

Original Research Article

Phytochemical Screening and Antimicrobial Activity of *Chromolaena odorata* Leaf Extract Against Selected Microorganisms

ABSTRACT

Aims: The increasing microbial drug resistance in recent times has necessitated the search for an alternative antimicrobial agents derived from natural sources. *Chromolaena odorata* L. (Asteraceae) is one of such natural sources that has been reported to possess healing properties. In this study, the phytochemical constituents and antimicrobial properties of *C. odorata* leaf extract were investigated.

Methodology: The leaves of *C. odorata* were collected from Babcock University garden, authenticated, prepared and extracted following standard procedures with methanol and ethyl-ether as extraction solvents. Phytochemical screening was carried out according standard protocol while antimicrobial screening was performed according to agar well diffusion method on the following organisms: *Pseudomonas aeruginosa* (ATCC19582), *Shigella flexneri* (KZN), *Klebsiella pneumoniae* (ATCC 10031), *Enterococcus faecalis* (ATCC 29212), *Enterococcus cloacae* (ATCC 13047), *Proteus vulgaris* ATCC 6830, *Escherichia coli* (ATCC 25922) *Enterococcus faecalis* (KZN) and *Neurospora crasa*.

Results: Of the nine phytochemicals screened (terpenoids, steroids, flavonoids, alkaloids, saponins, tanins, phlobatannin, phenols and anthraquinones), only one (phlobatannin) was absent in both solvent extracts. The antimicrobial activity of the extracts indicated least and highest zones of inhibition of 9 and 23 mm against *Shigella flexneri* (KZN) and *Shigella sonnei* (ATCC19930) respectively and fungicidal potency of 100% within 24 h on *Neurospora crasa*.

Conclusion: *Chromolaena odorata* extract possesses antimicrobial activity and thus, represents a promising source for medicines of which when carefully tapped and explored has enormous therapeutic potentials.

Keywords: Phytochemicals; Antmicrobials; *Chromolaena odorata*

1. INTRODUCTION

Since inception, plants have been recognized to contain natural products which serve as food [1] as well as medicine in the event of human infections [2, 3]. The presence of some phytochemicals determines the antimicrobial properties of any plant. They are also responsible for the plants flavors, odours, colors and majorly their system of defense or resistance against some pathogens [4]. There has been quest for search of an alternative antimicrobial agents from natural plant origin due to increasing microbial drug resistance [5, 6,7]. When new drug is introduced into clinical practice, microorganisms particularly bacteria develop resistance to such drug within short period of its introduction. In other words, new chemotherapeutic agents have always been accompanied by corresponding increase in drug resistance [8]. The problem of drug resistance has prompted researchers to turn their attention to folk medicines as alternative to conventional chemotherapeutic agents following several reports on the medicinal opportunities derived from higher plants [9]. One of these plants is *Chromolaena odorata* L. (King and Robinson) which belong to the family Asteraceae. This weed is found in tropical Africa, North America, and South and Southeast Asia. The common names include Siam weed, Christmas bush, and common floss flower [10]. Traditional medicinal uses include anti-diarrheal, astringent, antispasmodic, antihypertensive, antiinflammatory, diuretic tonic, antipyretic and heart tonic [11, 12, 13]. The phytochemical components or the secondary metabolites (Alkaloids, Tannins, Flavonoids and other Phenolic compounds) found in this plant have medical values. Generally, plant secondary metabolites exhibit their microbial actions by disrupting membrane function and structure, interrupting DNA/RNA synthesis, interfering with intermediary metabolism, inducing coagulation of cytoplasmic constituents and interrupting normal cell communication [14].

Pocket of studies have demonstrated biological activities of plants: phytopathogenic activity [15], anthelmintic activity [2], antiviral activity [16] as well as antiprotozoal activity [12] to mention few. Ijato and Tedela [17] demonstrated that *C. odorata* extract has inhibitory effect against fungal deteriorating agents of yam tubers. In addition, other authors also demonstrated that *C. odorata* extract exhibits bacterial activities and contains some phytochemicals [18, 19, 2, 20]. These later authors either use water, alcohol, ester or both as solvents as well as varied susceptibility test methods (minimum inhibitory concentration or disc diffusion). In this study however, different solvents combination (alcohol and ether), technique of phytochemical screening, and antibiogram method (agar well dilution) were employed to evaluate the presence of nine phytochemicals in and medicinal properties of *C. odorata* leaf extract on selected strains of microorganisms.

2. MATERIAL AND METHODS

2.1 Sample collection

Fresh leaves of *C. odorata* were collected from Babcock University behind the Science complex in June, 2015 and was properly authenticated by Prof. E.B. Esan, a plant scientist, Department of Basic Sciences, Babcock University, Ogun State, Nigeria.

2.1.1 Drying of the samples.

The leaves of *C. odorata* were subjected to hot air over (maker) at 40°C to a constant weight in the microbiology laboratory of the department of Biosciences and Biotechnology, Babcock University, Ilishan Remo, Ogun State, Nigeria.

2.1.1.1 Sample preparation

The dried leaves were manually grinded into uniform powder and transferred into a clean polythene bag. Two hundred gram (200 g) of the grinded powder was weighed into two separate one litre conical flasks each containing (750) ml of methanol and ethyl ether. The conical flasks were covered with sterile cotton wool and aluminum foil paper. The flasks with its contents were agitated at six-hour interval for three days and the contents were filtered using Whatman filter paper of 5 mm grade and labeled appropriately. The extracts were concentrated at 45°C using Rotary evaporator (Bioline Technologies, India).

2.2 Phytochemical screening

Plant extracts obtained with methanol and ethyl-ether were evaluated for the presence of terpenoids, steroids, flavonoids, alkaloids, Saponins, tannins, phlobatannins, phenols, and anthraquinones using Harbone method [21] as described below.

2.2.1 Test for Terpenoids

Five (5) ml of the extracts with 2 ml of chloroform was carefully added to 3 ml of concentrated H₂SO₄. A reddish brown color at the interface is an evidence of presence of terpenoids.

2.2.2 Test for Steroid

Exactly 0.5 ml of acetic anhydride and few drops of the concentrated H₂SO₄ were added into 1 ml of the extracts, and a bluish-green precipitate indicates the presence of steroids.

2.2.3 Test for Flavonoids

One (1) ml of extract was transferred into a 5 ml test tube. Two (2) ml of 10% NaOH was then added. A change in color from yellow to colorless on addition of dilute hydrochloric acid shows the presence of flavonoids.

2.2.4 Test for Alkaloids

One (1) ml of extract was transferred into a 5 ml test tube. Two (2) ml of chloroform and few drops of Wagner's reagent was added. A reddish brown precipitate indicates the presence of alkaloids.

2.2.5 Test for Saponins

One (1) ml of the extract was added to 5 ml of distilled water in test tube and shaken. Persistence fronting indicates the presence of saponins.

2.2.6 Test for Tannins

One (1) ml of extract was transferred into a 5 ml test tube. Three drops of 5% of Iron 111 Chloride was added to the extract. A greenish black precipitate shows the presence of tannins.

2.2.7 Test for phlobatanins

One (1) ml of extract was transferred into a 5 ml test tube. Few drops of 1% of hydrochloric acid was added and boiled. A red precipitate is as an evidence for the presence of Phylobatannins.

2.2.8 Test for Phenols

An equal volume of the extract and 1% Iron Chloride solution was mixed in a 5 ml test tube. A deep bluish green solution indicates the presence of phenols.

2.2.9 Tests for Anthraquinones

One (1) ml of extract was transferred into a 5 ml test tube followed by addition of 10 ml of chloroform. Thereafter, equal volume of extract-chloroform mixture and 10% ammonia solution was mixed. A pink violet or red color in the ammoniacal layer indicates the presence of anthraquinone.

2.3 Antimicrobial screening

2.3.1 Test organisms

Eight pure typed cultures of bacterial species and one fungus were collected from IITA Ibadan, Oyo State and Department of Biosciences and Biotechnology, Ilishan-Remo, Ogun State, Nigeria respectively. The bacteria include: *Pseudomonas aeruginosa* (ATCC19582), *Shigella flexneri* (KZN), *Klebsiella pneumoniae* (ATCC 10031), *Enterococcus faecalis* (ATCC 29212), *Enterococcus cloacae* (ATCC 13047), *Proteus vulgaris* ATCC 6830, *Escherichia coli* (ATCC 25922) and *Enterococcus faecalis* (KZN) while the fungus is *Neurospora crassa*.

2.3.2 Bacterial susceptibility

The inhibitory activity of the extract on the bacterial isolates was determine by agar well diffusion. Briefly, 1 ml of cell suspension of the test organism equivalent to McFarland turbidity standard was transferred into Petri dish followed by molten nutrient agar prepared according to the manufacturer's instruction. The plates were mixed well and allowed to solidify. Thereafter, sterile cork borer of diameter 8 mm was used to drill wells on each plate. With the aid of micro pipette, the wells were filled with 100 µg (0.1 ml) of plant extracts (methanolic and ethyl-ether extracts) of varying concentrations of 1 mg/ml, 500 µg /ml and 250 µg /ml. These plates were incubated at 37°C for 24 h and the zones of inhibition were measured in millimeters after incubation.

2.3.3 Anti-fungal screening

Potato Dextrose Agar was prepared according to the manufacturer's instruction and supplemented with 1 µg/ml of chloramphenicol to inhibit bacterial growth. Three different concentrations (4, 2 and 1 mg /ml) of methanolic and ethyl-ether extracts were prepared and seeded separately into the Petri plates. A sterile inoculating needle was used to inoculate the plates. Control Petri plates were prepared and inoculated with the organism without the extracts. All the plates were incubated at room temperature for complete five (5) days. The fungal growth was monitored and taken intermittently at every six (6) hour interval and was terminated at the end of one hundred and twenty hours (five days). The radial growth of fungus was measured using meter rule from the point of inoculation. The percentage inhibition level of aqueous plant extract was determined using the method of Okigbo [18]. Growth inhibition (%) = $(LC - LT)/LC \times 100$. Where LC = average length of uninfected portion of control, LT = average length of uninfected portion with treatment.

3. RESULTS AND DISCUSSION

3.1 Status of phytochemicals

The result of phytochemicals indicated that terpenoids, steroids, alkaloids and anthraquinones are highly present on both methanolic and ethyl-ether extract. Also, flavonoids were qualitatively and highly present on methanolic extract but fairly present on ethyl-ether extract. Terpenoids and phenols were fairly present both in methanolic and ethyl-ether extract. Meanwhile saponins was fairly present

on methanolic extract but was totally absent on ethyl-ether extract while Phlobatannin was totally absent in both extracts as shown in Table 1.

Table 1: Phytochemical constituents of methanolic and ethyl-ether extracts of *C. odorata* plant

Phytochemicals	Methanolic Extract	Ethyl-Ether Extract
Terpenoids	++	++
Steroids	++	++
Flavonoids	++	+
Alkaloids	++	++
Saponins	+	—
Tanins	+	+
Phlobatannin	—	—
Phenols	+	+
Anthraquinones	++	++

Keys: ++ = Highly present, + = Fairly present, - = Absent.

3.2 Antimicrobial activity

Different levels of inhibitions of the test organisms at concentrations of 100, 200 and 400 mg/ml, of both methanolic and ethyl-ether extracts were observed. At 400 mg/ml methanolic extract, zones of inhibitory activities of 22 mm, 20 mm, 19 mm, 17 mm and 17 mm against *Escherichia coli* (ATCC 25922), *Shigella flexneri* (KZN), *Proteus vulgaris* (ATCC 6830), *Enterococcus cloacae* (ATCC13047) and *Shigella sonnei* (ATCC19930) respectively were observed while in ethyl-ether extract, 23 mm, 20 mm, 19 mm and 17 mm against *Shigella sonnei* (ATCC19930), *Pseudomonas aeruginosa* (ATCC 19582) and *Enterococcus cloacae* (ATCC13047), *Klebsiella pneumoniae* (ATCC10031), *Proteus vulgaris* (ATCC 6830) and *Shigella flexneri* (KZN) were recorded in that order. However, the lowest zones of inhibition at concentration of 400 mg/ml were 14 mm against *Klebsiella pneumoniae* (ATCC10031), and 15 mm against *Enterococcus faecalis* (ATCC29212) and *Pseudomonas aeruginosa* (ATCC 19582) in methanolic extract while the lowest zones of inhibitions for ethyl-ether extract were 13 mm and 14 mm against *Enterococcus faecalis* (ATCC29212) and *Escherichia coli* (ATCC 25922) as showed in Table 2. At 200 mg/ml of methanolic extract, the highest zone of inhibition (16 mm) was observed for *Shigella flexneri* (KZN) and *Shigella sonnei* (ATCC19930), 15 mm for *Enterococcus cloacae* (ATCC13047) and *Proteus vulgaris* (ATCC 6830) while in ethyl-ether extract, *Enterococcus cloacae* (ATCC13047), *Klebsiella pneumoniae* (ATCC10031) showed the highest inhibitions of 17 mm followed by 15 mm against *Pseudomonas aeruginosa* (ATCC 19582), *Proteus vulgaris* (ATCC 6830), *Shigella flexneri* (KZN) and *Shigella sonnei* (ATCC19930). The lowest zones of inhibition at 200 mg/ml was 10 mm against *Enterococcus faecalis* (ATCC29212). The concentration of 100 mg/ml of methanolic extract was able to produce high levels of inhibitions of 16 mm, 14 mm, and 13 mm against *Shigella flexneri* (KZN), *Enterococcus cloacae* (ATCC13047) and *Proteus vulgaris* (ATCC 6830) respectively while in ethyl-ether extract produced 15 mm 14 mm and 13 mm against *Proteus vulgaris* (ATCC 6830), *Klebsiella pneumoniae* (ATCC10031) and *Enterococcus cloacae* (ATCC13047) as shown in Table 2.

At the concentration of 4 mg/ml, the fungicidal potency of methanol extract was able to subdue the growth of the *Neurospora crassa* by 100% within 24 h. At the concentration of 2 mg/ml and 1 mg/ml, the suppression growth ability was also achieved at 100% for 18 h (Fig 1). The growth suppression was also observed in ethyl-ether extract but with time variation (Fig 2).

Table 2: Zone of inhibition (mm) produced by methanolic and ethyl- ether extracts of *C. odoranta* at different concentrations on bacterial isolate.

Organism	Conc. methanolic extract (mg/ml)			Conc. Ethyl- ether extract (mg/ml)		
	100	200	400	100	200	400
<i>Escherichia coli</i> (ATCC 25922).	11	12	22	11	12	14
<i>Enterococcus faecalis</i> (ATCC29212).	10	10	15	10	13	13
<i>Pseudomonas aeruginosa</i> (ATCC 19582).	10	12	15	12	15	20
<i>Enterococcus cloacae</i> (ATCC13047).	14	15	17	13	17	20
<i>Klebsiella pneumoniae</i> (ATCC10031).	10	12	14	14	17	19
<i>Proteus vulgaris</i> (ATCC 6830).	13	15	19	15	15	17
<i>Shigella flexneri</i> (KZN).	16	16	20	9	15	17
<i>Shigella sonnei</i> (ATCC19930).	12	16	17	12	15	23

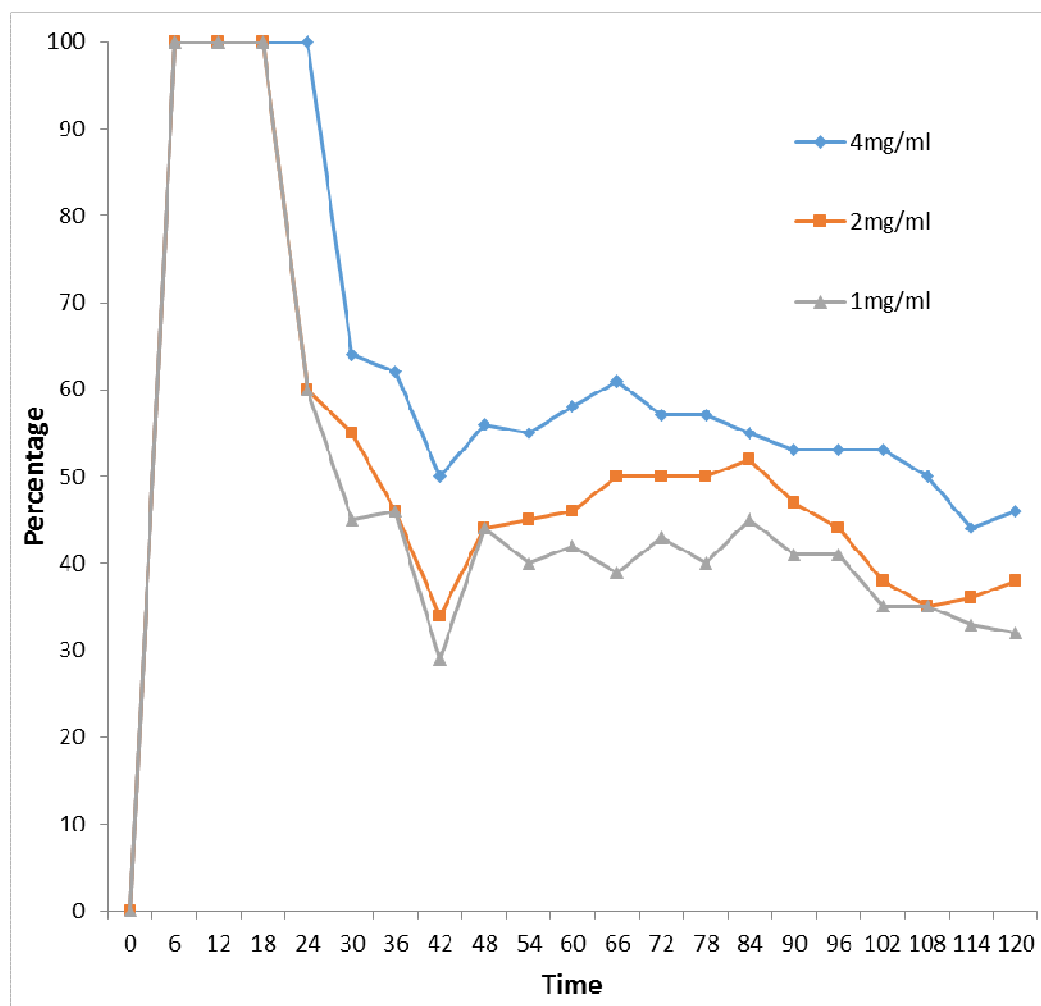


Figure 1. Fungicidal effect of methanol extract of *C. odorata* (in percentage) on *Neurospora crassa*

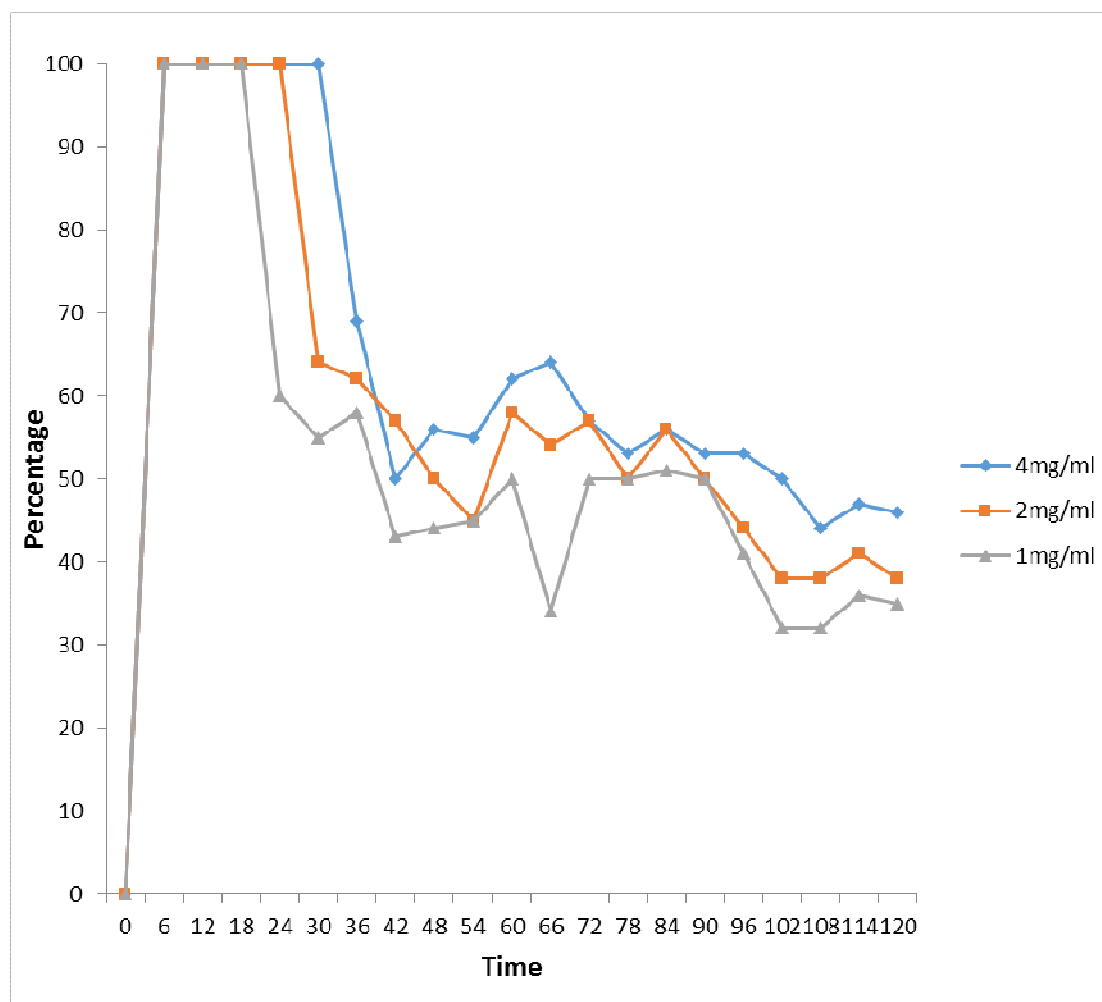


Figure 2. Fungicidal effect of ethyl-ether extract of *C. odorata* (in percentage) on *Neurospora crassa*

4. CONCLUSION

In this study, the level of flavonoids present in both methanolic and ethyl-ether extracts were variable. The variation in the degree of flavonoids present in ethyl-ether extract compared to methanolic extract may be due to difference in polarity of both solvents. It is imperative to note that the type of solvent used during extraction has a significant effect on the diversity of compound in the plant extract. Earlier report [22] indicated that the polarity of a solvent plays an important role in solubility of target phytochemicals. Methanol is more polar than ethyl-ether, hence, accounting for the observed variation. It is imperative to note that methanol will be a preferred solvent when flavonoids are the target phytochemicals of interest. The presence of terpenoids (a volatile oil) in this study was previously documented on its effect on plant, fungi and plant growth [23]. The detection of phenolics, alkaloids, steroids, saponins, flavonoids and tannins in *C. odorata* leaf was also comparable to similar studies by different authors [19, 24]. However, flavonoids and phenolics were not detected by other authors [25, 20, 26]. The abundance phytochemicals in this extract (terpenoids, steroids, alkaloids and anthraquinones) may probably be linked to its importance in protective function, physical characteristics and chemical characteristics of *C. odorata* plant. Phytochemicals protect plants from disease and damage, environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack as well as contribute to the plant's color, aroma and flavor (27-28).

The extended activities by both methanolic and ethyl-ether extracts on Gram negative bacteria can be attributed to the presence of secondary metabolites. Reports have it that Terpenoids exact inhibitory activity against Gram negative bacteria [29, 25]. The interaction of hydroxyl groups with the outer lipopolysaccharide membranes of Gram negative bacteria, results in disruption of the cell wall thus, allowing interaction with the microbial cytosolic enzymes and membranes [30-31]. Flavonoids (chrysin, abyssinone) have also been implicated in inactivation of microbial adhesins, enzymes, cell envelope transport proteins, and disrupt microbial membranes [32]. Phenols (catechol, epicatechin), tannins (ellagitannin) and alkanoids (berberine) exhibit microbial activity by substrate deprivation, membrane disruption, binding to proteins and intercalating into cell wall and/or DNA [33]. Since the extract has different phytochemicals of varied concentrations, high activity of the extracts may be attributed to synergistic effects of the various phytochemicals present in the leaf extract. The bioactivity of *C. odorata* extract in this study was similar to previous findings on the effect of *C. odorata* extract against various pathogens [2, 34-35, 26, 36-37, 24]. The most important aspect of bioactivity of this plant extract is its potency on both gram positive and gram negative bacteria which have over the years defiled therapeutic functions of chemosynthetic agents. However, two authors [18, 38] reported less potency of this extract on enteric pathogens. It has been shown in 2015 that leaf extract of this plant species was inhibitory against four human pathogens; *Bacillus cereus*, *Staphylococcus aureus*, *E. coli* and *Salmonella typhi* of which two of these are enteric pathogens as in this present study [17]. The potency of both solvent extracts of *C. odorata* leaf against *P. aeruginosa* in this study was commendable and this was contrary to result obtained in 2016 [24] using the same solvent (methanol) and plant extract. Difference in strains used might have accounted for such variation. Also, location of plant (geographical location) and differential power of technique might have also contributed to the disparity. There are many factors that could influence the potency of medicinal plants, these include the age of plant, extracting solvent, method of extraction, and even the time of harvesting the plant materials [18]. These and other factors could have accounted to aforementioned disparities.

The suppression of *N. crassa* of high growth rate (3 to 5 mm/h) is an indication that it may be a good agent against other fungi of medical importance. *Neurospora crassa* was deliberately chosen due to its rapid growth rate more than other known fungi of medical importance. This observation was recently documented in 2015 [17] on the phytotoxic potentials of *Chromolaena odorata* fungal deteriorating agents of yam tubers (*Aspergillus flavus*, *Aspergillus glaucus*, *Aspergillus niger* and *Botryodiplodia theobromae*) causing post-harvest rot disease of yam tubers. This inhibition may be attributed to the presence of fungi toxic substances; flavonoids, tannins and saponins found in the extract [19]. However, while the benefits of herbal-derived medicine are obvious and welcomed, there are however, subtle dangers or an underlying clinical problems associated with its use. The review on pyrrolizidine alkaloids in Chinese Herbal Medicines, indicated that *Chromolaena odorata* contains pyrrolizidine alkaloids which have been reported as hepatotoxic and tumorigenic compounds [39]. The authors further stated that production of dehydropyrrolizidine (pyrrolic) metabolites is the major metabolic pathway responsible for the genotoxic and tumorigenic activities of pyrrolizidine alkaloids, as evidenced by its ability to bind with macromolecules; DNA cross-linking, DNA-protein cross-linking, and DNA adduct formation. However, Heiss [40] demonstrated that *C. odorata* extract contains chromomoric acid C-I which is an Nrf2 (nuclear factor-erythroid 2-related factor 2) activator. Nrf2 is responsible for ameliorating effect experienced in diseases related to inflammation, increased oxidative stress, cancer, diabetes, and atherosclerosis.

5. SUMMARY

Chromolaena odorata extract possesses antimicrobial activity and thus, represents a promising source for medicines of which when carefully tapped and explored has enormous therapeutic potentials. It is also important to note that while herbal-derived medicines may be an alternative for combating microbial infections, care should be taken to minimize the risk associated with them.

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