

2 **Evaluation of Phytochemicals and Antimicrobial**
3 **Potentials of *Chromolaena odorata* (L.) on Selected**
4 **Human Pathogens**

5 **ABSTRACT**

6 **Aims:** This research was designed to evaluate the phytochemicals embedded in the leaf extracts of
7 *Chromolaena odorata* L. and its antimicrobial activities.

8 **Methodology:** The dried plant of *C. odorata* was pulverized and subsequently subjected to ethanolic and
9 aqueous extraction. The extracts were qualitatively and quantitatively screened for phytochemicals using
10 standard methods. The inhibitory activity of the leaf extracts were evaluated against clinical pathogens;
11 *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiella*
12 *pneumoniae*, *Proteus mirabilis* and *Candida albicans* using agar well diffusion technique at 100 mg/mL
13 and 200 mg/mL extract concentrations.

14 **Results:** The ethanolic extract of *C. odorata* had a better percentage yield of 5.49 g, followed by aqueous
15 extract (3.5g). The phytochemical screening conducted on the extracts revealed the presence of
16 flavonoid, alkaloid, saponin, cardiac glycoside, steroids, tannins and terpenoids. The ethanolic extract
17 exhibited better antimicrobial activity on *S. typhi*, *S. aureus*, *E. coli*, *Ps. aeruginosa* and *Candida albicans*
18 compared to the aqueous extract. This could be as a result of the higher extraction capability of the
19 ethanol to penetrate easily into the cellular membrane and dissolve the intracellular inclusions from the
20 plant materials than the aqueous solvent. The zones of inhibition of ethanolic extract at 100 mg/mL
21 ranges from 2.33±0.33 mm to 9.50±0.36 mm with the lowest efficacy observed on *P. mirabilis* and highest
22 on *S. aureus*. *S. typhi* was susceptible to the aqueous extract of the plant at this concentration with
23 inhibitory zone of 4.00±0.00 mm. The ethanolic extract of the plant was also effective against *C. albicans*
24 with inhibitory zone of 4.17±0.17 at 100 mg/mL. In comparison, chloramphenicol (antibiotic) inhibited all
25 the test bacteria with the highest efficacy on *E. coli* (16.33±0.03 mm) and ketoconazole at 25 mg/mL had
26 a better antifungal activity on *C. albicans* compared to the observed antifungal activities of the aqueous
27 and ethanolic extracts of *C. odorata* at 100 mg/mL. Furthermore, the test organisms were more
28 susceptible to the aqueous and ethanolic extracts of *C. odorata* at 200 mg/mL with zones of inhibition
29 ranging from 3.23±0.15 mm to 12.33±0.33 m. The lowest being observed on *E.coli* and highest on *S.*
30 *typhi* (ethanolic extract). *K. Pneumoniae* and *P. mirabilis* were resistant to the aqueous extract of *C.*
31 *odorata*. All the test bacteria were susceptible to the aqueous and ethanolic extracts of *C. odorata* at 200
32 mg/mL extracts concentration. Moreover, *C. albicans* was susceptible to the inhibitory effect of *C. odorata*
33 at this concentration with inhibitory zones of 3.00±0.00 mm and 5.33±0.33 mm on aqueous and ethanolic
34 extracts respectively.

35 **Conclusion:** The findings from this study revealed the antimicrobial activities of *C. odorata* leaf extracts
36 on the test pathogens which are in close proximity in comparison with the synthetic antimicrobial agents
37 and thus upon purification, can be harnessed as a lead for the development of natural products derived
38 antimicrobials in drug discovery against infections caused by these human pathogens evaluated in this
39 study.

40 **Key words:** Antimicrobial Potential, Phytochemicals, *Chromolaena odorata* L., Human pathogens.

41 **1.0 INTRODUCTION**

42 The emergence of pathogens resistant to antibiotics has increased in recent years due to indiscriminate
43 or misuse of drugs [1]. The plant *Chromolaena odorata* (Syn. *Eupatorium odoratum* Linn.) has been used
44 in folkloric medicine in western part of Nigeria in the treatment of burns, wounds and skin infections [2].
45 Traditionally, fresh leaves or a decoction of *C. odorata* is used in tropical countries for the treatment of
46 leech bite, soft tissue wounds, burn wounds and liver diseases [3]. Although synthetic antibiotics abound,
47 there is still need for continuous search on avenues to match the increased emergence of multiple
48 antibiotic resistant strains of pathogens [4].

49 Researchers are increasingly turning their attention to developing natural products antimicrobials as new
50 leads in complementary medicine against microbial infections, since many plants with antimicrobial
51 efficacy have bioactive compounds which presents opportunities for a new lead [5]. Natural products are
52 known by their active substances, for example, the phenolic compounds which are a part of the essential
53 oils [6] and tannins [7]. Medicinal values of plants is based on the abundance of their component
54 phytochemicals such as alkaloids, tannins, flavonoids and other phenols which gives definite
55 physiological action on the human body [8].

56 *Chromolaena odorata* (L.) belongs to the family Asteraceae (Compositae) and it is also called Siam weed;
57 it is a rapidly growing and scented perennial shrub commonly found in western Nigeria [9,10,11]. The
58 plant is used by traditional health care givers in the treatment of many ailments especially for dysentery,
59 headache and toothache [12]. Traditionally in some African communities, local dwellers apply crushed
60 leaves of *C. odorata* on fresh wound to facilitate healing [13].

61 Most of the synthetic antibiotics used in treating infections produce side effects and have varying toxicities
62 to humans [14,15]; more so, there have been continued reemergence of multiple antibiotic resistances

63 among pathogens of human infection which necessitates the use of natural products as alternative source
64 of antimicrobials. Hence, this study investigated the phytochemical constituents of *Chromolaena odorata*
65 as well as its antimicrobial efficacy against some selected human pathogens.

66 **2.0 Materials and Methods**

67 **2.1 Sample collection and Preparation**

68 Fresh leaves of *C. odorata* were collected within the Federal University of Technology, Akure campus.
69 The leaves were identified and authenticated by Prof. Y. A. Awodun at the Department of Crop Science
70 and Pest Management, The Federal University of Technology, Akure, Nigeria. The harvested leaves were
71 washed in distilled water to remove dirt, allowed to air dry and pulverized into smooth powder using a
72 grinder (type N model) and subsequently sieved with 1.18 sieve; they were stored in air tight plastic bags
73 before extraction was carried out.

74 **2.2 Preparation of Extracts and percentage yield**

75 **2.2.1 Preparation of Aqueous Extract**

76 A 100 g of powdered *C. odorata* was weighed and soaked in 1000 mL of distilled water in a conical flask,
77 swirled intermittently at an hour interval. After 72 hours, the mixture was filtered using Whatman No.1 filter
78 paper into a clean beaker and concentrated to dryness using water bath at 70°C for 24 hours [16]. The
79 extract obtained was stored at 4°C prior to analysis.

80 **2.2.2 Preparation of ethanolic extract**

81 A 100 g of powdered *C. odorata* was weighed and soaked in 1000 mL absolute ethanol contained in a
82 conical flask and swirled at every hour interval. After 72 hours, mixture was filtered using Whatman no.1
83 filter paper and membrane filter of pore size 0.45 micron to obtain sterile extract and this was stored at
84 4°C [17].

85 The recovery rate of each extracts was calculated using the formula below;

86

87 % Recovery of extract = $WA / IW \times 100$

88 Where WA = Weight of extracts recovered after extraction, IW = Initial weight of extracts.

89 **2.3 Phytochemical screening**

90 The aqueous and ethanolic extracts were qualitatively and quantitatively screened for phytochemicals as
91 described by Ayodele [18].

92 **2.4 Sterility Test of the extracts**

93 The extracts were filtered with Millipore membrane discs; a 2ml of sterile extracts was introduced into
94 10ml of sterile nutrient broth. This was incubated at 37°C for 24 hours; the absence of turbidity after the
95 incubation period denotes its sterility [19].

96 **2.5 Reconstitution of plant extracts**

97 The different concentrations of extracts were reconstituted by dissolving 2 g of the extract in 10 ml of 30%
98 Dimethyl Sulfoxide (DMSO) according to NCCLs [20] and a final concentration of 100 mg/mL of the
99 extracts is obtained according to method described by Hena [21].

100 **2.6 Source of Test pathogens**

101 The test organisms (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella*
102 *typhi*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Candida albicans*) were obtained from the
103 Microbiology Laboratory of the University College Hospital (UCH) Ibadan, Nigeria. The organisms were
104 confirmed by sub-culturing into sorbitol MacConkey agar and Nutrient agar and were identified using
105 standard biochemical tests (gram staining; indole test, Methyl red test, Citrate utilization, Voges
106 Proskauer test) etc and were further identified with reference to the Bergey's manual of systematic
107 bacteriology [22].

108 **2.7 Standardization of inoculum (Test organisms) for Antimicrobial Analysis**

109 A 0.5 McFarland standard was prepared by adding 0.5ml of 1% Barium chloride (BaCl₂) to 99.5ml of 1%
110 Sulphuric acid (H₂SO₄) solution. The turbidity of the 0.5McFarland standard was used to estimate bacterial
111 counts in broth culture after 24 hours of incubation at 37±1°C in order to obtain a standard bacterial
112 suspension of 1x10⁸ bacterial cells that was used for the antimicrobial assay [21,23].
113

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116 2.8 Antimicrobial assay of *Chromolaena odorata* extracts on test organisms

117 The susceptibility pattern of the test organisms to *C. odorata* aqueous and ethanolic leaf extracts was
118 carried out using agar well diffusion method as described by Douye [16]. A 1 ml of the standardized
119 inoculum of each test bacteria was pour-plated on freshly prepared Mueller-Hinton agar and Sabouraud
120 dextrose agar was used for the antifungal assay of extracts against test fungi. Different wells of 6 mm
121 wide were punched aseptically using sterile cork borer of 6 mm in diameter and 0.2 ml of different extract
122 concentrations was dispensed into the labeled wells. Chloramphenicol (250 mg/ml) and ketoconazole
123 were used as positive controls respectively for bacteria and fungi. The plates were allowed to set for 30
124 minutes ensuring diffusion and were incubated for 24 hours at 37±1°C for bacteria and 27±1°C for fungi,
125 the plates were examined and inhibition zone diameters were measured in millimeter.

126 2.9 Statistical Analysis

127 Data obtained are presented as mean ± SE (standard error), treatment groups were analyzed using one
128 way analysis of variance (ANOVA) and data means were compared with Duncan's New multiple range
129 tests at the level of P<0.05.

130

131 3.0 RESULTS

132 3.1 Percentage yield of *Chromolaena odorata* leaf extracts

133 The ethanol extract had significant percentage yield (5.49 g) after the extraction, while the aqueous
134 extract had a yield of 3.5 g determined by the formula;

135 % yield of extract = $WE/IW \times 100$; where WE = weight of extracts yielded, IW = Initial weight

136

137 **Table 1: Percentage yield of *Chromolaenaodorata* leaf extract**

Solvent	Original weight (g)	Weight of extract (g)	% yield
Ethanol	500	27.45	5.49
Aqueous	500	17.50	3.50

138

139 **3.2 Qualitative and quantitative phytochemical screening of *Chomolaena odorata* leaf**
140 **extract.**

141 The aqueous and ethanolic yields of the plant extracts were qualitatively and quantitatively screened for
142 phytochemicals which revealed the presence of saponins, tannins, flavonoids, steroids, terpenoids,
143 alkaloids and cardiac glycosides.

144 Findings from the study revealed that the aqueous solvent possesses low extractive potential for steroid
145 compared to the ethanolic solvent used for the extraction process. However, the ethanolic extract had the
146 highest extractive value for flavonoids, tannins and steroids than the aqueous extract.

147 The extract revealed higher flavonoid content of 26.18 ± 0.00 mg/g compared to the aqueous extract. The
148 aqueous extract showed significant extractive potential for flavonoid, alkaloid, saponin at varying
149 compositions than other phytochemicals present, however, not as much as the ethanolic extract.

150 The result also indicated that some phytochemicals were found to be absent. These include absence of
151 phlobatanin and anthraquinone.

152 **Table 2. Qualitative phytochemical composition of *Chromolaena odorata* leaf extract.**

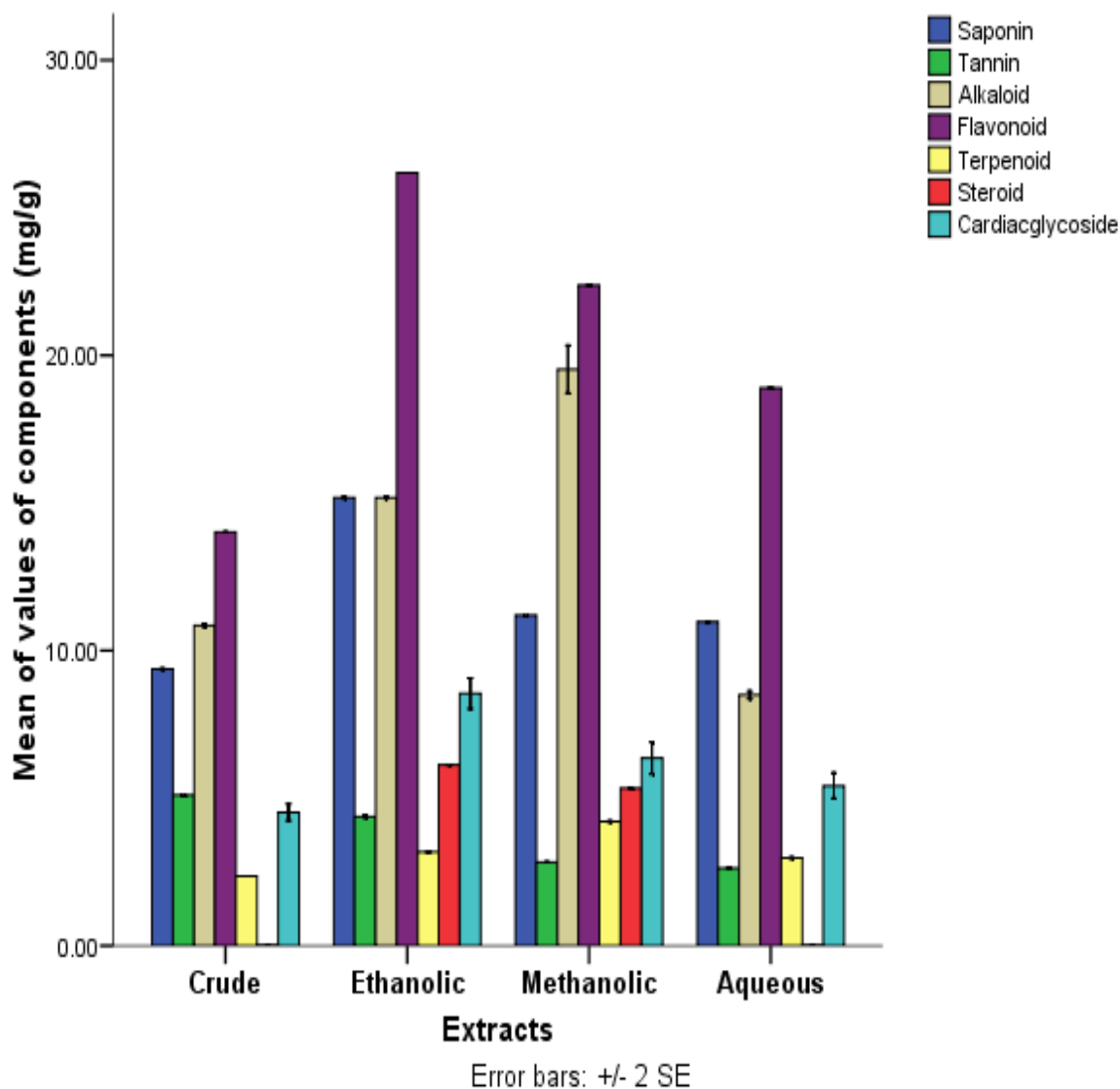
Phytochemical	Ethanol	Aqueous
Saponin	+	+
Tannin	+	+
Phlobatannin	-	-
Flavonoid	+	+
Steroid	+	-
Terpenoid	+	+
Alkaloid	+	+
Anthraquinone	-	-
Cardiac glycoside	+	+

153 Key: + = present. - = absent.
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159 Fig 1. Quantitative phytochemical composition of *Chromolaena odorata* revealing the
160 ethanolic and aqueous extraction potentials

161

162 3.3 Comparative antimicrobial activity of ethanolic and aqueous leaf extracts of 163 *Chromolaena odorata* L. at 100 mg/mL on Test organisms

164 The antimicrobial activities of *C. odorata* aqueous and ethanolic extracts at 100 mg/mL are presented in
165 Table 3. The zones of inhibition of ethanolic extract ranges from 2.33 ± 0.33 mm to 9.50 ± 0.36 mm with the
166 lowest efficacy observed on *P. mirabilis* and highest on *S. aureus* while only *S. typhi* was susceptible to
167 aqueous extract of *C. odorata* at this concentration with inhibitory zone of 4.00 ± 0.00 mm. The ethanolic
168 extract of *C. odorata* was also effective in inhibiting *C. albicans* with inhibitory zone of 4.17 ± 0.17 at 100

169 mg/mL. In comparison with the *C. odorata* aqueous and ethanolic extracts, chloramphenicol at 5 mg/mL
 170 inhibited all the test bacteria with the highest efficacy on *E. Coli* (16.33±0.03 mm). Also, ketoconazole at
 171 25 mg/mL had a better antifungal activity on *C. albicans* compared to the observed antifungal activities of
 172 aqueous and ethanolic extracts of *C. odorata* at 100 mg/mL.

173
 174 **3.4 Comparative antimicrobial activity of ethanolic and aqueous leaf extracts of**
 175 ***Chromolaena odorata* L. at 200 mg/mL on Test organisms**

176 The antimicrobial activities of *C. odorata* aqueous and ethanolic extracts at 200 mg/mL are presented in
 177 Table 4. The test organisms were more susceptible to the aqueous and ethanolic extracts of *C. odorata* at
 178 200 mg/mL with zones of inhibition that ranges from 3.23±0.15 mm to 12.33±0.33 mm with the lowest
 179 observed on *E. coli* (aqueous extract) and highest on *S. typhi* (ethanolic extract). It was observed that *K.*
 180 *Pneumoniae* and *P. mirabilis* were resistant to the aqueous extract of *C. odorata*. However, all other test
 181 bacteria were susceptible to the aqueous and ethanolic extracts at 200 mg/mL extracts concentration.
 182 Moreover, *C. albicans* was susceptible to the inhibitory effect of *C. odorata* at this concentration with
 183 inhibitory zones of 3.00±0.00 mm and 5.33±0.33 mm on aqueous and ethanolic extracts respectively
 184 while ketoconazole was most effective on the test fungi with inhibitory zone of 13.50±0.28 mm.

185
 186 **Table 3. Comparative antimicrobial activity of ethanolic and aqueous extracts of**
 187 ***Chromolaena odorata* L. leaf at 100 mg/mL on Test organisms in millimeter (mm).**

Test organisms	Extract		AB	AF
	Ethanolic	Aqueous	Chlo(5mg/mL)	Keto (25 mg/mL)
<i>Escherichia coli</i>	8.27±0.15 ^d	0.00±0.00 ^a	16.33±0.33 ^d	N.T
<i>Staphylococcus aureus</i>	9.50±0.36 ^d	0.00±0.00 ^a	14.33±0.33 ^e	N.T
<i>Pseudomonas aeruginosa</i>	8.17±0.17 ^c	0.00±0.00 ^a	13.53±0.29 ^d	N.T
<i>Salmonella typhi</i>	9.10±0.10 ^e	4.00±0.00 ^b	15.67±0.33 ^f	N.T
<i>Klebsiella pneumoniae</i>	4.17±0.16 ^b	0.00±0.00 ^a	10.17±0.17 ^c	N.T
<i>Proteus mirabilis</i>	2.33±0.33 ^b	0.00±0.00 ^a	10.33±0.33 ^c	N.T
<i>Candida albicans</i>	4.17±0.17 ^b	0.00±0.00 ^a	N.T	13.50±0.28 ^c

188 Key: Data are presented as Mean ± S.D (n=3). Values with the same superscript letter (s) along the same
 189 row are not significantly different (P<0.05). Chlo=Chloramphenicol; Keto=Ketoconazole, N.T= Not Tested,
 190 AB= Antibacterial agent, AF= Antifungal agent.

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193

194 **Table 4: Comparative antimicrobial activity of ethanolic, and aqueous extracts of *Chromolaena***
 195 ***odorata* L. leaf at 200mg/mL on Test organisms in millimeter (mm)**

Test organisms	Extract		AB	AF
	Ethanolic	Aqueous	Chlo (5mg/mL)	Keto (25 mg/mL)
<i>Escherichia coli</i>	9.33±0.33 ^d	3.23±0.15 ^b	16.33±0.33 ^e	N.T
<i>Staphylococcus aureus</i>	11.33±0.33 ^d	3.53±0.29 ^b	14.33±0.33 ^e	N.T
<i>Pseudomonas aeruginosa</i>	10.33±0.33 ^e	4.16±0.16 ^b	13.53±0.29 ^f	N.T
<i>Salmonella typhi</i>	12.33±0.33 ^e	6.17±0.17 ^b	15.67±0.33 ^f	N.T
<i>K. pneumonia</i>	6.33±0.33 ^d	0.00±0.00 ^a	10.17±0.17 ^e	N.T
<i>Proteus mirabilis</i>	6.27±0.27 ^c	0.00±0.00 ^a	10.33±0.33 ^d	N.T
<i>Candida albicans</i>	5.33±0.33 ^d	3.00±0.00 ^b	N.T	13.50±0.28 ^e

196 Key: Data are presented as Mean ± S.D (n=3). Values with the same superscript letter (s) along the same
 197 row are not significantly different (P<0.05). Chlo=Chloramphenicol; Keto=Ketoconazole, N.T= Not Tested,
 198 AB= Antibacterial agent, AF= Antifungal agent.

199
 200 **4.0. DISCUSSION**

201
 202 This research work has been able to establish the antimicrobial efficacy of *C. odorata*
 203 leaf extracts on human pathogens. The antimicrobial potential of medicinal plants and drugs varies in
 204 their inhibitory effect, depending on the concentration of crude extracts or synthetic drug, size of
 205 inoculums, temperature, rate of diffusion and the nature of organism [24]. The result of the extraction of
 206 *Chromolaena odorata* L. showed that the ethanolic extract had higher yield compared to aqueous extract.
 207 This result corroborate the work done by Tiwari [25] who submitted that ethanol has higher extraction
 208 capability than aqueous due to its ability to penetrate easily into the cellular membrane and dissolve the
 209 intracellular inclusions from the plant material. The limited ability of water to extract bioactive components
 210 from plant materials have also been shown by Ncube [26]. The plant extracts screened for
 211 photochemicals revealed the presence of saponins, tannins, flavonoids, phenols, glycosides,
 212 phlobatannins, alkaloids and steroids. These phytochemicals are common in plants although at varying
 213 quantities which have been reported by several researchers [26,27,28]. The variations in the presence of
 214 the phytochemicals may be due to the choice of solvent used in the extraction process; this may be that,
 215 during extraction, solvents may have diffused into the plant material and solubilized compounds with
 216 similar polarity [26].

217 The ethanolic extract revealed high flavonoid content of 26.18 ± 0.00 mg/g, The aqueous extract
218 showed significant extractive potential for flavonoid, alkaloid, saponin at varying compositions, however
219 ethanolic extract had a greater and better extraction capability on the phytochemicals present in *C.*
220 *odorata*. This result is in agreement with Sukanya [29] who reported that most of the compounds from
221 natural origin have positive property of being soluble in polar solvents. There was no significant
222 antimicrobial activity of *C. odorata* aqueous extract on the test organisms at 100 mg/mL except on *S.*
223 *typhi*. This may be as a result of insufficient phytochemicals in this extract and thus reducing its
224 antimicrobial efficiency. However, the comparative antimicrobial activities of the ethanolic and aqueous
225 extracts of *C. odorata* at 100mg/mL and 200mg/mL on the clinical test organisms indicated that the
226 extracts had better inhibitory effect on the test organisms at 200 mg/mL, with the ethanolic extract
227 showing higher inhibitory potential on *Salmonella typhi* (12.33 ± 0.33 mm), *Staphylococcus aureus*
228 (11.33 ± 0.33) and closely, followed by *Escherichia coli* with zones of inhibition of 9.33 ± 0.33 mm at 200
229 mg/mL extract concentration. Compared to the antimicrobial activities of *C. odorata* at 100 mg/mL, the
230 aqueous extract at 200 mg/mL demonstrated high inhibitory effect on the test organisms.

231 Noteworthy is the observation on some microbes such as *E. coli*, *S. aureus*, *Ps. aeruginosa*, *K.*
232 *Pneumoniae* and *C. albicans* which were resistant to aqueous extract of *C. odorata* at 100 mg/mL were
233 found to be susceptible to the extract at 200 mg/mL which indicated that the susceptibility pattern of the
234 pathogens to the extract was concentration dependent. This corroborates the findings of Owoyemi and
235 Oladunmoye [30]. The higher antimicrobial activities of the ethanolic extracts observed in this study may
236 be attributed to the presence of higher amounts of polyphenols in the ethanolic extract compared to the
237 aqueous extract. This implies that they are more efficient in cell walls and seeds degradation which have
238 unpolar character and cause polyphenols to be released from cells into the solvents [25] and this may be
239 responsible for the higher antimicrobial activity of *Chromolaena odorata*. Hence, high concentration of
240 bioactive compounds with inhibitory activities against the test organisms [31,32].

241 It was also reported by Negi and Jayaprakasha [33] who worked on the antibacterial and
242 antifungal effect of alcoholic extracts of *Punica granatum* and concluded that higher concentration of the
243 extracts were found in organic solvent and they exhibit better antibacterial activity. Similar conclusion was
244 drawn by Kokoska [34], who reported that the ethanolic extract of *S. officinalis* had high antibacterial

245 activity against *E. coli* and *S. aureus*. The conventional antimicrobial agent used in this study that include
246 Chloramphenicol and Ketoconazole were found to be very effective in inhibiting the test pathogens at low
247 concentrations of 5 mg/mL and 25 mg/mL respectively.

248 **5.0. CONCLUSION**

249 The phytochemical screening and antimicrobial activities of *C. odorata* leaf extracts analyzed in
250 this study revealed the presence of saponins, tannins, flavonoids, phenols, glycosides, phlobatannins,
251 alkaloids and steroids. The availability of these phytochemicals in *C. odorata* leaf extracts could be
252 responsible for the antimicrobial activity conferred on the tested pathogens at 200 mg/mL respectively.
253 Hence, *C. odorata* has plausible promise in the development of phytomedicines (drug discovery) with
254 great antimicrobial properties on human pathogens.

255 **COMPETING INTERESTS**

256 No competing interest exist

257

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