

Original Research Article

Phytochemical Properties and Antibacterial Activity of Leaf Extract of *Ocimum gratissimum* On *Salmonella* Species

Abstract

Aim: *Ocimum gratissimum* is commonly used as food and health purposes. This study is aimed at evaluating the bioactive compounds and anti-bacterial activity of leaf extract of *O. gratissimum* against *Salmonella* species.

Methodology: The Phytochemical screening of *O. gratissimum* was conducted using standard methods. Screening for antibacterial activity of the leaf extracts against *Salmonella* species was determinrd using agar well diffusion method. An in-vivo toxicity study was carried out with albino rats.

Results: The phytochemical screening revealed the presence of saponins, tannins, cardiac glycoside, flavonoid, glycosides, alkaloid, volatile oils and steroids. A zone of inhibition of 14mm was recorded against the organisms using ethanolic extract with a concentration of 100mg/ml and the lowest was recorded against *Salmonella paratyphi* with concentration of 25mg/ml of the ethanolic extract. Zone of inhibition of 9.00mm and 10.0mm was recorded against *S. typhi* and *S. paratyphi* on a concentration of 100mg/ml of the aqueous extract. A minimum inhibitory concentration of 100mg/ml and 25mg/ml of the aqueous and ethanolic extract of the leaf was recorded. After the toxicity test, no death was recorded after 2 (two) weeks.

Conclusion: The leaf extract of *O. gratissimum* shows promising potentials in the treatment of infectious diseases associated with *Salmonella typhi* and *Salmonella paratyphi*, due to it antimicrobial activity and low toxicity. However, further studies are needed to non-polar solvents to isolate other bioactive compounds as well as identify the active metabolites responsible for these activities.

KEY WORDS: *Ocimum gratissimum*, antibacterial activity, salmonella typhi, salmonella paratyphi, phytochemical, toxicity.

INTRODUCTION

Medicinal plants are known to contain, in one or more of its organs, substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs [1]. Many of such plants

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35 known to be used primitively to alleviate symptoms of illnesses have been screened to have
36 medicinal importance, some of which include: *Vernonia amygdalina* (bitter leaf), *Ocimum*
37 *gratissimum* (scent leaf), *Zingiber officinale* (ginger), *Azadirachta indica* (Dogonyaro), *Piper*
38 *guineese* (lyere), *Allium sativum* (garlic), Cotton leaf (*Gossypium* spp) etc. These plants have been
39 reportedly used in the traditional treatment of ailments such as stomach disorder, fever symptoms
40 and cough [2].

41 Researchers are increasingly turning their attention to natural products looking for new leads to
42 develop better drugs against cancer, as well as viral and microbial infections. The phytochemical
43 evaluation of *Ocimum gratissimum* shows that it is rich in alkaloid, tannins, oxalate, flavonoids and
44 essential oil [3]. In the coastal area of Nigeria, the plant *Ocimum gratissimum* is used in the
45 treatment of epilepsy, high fever and diarrhea [4]. *Ocimum gratissimum* (Scent leaf) is a perennial
46 plant which is widely distributed in the tropics of Africa and Asia. It belongs to the family Labiatae
47 and it as the most abundant of the genus *Ocimum*. In the southern part of Nigeria, it is called "Efirin
48 nla" by the Yoruba speaking tribe. "Nichonwu" in Igbo while in the northern part of Nigeria, it is
49 called "Daidoga" [5]. Leaf extract of *Ocimum gratissimum* and *Xylopia aethiopiae* were analyzed
50 against five pathogenic organisms. *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus*
51 *fecalis*, *Pseudomonas aeruginosa* and *Lactobacilli* [3]. The findings justifies the application of
52 *Ocimum gratissimum* in dermatological cream and indicate the effective doses could be achieved
53 at very low concentration and also shows that the aqueous fractions of both plants have more
54 potential as antimicrobial agents than their ethanolic fractions [3]. The findings of Silva *et al.* [6]
55 showed the extracts of *Ocimum gratissimum* to be active against human pathogenic dermatophytes.

56
57 A thousand years ago an extensive use of plants as medicines have been reported and were
58 initially taken in the form of crude drugs such as tinctures, elixirs, poultices, powders, and other
59 herbal formulations [7]. However, the use of herbal products should be based on scientific origin;
60 otherwise they would be useless and unsafe [7]. Furthermore, the irrational use of these herbal
61 products may cause serious toxicity for humans. Unfortunately, many people underestimate the
62 toxicity of natural products and do not realize that these agents could be as toxic as or more toxic
63 than synthetic drugs [7]. A typical example for a toxic herbal product are the leaves of *Atropa*
64 *Belladonna* and *Digitalis purpurea* [8], which show severe systemic toxicity if taken orally.

Comment [W5]: belladonna

65
66 Toxicology is the important aspect of pharmacology that deals with the adverse effect of bio active
67 substance on living organisms prior to the use as drug or chemical in clinical use [9], As per the
68 OECD 2001 guidelines, in order to establish the safety and efficiency of a new drug, toxicological
69 studies are very essential in animals like mice, rat, guinea pig, dog, rabbit, monkey etc under
70 various conditions of drug. Toxicological studies help to make decision whether a new drug should
71 be adopted for clinical use or not. OECD does not allow the use of drug clinically without its clinical

72 trial as well as toxicity studies. The aim of this present work therefore was to carry out
73 phytochemical screening of the leaf of *Ocimum gratissimum*, study the antibacterial effects of the
74 leaf extracts of *Ocimum gratissimum* on selected Enterobacteriaceae (*Salmonella* species) and to
75 estimate the toxic effects of aqueous and ethanolic extracts from *Ocimum gratissimum* in albino
76 Rats.

77 **MATERIALS AND METHODS**

78 **Collection and Identification of Leaf Materials**

79 *Ocimum gratissimum* (scent leaf) was obtained from Meat Market, Sokoto, Nigeria. The collected
80 leaf was identified and authenticated at the Herbarium Section of the Department of Biological
81 Sciences, Botany Unit of Usmanu Danfodiyo University Sokoto, Sokoto State, Nigeria. Voucher
82 specimen numbers UDUH/ANS/101 was obtained.

83 **Preparation and Extraction of Leaf Extracts**

84 The fresh leaves were allowed to dry completely at a room temperature before using them for this
85 study. The leaf material was pulverized using mortar and pestle into a fine powder. Two different
86 solvents were used for the extraction namely: water and ethanol. A 100g of powdered leaf was
87 soaked in 1000ml of each solvent in accordance with Udochukwu *et al.* [10]. Each solution was
88 stirred intermittently and allowed to stand for 48h, and then filtered by first, using a clean muslin
89 cloth and then, No. 1 Whatman filter paper. Sterilization of the solutions was made using membrane
90 filters. The sterile extract obtained was stored in sterile capped bottles and refrigerated [11].
91
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93 **Characterization and Identification of *Salmonella* Species**

94 **Source of Test Organism**

95 The test organisms for this study (*Salmonella* species) are members of the family
96 Enterobacteriaceae. The pure clinical isolates of *Samonella typhi* and *Samonella paratyphi* were
97 obtained from the Department of Medical Microbiology and Parasitology, Specialist Hospital Sokoto,
98 Nigeria. All the clinical isolates were checked for purity by sub-culturing the isolates onto
99 Salmonella-Shigella Agar medium. After 24hrs of incubation, there were growths of the isolates and
100 they were maintained on nutrient agar slants at 4⁰C in the refrigerator until required for further use.

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103 **Biochemical Confirmation and Serotyping of *Salmonella***

104 The ISO-6579 [12], standard recommendation was used for biochemical confirmation of *Salmonella*.
105 Subculture of characteristic colonies from each Petri dish of Salmonella-Shigella agar medium was
106 made. The triple sugar iron agar (TSI agar), Urea agar/broth, L-lysine decarboxylase, β -
107 galactosidase (ONPG), Voges Proskauer and Indole tests were followed in this order.
108
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110 In serotyping, subculture of characteristic colonies from each Petri dish of Salmonella-Shigella agar
111 was transferred onto nutrient agar slopes and incubated overnight at 37°C. Using a wire loop, 3
112 separate drops (each 0.02 ml) of saline solution were placed onto a clean microscope slide. Growth
113 from the agar slope was added and emulsified to produce homogeneous suspension. A loopful of
114 *Salmonella* polyvalent 'O' (PSO) anti-serum was mixed with the first drop of suspension and a
115 loopful of *Salmonella* polyvalent 'H' (PSH) anti-serum with the second drop. It was rocked gently
116 back and forth and examined for agglutination against a black background. Positive results were
117 recorded if agglutination occurred within 20 min after shaking against dark background. In order to
118 exclude any spontaneous agglutination (auto-agglutination), a negative control (using physiological
119 saline solution and bacterial colony to be tested) was included in the test.

120

121 **Standardization of Bacteria Cell Suspension**

122 The nutrient broth cultures of the organisms for this study were taken and inoculated at 37°C on a
123 fresh agar plate of nutrient agar for 24 hours. Sterile distilled water (2ml) was poured on it and then
124 mixed with the inoculums, 1ml of each was taken and transferred into 9ml of sterile distilled water
125 and diluted to 0.5 Macfarland Standard giving a load of 10⁵- 10⁶ organisms/ml. One hundred
126 microlitres of these were taken and poured onto the surface of the agar and then spread evenly with
127 the use of a spreader on the plate to be used for the study [13].

128

129 **Preparation of Extracts Concentration**

130 The different extracts of the sample were reconstituted with sterile distilled water. The initial
131 concentration of each plant extracts (1g) was diluted using 10ml of sterile water to obtain the stock
132 culture. From this stock culture, different concentrations were gotten which were 100mg/ml,
133 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and 3.125mg/ml for each of the extracts (water and
134 ethanol).

135

136 **Determination of Antibacterial Activities of Leaf Extracts**

137

138 Agar-well diffusion Method was employed for the antibacterial testing [14]. The antibacterial
139 screening of the extracts was done as described by Perex *et al.* [14]. One (1) gram of each crude
140 extract (aqueous and ethanolic) was poured into 10ml water. From this stock culture, different
141 concentrations were gotten which were 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, and
142 3.125mg/ml for each of the extracts (aqueous and ethanolic). Nutrient agar was poured in sterile Petri
143 dishes and was allowed to solidify. A loopful of the test culture of MacFarland standard was dropped
144 on the solidified agar and the organism was spread all over the surface of the agar using a spreader
145 (wire loop). The inoculated plates was allowed to dry after which wells of approximately 5mm in
146 diameter were made on the surface of the agar medium using a sterile cork borer. Then, 0.2ml of
147 different concentrations of the extract was separately introduced into the different wells that have
148 been labeled accordingly. This procedure was repeated in triplicate and allowed to stay for 30mins

149 on the bench after which they were incubated for 24h at 37°C. At the end of incubation, observed
150 zones of inhibition were measured and recorded to the nearest millimeter.

151
152 **Determination of Minimum Inhibitory Concentration of the Extracts**
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154 This was carried out using agar diffusion method following the recommendations of the Clinical and
155 Laboratory Standard Institute [15]. Different concentrations 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml
156 of the extracts were prepared and 1ml from each of the concentrations of the extracts was added
157 onto molten nutrient agar and was mixed thoroughly. Then, 1µml of an overnight nutrient broth
158 culture of the test isolates were added to each plate of the Mueller-Hinton agar containing the
159 extracts and incubated at 37°C for 24 h. The experiment was conducted in triplicate for all the test
160 isolate. Plates without visible growth of the organisms in each concentration were taken as the MIC
161 [11].
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Comment [W6]: microlitre or milli litre?

163 **Phytochemical Screening of Leaf of *Ocimum gratissimum***

164 The pulverized leaf obtained was subjected to phytochemical screening to determine the presence
165 of bioactive compounds.
166

167 **Test for Tannins**

168 Five percents (5%) ferric chloride were added drop by drop to 3ml of each extract and observed for
169 brownish green or a blue black colouration [16].
170

171 **Test for Saponins**

172 Two grams (2g) of the powdered sample of each extract was boiled in 20ml of distilled water in a
173 water bath and filtered. Then, 10ml of the filtrate was mixed with 5ml of distilled water and shaken
174 vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil, shaken
175 vigorously and then observed for the formation of emulsion [17].
176

177 **Test for Flavonoids**

178 One milliliter (1ml) of 10% NaOH solution was added to a portion of the aqueous filtrate of the each
179 plant extract, followed by addition of concentrated H₂SO₄. A yellow coloration observed in the
180 extract indicated the presence of flavonoids [16].
181

182 **Test for Cardiac Glycosides**

183 Five milliliters (5ml) of each extract was treated with 2ml of glacial acetic acid containing 1 drop of
184 ferric chloride solution (3.5%). The content was allowed to stand for one minute. One milliliter (1ml)
185 of concentrated H₂SO₄ was carefully poured down the wall of the tube. A reddish brown ring of the
186 interface indicated a deoxysugar characteristic of cardenolides [18].
187

188 **Test for Alkaloid**

189 Two milliliter (2ml) of each extract was stirred with 2ml of 10% dilute hydrochloric acid. Then, 1ml
190 was treated with a few drops of Wagner's reagent and second 1ml portion treated with Mayer's
191 reagent. A deep brown precipitation indicated a positive test [18].

192
193 **Test for Glycosides**

194 The 2.5ml of 50% H₂SO₄ was added to 5ml of each of the extracts in test tubes. The mixture was
195 heated in boiling water for 15minutes. Cooled and neutralized with 10% NaOH, 5ml of Fehling's
196 solution was added and the mixture was boiled again. A brick-red precipitate was observed, which
197 indicated the presence of glycosides [18].

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199 **Test for Steroids**

200 This was carried out according to the method of Harborne [18]. One (1) ml of the each leaf extract
201 was added in 2ml of chloroform, and 2ml of sulphuric acid (H₂SO₄) was added thereafter. A red
202 colouration confirmed the presence of steroids.

203
204 **Test for Volatile oils**

205 One milliliter (1ml) of each of the extract fractions was mixed with 5ml of dilute HCL. A white
206 precipitate was formed, which indicated the presence of volatile oils [17].

207
208 **Toxicity Study of the Leaf Extracts of *Ocimum gratissimum***

209 Acute oral toxicity test was carried out using the procedure of the Organization for Economic
210 Cooperation and Development [19]. Ten (10) randomly selected Albino rats were used. The rats of
211 both sexes weighing 160-200g were used for the study. The animals were obtained from the Faculty
212 of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. The animals were acclimatized for a
213 period of seven days. All animals were housed, caged and allowed free access to food and water
214 before they were used for the experiment. The animals' weights were taken and starved of food.
215 Then 5000mg/kg body weight of the extract was administered in a single concentration.
216 Concentrations were calculated according to the body weight of the animals. Oral administration of
217 extracts was done using a graduated syringe and cannula. They were placed under observation for
218 48 hours for behavioral changes and daily for 14days for mortality [19], upon which the number of
219 deaths and LD₅₀ were determined.
220

221
222 **RESULTS AND DISCUSSION**

223
224 The results of phytochemical screening of *O. gratissimum* leaves revealed the presence of the
225 following secondary metabolites; tannins, saponins, flavonoid, steroid, cardiac glycoside,
226 glycosides, alkaloid, and volatile oil. This is similar to the findings of Nweze *et al.* [20] who reported

Comment [W7]: Please mention whether the study was approved? If yes, mention the ethical status.

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227 the presence of alkaloids, tannins, glycoside, saponin, cardiac glycoside, steroid and flavonoids in
 228 *O. gratissimum* which is similar to the results obtained in this study.
 229 The results revealed that the aqueous extract of *Ocimum gratissimum* had less inhibitory activity on
 230 the test organisms (Table 2), while the ethanolic extracts of *Ocimum gratissimum* (Table 3) had
 231 antibacterial activity against the isolates tested. At 100mg/ml concentration, the ethanolic extracts
 232 showed greater antibacterial activity than the aqueous extracts as indicated by zones of inhibition.
 233 At 12.5mg/ml – 3.125mg/ml, the ethanolic extracts of *Ocimum gratissimum* (Table 3) was not
 234 effective on the isolates. While at 50mg/ml – 3.125mg/ml the aqueous extracts of *Ocimum*
 235 *gratissimum* (Table 2) was not effective on the isolates. This indicates that the antibacterial activity
 236 of this leaf extracts is concentration dependent. Ethanolic extract showed high inhibitory zones than
 237 aqueous extracts and when compared to standard antibiotic such as Pemaclav drug had an
 238 appreciable zone of inhibition of the test organisms. The result of this work showed that the
 239 ethanolic extract showed high inhibitory zones than aqueous extracts. This observed difference
 240 between these plants extracts may be due to insolubility of active compounds in water or the
 241 presence of inhibitors to the antimicrobial components Okigbo and Ogbonnanya [21], Amadioha and
 242 Obi [1999], Okigbo and Ajale [2005]. They have attributed this observation to the high volatility of
 243 ethanol which tends to extract more active compound from the sample than water, hence, this study
 244 follow similar trends. The aqueous extract of *O. gratissimum* showed a decrease in the level of
 245 inhibition against isolates at the highest concentration compared to the positive control, inhibition
 246 zones ranging from 9.0 to 10.0 mm.

Comment [W9]: Mention the generic name

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 249 **Table 1. Phytochemical properties of the leaf of *O. gratissimum* Leaf Extract.**

Comment [W10]: constituents

Phytochemical	<i>O. gratissimum</i>
Tannins	+
Saponins	+
Flavonoid	+
Cardiac glycoside	+
Alkaloid	+
Glycosides	+
Steroid	+
Volatile oil	+

Comment [W11]: This is for ethanol extract or aqueous extract?

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KEY:- = Not detected, + = Detected

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Table 2. The Antibacterial Activities of Aqueous Leaf Extracts of *O. gratissimum*

Test Organism	Zone of inhibition(mm)						
	<i>O. gratissimum</i>						
Concentration (mg/ml)	100	50	25	12.5	6.25	3.125	+ve Control
<i>Salmonella typhi</i>	09.0	x	x	x	x	x	20.0
<i>Salmonella paratyphi</i>	10.0	x	x	x	x	x	20.0

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Key:

Values are mean of three replicates (n=3)
x = No zone of inhibition
+ve control = Pemaclav drug (10mg/ml)

Table 3. The Antibacterial Activities of Ethanolic Leaf Extracts of *O. gratissimum*

Test Organism	Zone of inhibition(mm)						
	<i>O. gratissimum</i>						
Concentration (mg/ml)	100	50	25	12.5	6.25	3.125	+ve control
<i>Salmonella typhi</i>	14.0	13.0	12.0	x	x	x	20.0
<i>Salmonella paratyphi</i>	14.0	11.0	07.0	x	x	x	20.0

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Key:

Values are mean of three replicates (n=3)
x = No zone of inhibition
+ve control = Pemaclav drug (10mg/ml)

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The minimum inhibitory concentrations (MIC) of aqueous and ethanolic leaf extracts on the test organisms ranged between 25mg/ml –100mg/ml. The minimum inhibitory concentrations of ethanolic extracts of *O. gratissimum* as 25mg/ml while aqueous leaf extracts of *O. gratissimum* had their MIC as 100mg/ml. Minimum inhibitory concentrations (MICs) of both aqueous and ethanolic extracts on test organisms using agar dilution method revealed low MIC, which is an indication of high efficacy of the leaf extracts while high MIC may indicates low efficacy or possible development of resistance by the microorganisms to the antimicrobial [24]. The aqueous extract showed its MIC at high concentration of 100mg/ml while ethanolic extract showed its MIC at 25mg/ml.

Oral administration of a single dose of ethanol and aqueous extracts of *O. gratissimum* of 5000mg/kg body weight of the test animals produced no mortality in them. The general signs and symptoms of toxicity were observed for a period of 14 days after administration of the extracts. However, the following observations were made during the exposure period; slow movement, scratching of hair and mouth, tremor, raised hair coat and weakness. Thus, the median dose (LD₅₀)

286 of the leaf extracts was estimated to be greater than 5000mg/kg because 5000mg/kg is the highest
 287 dose according OECD [19]. From the experiment performed as per the OECD Guidelines 2001, the
 288 results reveal that the both aqueous and ethanolic extract of *Ocimum gratissimum* have been found
 289 nontoxic at 5000 mg/kg body weight of experimental animals as in the first 1 hour of observation, no
 290 morbidity was observed but weakness, slow movement, scratching of mouth, fur and body, tremor
 291 were observed and in the next 48 hours of observation mortality were not found and all that
 292 parameters used for evaluation of toxicity were found to be normal. No significant changes were
 293 observed in body weight. In the last 2 weeks of observation, no death rate recorded. As per
 294 observations and calculations from Acute Oral Toxicity (OECD Guidelines 2001), the LD₅₀ value of
 295 aqueous and ethanolic Extract of *Ocimum gratissimum* were found to be more than 5000 mg/kg
 296 body weight of the rats.

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Table 4 Minimum Inhibitory Concentration of the Aqueous and Ethanol Leaf Extracts of *O. gratissimum* against *Salmonella* spp

Bacterial Isolates	Aqueous extract MIC (mg/ml)	Ethanol extract MIC (mg/ml)	Pemaclav drug (Amoxicillin combination) MIC (mg/ml)
<i>Salmonella typhi</i>	100	25	12
<i>Salmonella paratyphi</i>	100	25	12

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Values are mean of three replicates (n=3)

Table 5. Acute Toxicity Results on Twenty Randomly Selected Albino Rats.

Dose (mg/kg)	Time Duration	No. of Animals	No of Deaths	Observation Signs
5000	0-30 minutes	5	0	Weakness, slow movement immediately after administration.
	1 hour			Continuously scratching of mouth part, fur and body, tremor.
	24 hours			Ruffled fur, scratching of their nostril.
	48 hours			Normal movement and less scratching of body part.
5000	0-30 minutes	5	0	No death rate recorded.
	1 hour			Increased breathing
	24 hours			Scratching of mouth and body parts
	48 hours			Ruffled fur No scratching of body part

2 weeks

No death rate recorded

307 Key:

308 a) The first 5 rats were given aqueous leaf extracts of *O. gratissimum*

309 b) The last two 5 rats were given ethanolic leaf extracts of *O. gratissimum*

310

311 Conclusion

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313 From this study, it was observed that ethanol extracts exhibited high inhibitory activity on the test
314 organisms. This can be deduced to the ability of ethanol to extract more of the essential oils and
315 secondary plant metabolites which are believed to exert antibacterial activity on the test organisms.
316 This suggests the possibility of using the ethanol extracts of *O. gratissimum* in treating the diseases
317 caused by the test organisms. Aqueous and ethanolic extracts of *Ocimum gratissimum* exhibit no
318 toxic effects when given orally at concentration of 5000mg/kg body weight. However the normalcy
319 and insignificant changes in toxicity parameters and body weights reveals the safety of aqueous
320 and ethanolic extract at a dose of 5000 mg/kg body weight. This study however can justify the use
321 of the leaf in traditional medicine practice as a therapeutic agent and can explain the long history
322 use of these plants.

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