Original Research Article

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

ABSTRACT

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

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

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

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

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

1.0 INTRODUCTION

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

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

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

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

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

2.0 Materials and Methods

2.1 Sample collection and Preparation

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

2.2 Preparation of Extracts and percentage yield

2.2.1 Preparation of Aqueous Extract

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

2.2.2 Preparation of ethanolic extract

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

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

% Recovery of extract = WA / IW x 100

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

2.3 Phytochemical screening

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

2.4 Sterility Test of the extracts

The extracts were filtered with Millipore membrane discs; a 2ml of sterile extracts was introduced into 10ml of sterile nutrient broth. This was incubated at 37^0C for 24 hours; the absence of turbidity after the incubation period denotes its sterility [19].

2.5 Reconstitution of plant extracts

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

2.6 Source of Test pathogens

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

2.7 Standardization of inoculum (Test organisms) for Antimicrobial Analysis

A 0.5 McFarland standard was prepared by adding 0.5ml of 1% Barium chloride ($Bacl_2$) to 99.5ml of 1% Sulphuric acid (H_2S0_4) solution. The turbidity of the 0.5McFarland standard was used to estimate bacterial counts in broth culture after 24 hours of incubation at 37 ± 1^oC in order to obtain a standard bacterial suspension of $1x10^8$ bacterial cells that was used for the antimicrobial assay [21,23].

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

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

2.9 Statistical Analysis

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

3.0 RESULTS

3.1 Percentage yield of *Chromolaena odorata* leaf extracts

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

% yield of extract = WE/IW X 100; where WE = weight of extracts yielded, IW = Initial weight

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

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

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

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

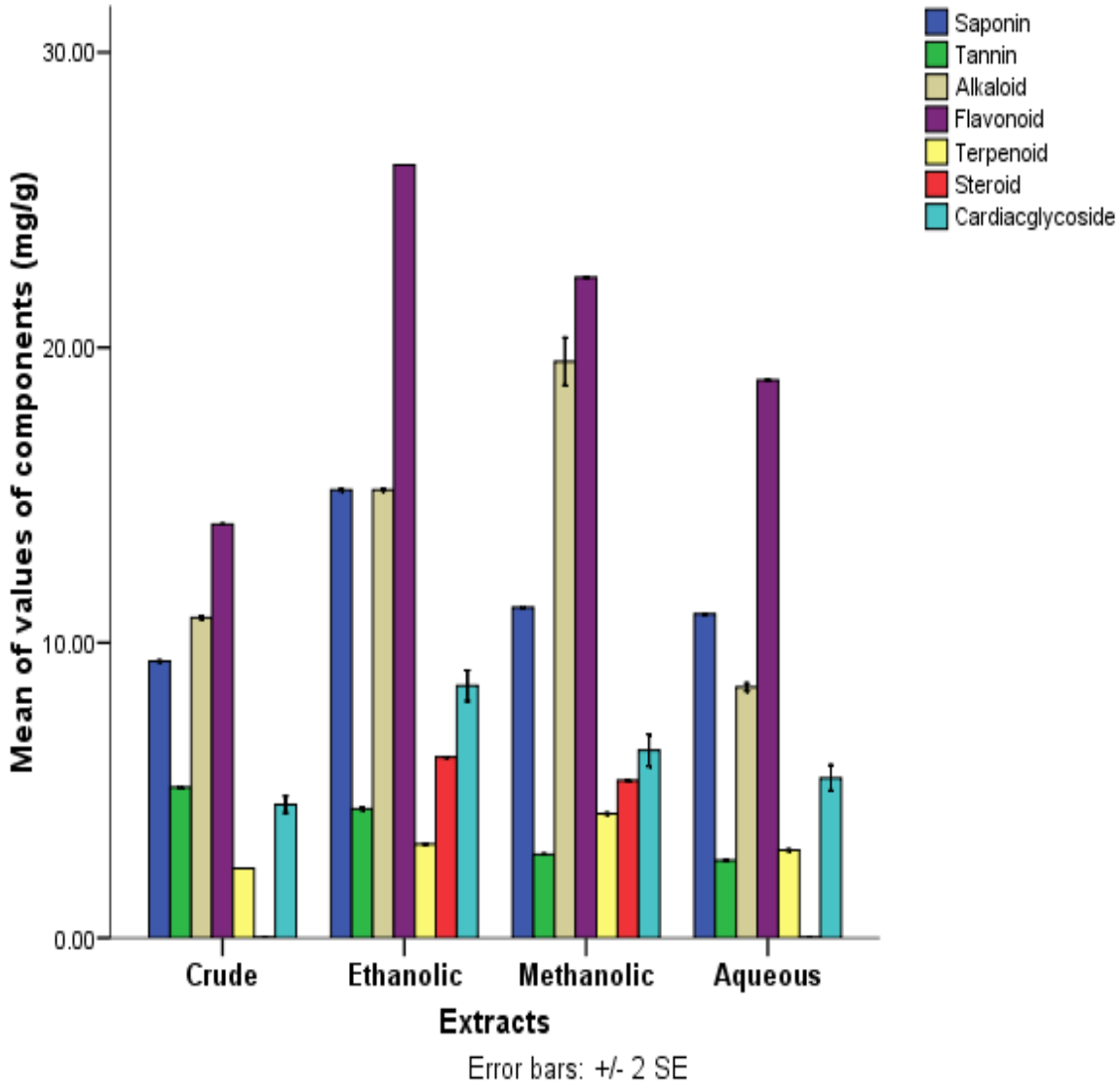
The extract revealed higher flavonoid content of 26.18±0.00 mg/g compared to the aqueous extract. The aqueous extract showed significant extractive potential for flavonoid, alkaloid, saponin at varying compositions than other phytochemicals present, however, not as much as the ethanolic extract.

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

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

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

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

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

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

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

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

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

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

Table 4: Comparative antimicrobial activity of ethanolic, and aqueous extracts of *Chromolaena 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)
Escherichia coli	9.33±0.33^{d}	3.23±0.15^{b}	16.33±0.33^{e}	N.T
Staphylococcus aureus	11.33±0.33^{d}	3.53±0.29^{b}	14.33±0.33^{e}	N.T
Pseudomonas aeruginosa	10.33±0.33^{e}	4.16±0.16^{b}	13.53±0.29^{f}	N.T
Salmonella typhi	12.33±0.33^{e}	6.17±0.17^{b}	15.67±0.33^{f}	N.T
K. pneumonia	6.33±0.33^{d}	0.00±0.00^{a}	10.17±0.17^{e}	N.T
Proteus mirabilis	6.27±0.27^{c}	0.00±0.00^{a}	10.33±0.33^{d}	N.T
Candida albicans	5.33±0.33^{d}	3.00±0.00^{b}	N.T	13.50±0.28^{e}

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

4.0. DISCUSSION

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

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

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

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

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

5.0. CONCLUSION

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

COMPETING INTERESTS

No competing interest exist

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