market, Zaria, and taxonomically characterized at the herbarium section of Biological Science Department, Usmanu Danfodiyo University, Sokoto with the aid of botanical keys [11]. The leaves were thoroughly washed through running water and dried under shade for 4-6 days. The parts of the plants were collected fresh (Fig. 1), healthy and free from organic contaminants that may interfere with the substances of interest by washing them with clean water [12]. The dried leaves were ground into powdered form. The leaf powder was stored and sealed in labeled sterile reagent bottles for further use. The bioactive components were extracted using the methods of Akerele et al. [13].

2.2 Preparation of Plant Extract

Plant extracts was prepared by the method of Alade and Irobi [14] with minor modifications. To study the antibacterial potential of *Ocimum gratissimum* polar solvent such as methanol and non-polar solvent such as chloroform were used. A mass of 30 g of dried powder was weighed on a weighing balance (Mettle 166®) and was extracted by 100 ml of each solvent by using Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent until the aqueous content evaporated completely as adopted by Lin et al. [15]. At the end of extraction, the extract was filtered through Whatman No. 1 filter paper. The dry extract was collected and weighed in varying concentration and kept in an airtight container at 4°C.

Fig. 1: Fresh scent leaf (*Ocimum gratissimum* L.) leaves

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical investigation was carried out for methanolic and chloroform extracts of scent leaf (*Ocimum gratissimum*) for the detection of various phyto-constituents by using standard procedures to identify the constituents [16-18].

2.3.1. Test for the presence of Alkaloids (Wagner's test)

Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of KI in 100 ml of water. The plant extract was prepared by taking 500 mg of plant material in 500 ml of methanol for 20 minutes, on a water bath. The extract was then be filtered off and allowed to cool. The 2 ml plant extract was then taken and treated with few drops of Wagner's reagent. A reddish brown coloured precipitate indicates the presence of alkaloids.

2.3.2. Test for the presence of Anthraquinone (Borntrager's test)

About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It will be filtered and allowed to cool. Equal volume of chloroform was added to the filtrate. Few drops of 10 percent ammonia will be added to the mixture and heated. Formation of rose-pink colour indicates the presence of anthraquinones.

2.3.3. Test for the presence of Flavonoids

The crude powder of dried plant was heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution and a yellow colouration of solution was observed.

2.3.4. Test for the presence of Phlobatannins

An aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid (HCl) to observe the deposition of red precipitate.

2.3.5. Test for the presence of Glycosides (Fehling's test)

The crude plant powder of 0.5 g was dissolved in 5 ml of methanol. The 2 ml of this solution was taken and to it 10 ml of 50% HCl was added. The mixture was heated in a boiling water bath for 30 min. To the mixture 5 ml of Fehling's solution was added and the mixture was boiled for another 5 min to observe a brick red precipitate as an indication for the presence of glycosides.

2.3.6. Test for the presence of Saponins (Frothing test)

About 0.2 g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy layer of small bubbles) showed the presence of saponins.

2.3.7. Test for the presence of Steroids (Salkowski test)

The 1 ml of plant extract was taken and to it few drops of concentrated sulphuric was added. The presence of red colouration indicates the presence of steroids.

2.3.8. Test for the presence of Tannins (Ferric chloride test)

The presence of tannins was tested in 0.5 g of the crude plant powder and was stirred with 10 ml of distilled water. The extract was filtered and ferric chloride reagent was added to the filtrate, a blue-black precipitate was taken as an evidence for the presence of tannin.

2.3.9. Test for the presence of Terpenoids (Salkowski test)

The presence of terpenoids was tested in 0.2 g of the extract of the plant sample and mixed with 2 ml of chloroform and concentrated sulphuric acid (3 ml H_2SO_4) was added carefully to

form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

2.4 Cultivation and Management of Bacterial Pathogens

Pre-characterized clinical isolates of *Candida albicans*, *Bacillus subtilis, Salmonella Typhi, Staphylococcus aureus* and *Escherichia coli* were collected from Medical Microbiology laboratory, Ahmadu Bello University Teaching Hospital, Zaria. All the isolates was checked for purity and maintained in slants of nutrient agar for the bacteria and Sabouraud's Dextrose agar for the fungi.

2.5 Antimicrobial Assay of the Extract

The antimicrobial assay of the extract was carried out in a microbiology laboratory under a standard and controlled experimental condition. This was done to ascertain which of the extracts will have a maximum microorganism inhibitory property which can serve as a tool to determine which of the extract can be potentially active in the synthesis of antimicrobial drugs to combat diseases caused by microorganism. Various bacterial isolates were used for the study which includes: *Staphylococcus aureus, Bacillus subtilis, Salmonella Typhi, Escherichia coli* and *Candida albicans*.

A mass of 0.5g of the extract was weighed and dissolved in $10cm^3$ of distilled water so as to obtain a concentration of 50mg/ml of the extract. This was the initial concentration of the extract used to check the antimicrobial effects of the extracts. Mueller Hinton agar was used as the growth medium for the bacterial species; while, Sabouraud's Dextrose agar was used for *Candida albicans*. The media were prepared according to manufacturer's instructions, sterilized at 121°C for 15minutes [19]. The sterilized molten media were then poured into sterilized Petri dishes, the plates were allowed to cool and solidify in accordance with CLSI [20] specifications.

Well-diffusion method was used for screening the extracts. The sterilized medium was seeded with $0.1cm^3$ of the standard inoculums. It was then spread evenly over the surface of the medium by using a sterile swab. Using a sterilized cork-borer of 6mm in diameter, a well was excavated at the center of each inoculated medium. A volume of 0.1ml of the solution of the extract of concentration of 50mg/ml was then introduced into each of the dug wells on the medium. The inoculated medium was allowed to absorb the extract and then incubated at 35°C for 24 hours, after which each plates was observed for zone of inhibition, the zone was measured with a transparent meter rule and the results was recorded in millimetres [20]. The same procedure was used for the other extracts using different concentrations. Ciprofloxacin (30µg) was used as positive control for bacteria, while Econazole (30µg) was used as positive control for *Candida albicans*.

2.6 Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined using the broth dilution method. Mueller Hinton agar was prepared and $10cm^3$ was dispensed into a test tube, sterilized at 121°C for 15 minutes and then allowed to cool. McFarland's turbidity standard scale number 0.5 was used to give a turbid solution. Normal saline was prepared, and $100cm^3$ was dispensed into sterile test tube and the test microbe was inoculated at 37°C for 6 hours. Dilution of the test microbe was done in the normal saline until the turbidity matches that of the McFarland's scale by visual comparison [19]. At this point, the test microbes might have a concentration of about 1.5×10^8 cfu/ml. Two fold serial dilution of the extract in the sterile broth was made to obtain the concentration of 50mg/ml, 25mg/ml and 12.5mg/ml. The initial concentration was obtained by dissolving 0.5g of the extract in the broths; $0.1cm^3$ of the test

microbes in the normal saline was then introduced into the different concentrations of the extract in the broth which was incubated at 37°C for 24 hours after which each test tube was observed for turbidity. The lowest concentration of the extracts in the broth which might show no turbidity was recorded as the minimum inhibitory concentration [21].

2.7 Animal Experimentation (in vivo Toxicity Testing)

Exactly 18 albino rats of both sexes weighing between 125-375g weights were obtained from the animal house, Ahmadu Bello University, Zaria. They were kept in plastic cages with iron nettings at the experimental laboratory, Nigeria Institute of Leather and Science Technology Samaru, Zaria. They were allowed to acclimatize for a period of two weeks and fed with growers mash. They were also given tap water at pleasure using water bottles.

2.8 Administration of Extract

Following reception of ethical approval from the Ethical Committee of Regulating Animal Vivisection, Ahmadu Bello University, Zaria. The albino rats were grouped into six groups, with each group comprising of three animals according to the bodyweight of the rats. Group 5 serves as normal control, while groups 1, 2, 3 and 4 received the crude extracts of *Ocimum gratissimum* at various concentrations (100mg/ml, 50mg/ml and 25mg/ml) at doses of 5ml/kg, 3ml/kg and 2ml/kg body weights respectively. Group 6 served as positive control. Administration of extract was carried out orally by the use of calibrated syringes in order to determine the actual administered dose. Group 5 animals were given nothing except normal feed and water, while, group 6 were given hepatotoxic substance, acetaminophen (200mg/kg of bodyweight) to serve as positive control. The animals that died were dissected and their visceral organs were observed for damage. The animals also received their doses 3 times weekly for a period of three weeks and their behaviors were observed for any physiological changes as adopted by Sanmugapriya et al. [22]. After three weeks analysis, the number of laboratory animals survived were noted and recorded. The principles of laboratory animal care (NIH publication no. 85-23, revised 1985) were duly followed throughout the period of the experimentations.

3. RESULTS AND DISCUSSION

Table 1 showed the phytochemical screening of scent leaf extract. Phytochemicals such as anthraquinone, saponins, tannins, terpenoids and alkaloids, were detected in methanol extracts analyzed. But, flavonoids,glycosides, phlobatannins and steroids were not in the methanol extracts analyzed. While, only flavonoids was detected in chloroform extract. All other phytochemicals were absent.

Table 1. Preliminary qualitative phytochemical analysis of *Ocimum gratissimum* extracts

Phytochemicals	Methanol	Chloroform
Anthraquinone	+	−
Flavonoids	−	+
Saponins	+	−
Tannins	+	−
Phlobatannins	−	−

Steroids	−	−
Terpenoids	+	−
Glycosides	−	−
Alkaloids	+	−

*+ = *presence; − = absence*

Based on the phytochemical analysis findings of the research study, it was found out that phytochemicals such as anthraquinone, saponins, tannins, terpenoids and alkaloids, were detected in methanol extracts analyzed. But, flavonoids, glycosides, phlobatannins and steroids were not in the methanol extracts analyzed. While, only flavonoid was detected in chloroform extract. All other phytochemicals were absent (Table 1). This may be due to polarity and higher colour intensity usually observed in the methanolic extracts that indicates presence of terpenoids and saponins with absence of flavonoids, steroids and phlobatannins [23].

Table 2 showed the effect of scent leaf methanol and chloroform extracts at different concentration recorded high antimicrobial effect against *Staphylococcus aureus, Bacillus subtilis Salmonella Typhi* and *Escherichia coli*. But, the extracts showed no effect against *Candida albicans*.

Based on the antimicrobial activities of methanol and chloroform extracts of *Ocimum gratissimum*, at different concentrations, the extracts recorded high antimicrobial effect against *Staphylococcus aureus, Bacillus subtilis Salmonella Typhi* and *Escherichia coli*. But, the extracts showed no effect against *Candida albicans* (Table 2). A slight antimicrobial activity of the extracts of *Ocimum gratissimum* against *Escherichia coli* may be due to the polarity nature of chloroform used for the extraction. This agrees with the previous study by Parek et al. [24], who reported that most plants are extracted by traditional healers using various solvents, and such solvent extracts prominently exhibit antibacterial activity more than the others.

298 **Table 2. Antimicrobial effects of *Ocimum gratissimum* extracts in millimetres (mm)**
299

TEST ORGANISMS	Methanol extract (mg/ml)				Chloroform extract (mg/ml)				Controls (mg/ml)	
	100	**50**	**25**	**12.5**	**100**	**50**	**25**	**12.5**	**Ciprofloxacin (10µg)**	**Econazole (30µg)**
Staphylococcus aureus	23	20	18	16	22	18	16	14	35	−
Salmonella Typhi	20	18	−	−	18	14	−	−	38	−
Bacillus subtilis	19	16	14	12	21	18	15	13	32	−
Escherichia coli	17	13	−	−	19	13	−	−	37	−
Candida albicans	−	−	−	−	−	−	−	−	−	37

300 − = not determined

Table 3 showed that the minimum inhibitory concentration of methanol and chloroform extracts were determined with *Staphylococcus aureus* and *Bacillus cereus* recording MICs at lowest concentration (12.5mg/ml) of the methanol and chloroform extracts used. While, methanol and chloroform extracts were found to have recorded moderate activity *Salmonella Typhi* and *Escherichia coli* at the MIC of 50mg/ml. The methanol and chloroform extract recorded MBC of 50mg/ml on *Bacillus subtilis* and *Salmonella Typhi*. However, *Salmonella Typhi* was inhibited at the concentration of 100mg/ml of chloroform scent leaf extract. The MICs of *Candida albicans* were not determined in the methanol and chloroform scent leaf extract analyzed.

The minimum inhibitory concentration of methanol and chloroform extracts were determined with *Staphylococcus aureus* and *Bacillus cereus* recording MICs at lowest concentration (12.5mg/ml) of the methanol and chloroform extracts used. While, methanol and chloroform extracts were found to have recorded moderate activity *Salmonella Typhi* and *Escherichia coli* at the MIC of 50mg/ml. The methanol and chloroform extract recorded MBC of 50mg/ml on *Bacillus subtilis* and *Salmonella Typhi* (Table 3). This result confirmed the findings of Pawar and Pandit [25] who worked on antibacterial activity of leaf extracts of *Ocimum sanctum* L. against gram positive bacteria.

Table 3. Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Extract against the Test Organisms

	MIC (mg/ml)		MBC (mg/ml)	
TEST ORGANISMS	Methanol extract	Chloroform extract	Methanol extract	Chloroform extract
Staphylococcus aureus	12.5	12.5	25	−
Salmonella Typhi	50	50	50	100
Escherichia coli	50	50	−	−
Bacillus subtilis	12.5	12.5	50	50
**Candida albicans*	ND	ND	ND	ND

ND = not determined; − = absence; MBC = Minimum Bactericidal Concentration; MIC = Minimum Inhibitory Concentration; * = Minimum Fungicidal Concentration

Table 4 shows the in vivo toxicity of *Ocimum gratissimum* extracts against albino rats. The plant extracts were found to exhibit mild toxicity at higher doses, but the overall remark showed that the plant extract was safe at various concentrations used when compared to hepatotoxic substance such as acetaminophen. All the animals tested with the crude extract of *Ocimum gratissimum* survived.

The in vivo toxicity of *Ocimum gratissimum* extracts against albino rats. The plant extracts were found to exhibit mild toxicity at higher doses, but the overall remark showed that the plant extract was safe at various concentrations (Table 4). This is in conformity with the findings of Rasayana [26], who reported that *Ocimum gratissimum* is harmless edible plant and it is considered in Ayurvedic system as a type of “elixir of life”.

336 **Table 4. In vivo Toxicological Testing of *Ocimum gratissimum* extracts**
337

Groups	Number of albiono rats	Dosage (mg/ml) Administered according to body weight	Observations	After three weeks of observations
Group 1	3	100, 50 and 25	After administration of 100mg/ml, they became weak. While, with 50mg/ml and 25mg/ml, they showed no effect	They all survived
Group 2	3	100, 50 and 25	Administration of 100mg/l resulted into salivation. While, 50mg/ml and 25mg/ml showed no effects	They all survived
Group 3	3	100, 50 and 25	After administration of 100mg/ml and 50mg/ml, they showed sluggish movement. While, after administration of the extract at 25mg/ml, they showed no effect	They all survived
Group 4	3	100, 50 and 25	No any noticeable sign	They all survived
Normal Control	3	Normal feed and water given	Normal. No any noticeable sign	They all survived
Positive control	3	Oral administration of 200mg/ml of acetaminophen	Sluggishness, bloody stooling and restlessness	They all died within the week with liver damage

4. CONCLUSION

Herbal medicines that are in use since ancient times can be used to fight microbial diseases and infections. The uses of indigenous medicinal plants have played an important role in the traditional therapy. A lot of work has been done on scent leaf, still researchers are engaged to investigate antimicrobial effect of scent leaf on different pathogenic strains. As nowadays antimicrobial resistance is a big and serious problem for researchers, so the medicinal plants, herbs and aromatic plants could be used as best alternate of medicine, and having ability to kill different pathogenic bacterial strains. Main advantage of using herbs is that they don't have any side effect as allopathic medicine has. Not only a single part of any plant is useful but whole plant has medicinal properties such as *Ocimum gratissimum* leaves having ability to kill different bacterial strains. The extract has less toxicity compared to other orthodox antimicrobials whose overdose can cause hepatotoxicity such as acetaminophen.

This work has successfully revealed the presence of phytochemicals that are present in *Ocimum gratissimum* plant, which displayed high antimicrobial activity at lower dosage with mild or no toxicity to the recipient laboratory animals. Other solvents should be used in the future studies in order to extract more active phytochemicals that possess antimicrobial activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

REFERENCES

1. Nnaemeka, Achinewu S. C., Aniena M. I. Studies of spices of food value in the South Eastern states of Nigeria: Journal of African Medicinal Plants, 18; 1991: 135-139.
2. Umar M., Zubairu A., Hamisu H. S., Mohammed I. B., Oko J. O., Abdulkarim I. M., Salisu A., Yaya A. A. and Aliko A. A. Evaluation of Phytochemical and in vitro Antimicrobial Effects of Solanum lycopersicum Linn. (Tomato) on Oral Thrush and Human Cariogenic Pathogens. *Journal of Advances in Medical and Pharmaceutical Sciences*. 2016; 11(4): 1-9.
3. Moss M.O. and Adams, M.R. Food Microbiology and the Royal Society of Chemistry. Thomas Graham House. 2002
4. Kamboj V.P. Edeoga H. O., Eriata D.O. Alkaloids,Tannins and contents of some Nigerian medicinal plants: Journal of Medicinal Aromatic Plants Science; 2000; 23: 21-25.
5. Effraim K.D, Jacks T. W and Sodipo O. A. Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of Rabbits. African Journal of Biomedical Research. 2001; 6: 21-25.
6. Wagner H. Pharmazeutische biologic 5.(ed), AUFI.15 BN 3 – 437 – 20 498 –X. Gustav fisher Vwelag, Stuttgart, Germany; 1993.
7. Ezekwesili C.N., Obiora O. P. and Ugwu O.P. Antidiarrheal property of crude aqueous extract of *Ocimum gratissimum* L. (Labiatae) in rats. *Biokemistri*, 2004; 16(2): 122-131.
8. Sofowora, A.E. Medicinal Plants and Traditional Medicines in Africa. 2nd Edition. Spectrum Books, Ibadan, Nigeria. 1993: 289.
9. Edeoga H.O. and Eriata D.O. Alkaloid, tannin and saponin contents of some Nigerian medicinal plants. Journal of Medicinal and Aromatic Plant Science. 2001; 23: 344-349.
10. Neergheen-Bhujun V.S. Underestimating the toxicological challenges associated with the use of herbal medicinal products in developing countries. BioMed Research International, 2013; 1-9. doi: 10.1155/2013/804086
11. Arber A. Water Plants: A Study of Aquatic Angiosperm. Welden Wisely Limited. 1972: 436.
12. Onoruvwe O. and Olorunfemi P. O. Antimicrobial Screening and Pharmagnostical Evaluation of *Dischrostachys cinerea* Root; West Africa Journal of Biol. Science. 1998; 7: 91-99.
13. Akerele, Z. O., Obasuyi, O., Ebomoyi, M. I., Oboh, I. E., and Uwumarongie. O. H. African Journal of Biotechnology. 2008; 7(2): 169-172
14. Alade, P.I. and O.N. Irobi, Antimicrobial Activities of Crude Leaf Extracts of Acalypha wilkesiana. J. Ethnopharmacol. 1993; 39, 171-174.

15. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK, Staden (2015). Available at: www.globalresearchonline.netAlso on: www.edu-science-com/10/comparative-study-on-phytochemical.html?m=1
16. Tiwari, P., Kumar, B., Kaur M, Kaur G & Kaur, H. Phyto-chemical Screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011; 1 (1): 98-106.
17. Mir, M.A., Sawhney, S.S. & Jassal, M.M.S. Qualitative and Quantitative Analysis of Phyto-chemicals of Taraxacum officinale. Wudpecker JPPS. 2013; 2 (1): 001-5.
18. Umar M., Mohammed I. B., Oko J. O., Tafinta I. Y., Aliko A. A. and Jobbi D. Y. Phytochemical Analysis and Antimicrobial Effect of Lemon Grass (*Cymbopogon citratus*) Obtained From Zaria, Kaduna State, Nigeria. *Journal of Complementary and Alternative Medical Research*. 2016; 1(2): 1-8.
19. Cheesbrough, M. District laboratory practice in tropical countries, Part 2. Cambridge University Press. 2000; 153.
20. CLSI. CLSI document M100-S23 (M02-A11) "Disc diffusion supplemental tables'' Performance standards for antimicrobial susceptibility testing. The complete standard may be obtained from the Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19807. 2013.
21. Wayne A. (2006). Spinach Health Benefits, Nutrition Facts. Retrieved from: www.almanac.com/plant/spinach.
22. Sanmugapriya E. and Venkataraman S. Toxicological investigations on *Strychnos potatorum* seeds in experimental models. Journal of Health Sciences, 2006; 52: 339-343.
23. Asguit TN, Butler LG. Interaction of condense tannin with selected proteins. *Phytochemistry*. 1996; 25 (7) 1591-15-93.
24. Parek J, Jadija D, Chanda S. Efficacy of Aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. Turkey Journal of Biology. 2005; 29; 203; 10.
25. Pawar BT and Pandit BD. Antibacterial activity of leaf extracts of Ocimum sanctum L. against Xanthomonas campestris pv. mangiferaeindicae. Research Journal of Recent Sciences. 2003; 3 (2013): 291-294.
26. Rasayana PHS. Ayurvedic Herbs for longevity and rejuvenation. CRC Press Rajeshwari S. (1992) ed. *Ocimum sanctum*. The Indian home remedy. Current Medical Scene. March-April published by Cipla Ltd., Bombay Central, Bombay. 1992