

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* L.) were investigated.

Methods: All the analyses were carried out using standard scientific procedures using Soxhlet extraction, well-diffusion agar antimicrobial testing and *in vivo* acute toxicity testing.

Results: The phytochemical analysis according to standard screening tests using conventional protocols revealed the presence of anthraquinone, saponins, tannins, terpenoids and alkaloids, which were detected in methanol extract analyzed. But, flavonoids, glycosides, phlobatannins and steroids were not detected in the methanol extracts analyzed. While only flavonoids were 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 *O. gratissimum* was determined with *S. aureus* and *B. cereus* recording MICs at the lowest concentration (12.5mg/mL) of the methanol and chloroform extracts used. While methanol and chloroform extracts were found to have recorded moderate activity *S. Typhi* and *E. coli* at the MIC of 50mg/mL. The methanol and chloroform extract recorded MBC of 50mg/mL on *B. subtilis* and *S. Typhi*. However, *Salmonella Typhi* was inhibited at the concentration of 100mg/mL of chloroform scent leaf extract. The MICs of *C. albicans* were not determined in the methanol and chloroform scent leaf extracts analyzed. The *in vivo* toxicity of *O. 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, and the plant has less toxicity when administered orally.

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 of 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

[1].

24 Research on herbs, spices and medicinal plants originated with our ancestors thousands of
25 years ago it's now a popular subject that appeals to life scientist due to the problems of
26 drugs resistance and cost of drugs. Scientist in Africa and other developing countries are
27 conducting researches into local plants which are used in traditional medicine [2].

28 Majority of chemically synthesized drugs have serious adverse effects to the recipients,
29 which may lead to temporary or permanent disability and incapacitations. Also,
30 gastrointestinal disorders, dysentery, diarrhoea and candidiasis are very serious infections
31 that can lead to frequent morbidity and mortality in tropical countries like Nigeria. These
32 disorders are serious diseases that can affect many people at various stages of their lives
33 causing distress and discomfort [1]. Sometimes, the disorder can even lead to
34 hospitalization. Majority of the etiologic agents of gastroenteritis were found to be resistant to
35 variety orthodox drugs, as such complementary and alternative therapy is the only future to
36 the success of pharmacology [3].

37
38 Treatment of diseases has always been associated with the administration of drugs gotten
39 from plants, animals and mineral sources. The use of plants or herbs extract in the treatment
40 of human ailments is a very ancient art. Investigation of African medicinal plants for
41 antimicrobial activities ranks highest among biological test carried in many plants and their
42 extracts [4]. Herbal medicines tend to look primitive and unscientific when compared to
43 synthetic (conventional) drugs, which are thought to be more reliable than those made from
44 plants. Herbal medicine is still the mainstay of about 75-80% of the world population, mainly
45 in developing countries for primary health care [5].

46
47 The perennial plant *O. gratissimum* (scent leaf) is widely distributed in the tropics of Africa
48 and Asia. It belongs to the family Labiatae and it is the most abundant of the genus
49 *Ocimum*. In the southern part of Nigeria, the plant is called “*effinrin-nia*” by the Yoruba
50 speaking tribe, “*Nchonwu*” in Igbo, while in the northern part of Nigeria, the Hausas call it
51 “*Daidoya*” [6]. These perennial plants are woody at the base. The plants are found to grow
52 somewhere around 1-3 metres in height. The stems of these plants are dark brown in colour
53 bearing leaves from top to bottom. The leaves are narrow and oval in shape growing 5-13
54 cm in length and have 3-9 cm width, but sometimes the leaves are green in colour. The
55 flowers are pale yellow in colour and the plants give out a sweet scent of camphor [7].

56
57 A lot of research has been carried out on the herb, *O. gratissimum*. Though, literature search
58 has not revealed any study on the effect of *O. gratissimum* on the histology of the lung of the
59 albino rat, some works closely related to it has been documented. This plant is used by
60 herbalists to treat a variety of maladies, from bacterial infections and diabetes to pain and
61 liver damage. Several studies have been performed that lend credence to herbalist use of
62 this plant for treating diarrhoea and other gastrointestinal infections [8].

63
64 In folk medicine, *O. gratissimum* is extensively used throughout West Africa as a febrifuge,
65 anti-malarial and anti-convulsant. The crushed leaf juice is used in the treatment of
66 convulsion, stomach pain and catarrh. Oil from the leaves has been found to possess
67 antiseptics, antibacterial and antifungal activities [8]. In the coastal area of Nigeria, the plant
68 is used in the treatment of epilepsy, high fever and diarrhoea. While in the savannah areas
69 decoctions of the leaves are used to treat mental illness [9].

70 *Ocimum gratissimum* is used by the Igbos of southern Nigeria in the management of the
71 baby's cord. It is believed to keep the baby's cord and wound surface sterile. It is used in the
72 treatment of fungal infections, fever, cold and catarrh [9]. Clinical trials in creams formulated
73 against dermatological disease have yielded favourable results. Several tonics are produced
74 from these herbs and used in treating 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 [10].

Ocimum tea is an infused form of the herb used in treating fever and diaphoresis. The volatile oil acts as a good antimicrobial agent and nutritional importance of this plant centres on its usefulness as a seasoning because of its aromatic flavour. It is used as a flavour in spicing meat products [8].

In Congo, a scent leaf decoction is used for diarrhoea, gonorrhoea infection, vaginal douches for vaginitis and used in the treatment of mental illness. Extracts of scent leaves have also been reported to have lower blood pressure, strong insect repellent effects and kill many micro-organisms that cause diseases, including candida. Several studies have confirmed the efficacy of *O. 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 [8]. Studies suggest that *O. 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 *O. 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 [8].

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, diarrhoea 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, diarrhoea 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 [11]. Therefore this research was designed to determine the antimicrobial effect of scent leaf (*O. 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 [12]. 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 [13]. The dried leaves were ground into powdered form. The leaf powder was stored and sealed in labelled sterile reagent bottles for further use. The bioactive components were extracted using the methods of Akerele et al. [14].

2.2 Preparation of Plant Extract

125 Plant extracts were prepared by the method of Alade and Irobi [15] with minor modifications.
126 To study the antibacterial potential of *O. gratissimum* polar solvents such as methanol and a
127 non-polar solvent such as chloroform were used. A mass of 30 g of dried powder was
128 weighed on a weighing balance (Mettler 166®) and was extracted by 100 mL of each solvent
129 by using Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of
130 the solvent until the aqueous content evaporated completely as adopted by Lin et al. [16]. At
131 the end of extraction, the extract was filtered through Whatman No. 1 filter paper. The dry
132 extract was collected and weighed in varying concentration and kept in an airtight container
133 at 4°C.

134



135
136 Fig. 1: Fresh scent leaf (*O. gratissimum* L.) leaves

137

138 2.3 Preliminary Phytochemical Screening

139 The preliminary phytochemical investigation was carried out for methanolic and chloroform
140 extracts of scent leaf (*O. gratissimum*) for the detection of various phyto-constituents by
141 using standard procedures to identify the constituents [17-19].

142 2.3.1. Test for the presence of Alkaloids (Wagner's test)

143 Wagner's reagent was prepared by dissolving 2 g of iodine and 6 g of KI in 100 mL of water.
144 The plant extract was prepared by taking 500 mg of plant material in 500 mL of methanol for

145 20 minutes, on a water bath. The extract was then be filtered off and allowed to cool. The 2
146 mL plant extract was then taken and treated with few drops of Wagner's reagent. A reddish
147 brown coloured precipitate indicates the presence of alkaloids.

148 **2.3.2. Test for the presence of Anthraquinone (Borntrager's test)**

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

153 **2.3.3. Test for the presence of Flavonoids**

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

157 **2.3.4. Test for the presence of Phlobatannins**

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

160 **2.3.5. Test for the presence of Glycosides (Fehling's test)**

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

166 **2.3.6. Test for the presence of Saponins (Frothing test)**

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

170 **2.3.7. Test for the presence of Steroids (Salkowski test)**

171 The 1 mL of plant extract was taken and to it, few drops of concentrated sulphuric were
172 added. The presence of red colouration indicates the presence of steroids.

173 **2.3.8. Test for the presence of Tannins (Ferric chloride test)**

174 The presence of tannins was tested in 0.5 g of the crude plant powder and was stirred with
175 10 mL of distilled water. The extract was filtered and ferric chloride reagent was added to the
176 filtrate, a blue-black precipitate was taken as evidence for the presence of tannin.

177 **2.3.9. Test for the presence of Terpenoids (Salkowski test)**

178 The presence of terpenoids was tested in 0.2 g of the extract of the plant sample and mixed
179 with 2 mL of chloroform and concentrated sulphuric acid (3 mL H₂SO₄) was added carefully
180 to form a layer. A reddish brown colouration of the interface was formed to indicate positive
181 results for the presence of terpenoids.

182 2.4 Cultivation and Management of Bacterial Pathogens

183 Pre-characterized clinical isolates of *C. albicans*, *B. subtilis*, *S. Typhi*, *S. aureus* and *E. coli*
184 were collected from Medical Microbiology laboratory, Ahmadu Bello University Teaching
185 Hospital, Zaria. All the isolates were checked for purity and maintained in slants of nutrient
186 agar for the bacteria and Sabouraud's Dextrose agar for the fungi.

188 2.5 Antimicrobial Assay of the Extract

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

196 A mass of 0.5g of the extract was weighed and dissolved in 10cm³ of distilled water so as to
197 obtain a concentration of 50mg/mL of the extract. This was the initial concentration of the
198 extract used to check the antimicrobial effects of the extracts. Mueller Hinton agar was used
199 as the growth medium for the bacterial species; while, Sabouraud's Dextrose agar was used
200 for *C. albicans*. The media were prepared according to the manufacturer's instructions,
201 sterilized at 121°C for 15minutes [20]. The sterilized molten media were then poured into
202 sterilized Petri dishes, the plates were allowed to cool and solidify in accordance with CLSI
203 [21] specifications.

204 The well-diffusion method was used for screening the extracts. The sterilized medium was
205 seeded with 0.1cm³ of the standard inoculums. It was then spread evenly over the surface of
206 the medium by using a sterile swab. Using a sterilized cork-borer of 6mm in diameter, a well
207 was excavated at the centre of each inoculated medium. A volume of 0.1mL of the solution
208 of the extract of concentration of 50mg/mL was then introduced into each of the dug wells on
209 the medium. The inoculated medium was allowed to absorb the extract and then incubated
210 at 35°C for 24 hours, after which each plate was observed for the zone of inhibition, the zone
211 was measured with a transparent meter rule and the results were recorded in millimetres
212 [21]. The same procedure was used for the other extracts using different concentrations.
213 Ciprofloxacin (30µg) was used as a positive control for bacteria, while Econazole (30µg) was
214 used as a positive control for *C. albicans*.

216 2.6 Determination of Minimum Inhibitory Concentration

217 The minimum inhibitory concentration was determined using the broth dilution method.
218 Mueller Hinton agar was prepared and 10cm³ was dispensed into a test tube, sterilized at
219 121°C for 15 minutes and then allowed to cool. McFarland's turbidity standard scale number
220 0.5 was used to give a turbid solution. Normal saline was prepared, and 100cm³ was
221 dispensed into a sterile test tube and the test microbe was inoculated at 37°C for 6 hours.
222 Dilution of the test microbe was done in the normal saline until the turbidity matches that of
223 the McFarland's scale by visual comparison [20, 22]. At this point, the test microbes might
224 have a concentration of about 1.5×10^8 cfu/ml. Two-fold serial dilution of the extract in the
225 sterile broth was made to obtain the concentration of 50mg/mL, 25mg/mL and 12.5mg/mL.
226 The initial concentration was obtained by dissolving 0.5g of the extract in the broths; 0.1cm³
227 of the test microbes in the normal saline was then introduced into the different
228 concentrations of the extract in the broth which was incubated at 37°C for 24 hours after
229 which each test tube was observed for turbidity. The lowest concentration of the extracts in
230 the broth which might show no turbidity was recorded as the minimum inhibitory
231 concentration [23].

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 the 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 body weight of the rats. Group 5 serves as normal control, while groups 1, 2, 3 and 4 received the crude extracts of *O. gratissimum* at various concentrations (100mg/mL, 50mg/mL and 25mg/mL) at doses of 100mg/mL, 50mg/mL and 25mg/mL respectively, each dissolved in 0.5ml normal saline in relation to the animal's bodyweights respectively [24, 25]. Group 6 served as the 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 body weight) to serve as the 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 behaviours were observed for any physiological changes as adopted by Sanmugapriya et al. [26]. After three weeks of analysis, the number of laboratory animals survived was 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 were detected in chloroform extract. All other phytochemicals were absent.

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

Phytochemicals	Methanol	Chloroform
Anthraquinone	+	-
Flavonoids	-	+
Saponins	+	-
Tannins	+	-
Phlobatannins	-	-
Steroids	-	-
Terpenoids	+	-
Glycosides	-	-
Alkaloids	+	-

*+ = presence; - = absence

275 Based on the phytochemical analysis findings of the research study, it was found out that
276 phytochemicals such as anthraquinone, saponins, tannins, terpenoids and alkaloids, were
277 detected in methanol extracts analyzed. But, flavonoids, glycosides, phlobatannins and
278 steroids were not in the methanol extracts analyzed. While the only flavonoid was detected
279 in chloroform extract. All other phytochemicals were absent (Table 1). This may be due to
280 polarity and higher colour intensity usually observed in the methanolic extracts that indicate
281 the presence of terpenoids and saponins with the absence of flavonoids, steroids and
282 phlobatannins [27].

283 Table 2 showed the effect of scent leaf methanol and chloroform extracts at different
284 concentration recorded high antimicrobial effect against *S. aureus*, *B. subtilis* *S. Typhi* and *E.*
285 *coli*. But, the extracts showed no effect against *C. albicans*.
286

287 Based on the antimicrobial activities of methanol and chloroform extracts of *O. gratissimum*,
288 at different concentrations, the extracts recorded high antimicrobial effect against *S. aureus*,
289 *B. subtilis* *S. Typhi* and *E. coli*. But, the extracts showed no effect against *C. albicans* (Table
290 2). A slight antimicrobial activity of the extracts of *O. gratissimum* against *E. coli* may be due
291 to the polarity nature of chloroform used for the extraction. This agrees with the previous
292 study by Parek *et al.* [28], who reported that most plants are extracted by traditional healers
293 using various solvents, and such solvent extracts prominently exhibit antibacterial activity
294 more than the others. To confirm the antimicrobial potentiality of plants, Prosper-Cabral *et al.*
295 [29] reported that the importance of medicinal plants, and the contribution of phytomedicine
296 to the well-being of a significant number of the world's population, has attracted interest from
297 a variety of disciplines.

298 **Table 2. Antimicrobial effects of *O. 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)
<i>Staphylococcus aureus</i>	23	20	18	16	22	18	16	14	35	–
<i>Salmonella</i> Typhi	20	18	–	–	18	14	–	–	38	–
<i>Bacillus subtilis</i>	19	16	14	12	21	18	15	13	32	–
<i>Escherichia coli</i>	17	13	–	–	19	13	–	–	37	–
<i>Candida albicans</i>	–	–	–	–	–	–	–	–	–	37

300 – = not determined

Table 3 showed that the minimum inhibitory concentration of methanol and chloroform extracts were determined with *S. aureus* and *B. cereus* recording MICs at the lowest concentration (12.5mg/mL) of the methanol and chloroform extracts used. While methanol and chloroform extracts were found to have recorded moderate activity *S. Typhi* and *E. coli* at the MIC of 50mg/mL. The methanol and chloroform extract recorded MBC of 50mg/mL on *B. subtilis* and *S. Typhi*. However, *S. 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 concentrations of methanol and chloroform extracts were determined with *S. aureus* and *B. cereus* recording MICs at the lowest concentration (12.5mg/mL) of the methanol and chloroform extracts used. While methanol and chloroform extracts were found to have recorded moderate activity *S. Typhi* and *E. coli* at the MIC of 50mg/mL. The methanol and chloroform extract recorded MBC of 50mg/mL on *B. subtilis* and *S. Typhi* (Table 3). This result confirmed the findings of Pawar and Pandit [30] who worked on antibacterial activity of leaf extracts of *Ocimum sanctum* L. against gram-positive bacteria. The effectiveness of this plant extract against the test organisms may be due to the presence of phytochemical components such as alkaloids, tannins, anthraquinones, glycosides and steroids which possess antimicrobial activities. This agreed with the findings of Harborne [31], who reported that antimicrobial activity is concerned with the enormous variety of organic substances such as alkaloids, carotenoids, steroids, flavonoids, terpenoid, etc., which are elaborated and accumulated by plants and deal with the chemical structures of various microorganisms.

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

TEST ORGANISMS	MIC (mg/mL)		MBC (mg/mL)	
	Methanol extract	Chloroform extract	Methanol extract	Chloroform extract
<i>Staphylococcus aureus</i>	12.5	12.5	25	–
<i>Salmonella Typhi</i>	50	50	50	100
<i>Escherichia coli</i>	50	50	–	–
<i>Bacillus subtilis</i>	12.5	12.5	50	50
* <i>Candida albicans</i>	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 *O. 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 a hepatotoxic substance such as acetaminophen. All the animals tested with the crude extract of *O. gratissimum* survived.

The in vivo toxicity of *O. 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 [32], who reported that *O. gratissimum* is harmless edible plant and it is considered in the Ayurvedic system as a type of “elixir of life”. Jayaprasad *et al.* [25], also reported that the plant kingdom is a treasure house of potential drugs and in recent years

342 there has been an increasing awareness about the importance of medicinal plants. Drugs
343 from the plants are easily available, less expensive, safe, and efficient and rarely have side
344 effects. The toxic effects may take place prior to the binding of the toxicants to the vital
345 organs such as the liver and kidneys. Hence, evaluation of toxic properties of a substance is
346 crucial when considering public health protection because exposure to chemicals can be
347 hazardous and results in adverse effects on the human being.

348 The absence of toxicity in plant extracts was also reported by Khan and Akhtar [24], who
349 reported the absence of toxicity or mortality by plant extracts containing alkaloids,
350 glycosides, saponins, tannins and anthraquinones even at high doses.

UNDER PEER REVIEW

351
352

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

Groups	Number of albino 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/mL resulted in salvation. 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 have 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 traditional therapy. A lot of work has been done on scent leaf, still, researchers are engaged to investigate the 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 the best alternate of medicine, and having the ability to kill different pathogenic bacterial strains. The 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 the whole plant has medicinal properties such as *Ocimum gratissimum* leaves having the 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 *O. gratissimum* plant, which displayed high antimicrobial activity at a lower dosage with mild or no toxicity to the recipient laboratory animals. Other solvents should be used in 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

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