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Research Article

# Quantitative Phytochemical Analysis and Antimicrobial Potential of the Ethanol and Aqueous Extracts of the Leaf, Stem and Root of *Chromolaena odorata* (Asteraceae)

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**ABSTRACT**

Background: Plants contain secondary metabolites or phytochemicals, which when consumed by humans give therapeutic effect. This study therefore analyzed the phytochemical composition of *Chromolaena odorata* so as to give an idea of its possible pharmacological potentials. An antimicrobial assay was also carried out to verify claims on its use in the treatment of infectious diseases. Methods: The experimental procedure involved collection of the leaf, stem and root of the plant from the wild, authenticating the samples and drying under shade to facilitate pulverization. Preliminary qualitative and quantitative phytochemical analyses were done using standard methods to reveal the presence and percentage composition of basic phytochemicals. The powder was also macerated in ethanol and water to produce ethanol and aqueous crude extracts that were reconstituted in normal saline to concentrations (mg/ml) of 150, 100, 75 and 50. Clinical isolates of *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Aspergillus niger* and *Candida albicans* were screened for sensitivity to the extracts using the agar well diffusion method. Results: The plant parts contain alkaloids, tannins, flavonoids, saponins, terpenoids, phenols, cardiac glycosides, and sterols at varying concentrations. The leaf however had highest concentration of almost all phytochemicals present. The antimicrobial activity of the plant was concentration-dependent in all parts of the plant and both extracts. *Aspergillus niger* and *Candida albicans* were most susceptible while *Salmonella typhi* and *Escherichia coli* were least susceptible with an average Inhibition Zone Diameter (IZD) of about 8 – 10 mm. Conclusion: The substantial quantity of the basic phytochemicals in *Chromolaena odorata* could render it a utility plant in therapeutic use. Due to the profound antimicrobial effect as revealed by the IZDs, the plant could be classified as a broad spectrum antimicrobial agent. Thus, the claim on its potency in treatment of infectious diseases by traditional medical practitioners could be said to be justified.

**Keywords:** *Chromolaena odorata*, phytochemical, clinical isolates, Inhibition, pharmacological.

## INTRODUCTION

Plants are composed of an array of relatively complex mixtures of secondary metabolites, which exhibit therapeutic effect. These are called phytochemicals and include alkaloids, glycosides, terpenoids and phenols[1]. Phytochemicals in medicinal plants can be used directly for therapeutic purposes or as a precursor for the synthesis of pharmaceuticals[2]. Some of these medicinal plants are variously used as food and mostly cultivated, while a number of plants growing in the wild are strictly used for therapeutic applications. Such wild plants are sometimes added to foods for medicinal purpose[3]. Alkaloids exhibit important pharmacological uses as analgesics, antibacterial, anti-malarial, anticancer, anti-hypertensive[4]. Tannins are antiseptic in nature and hasten healing of wounds[5]. Flavonoids have been shown to exhibit pharmacological effects as anti-allergic, anti-inflammatory, antioxidant, anti-cancer[6], antibacterial[7,8], antifungal[9,10] and antiviral[9] agents. Saponins defend plants against microbial attack, hence serve as antimicrobial and antifungal agents[11] to humans and are applied as diuretics and cough remedy. Terpenoids are extensively aromatic and are used in food and pharmaceutical industries for flavor, and odour improver[12]. Phenols are a precursor to a large collection of drugs, most notably aspirin, and also many herbicides and pharmaceutical drugs as oral anaesthetic/analgesic, being the active ingredient in oral analgesics such as Chloraseptic spray and Carmex, commonly used to temporarily treat pharyngitis[13,14]. Therapeutic uses of cardiac glycosides primarily involve the treatment of cardiac failure[15]. Phytosterols have been shown in clinical trials to block cholesterol absorption sites in the human intestine, thus helping to reduce cholesterol in humans[16].

*Chromolaena odorata*, commonly called Siam Weed[18] or Awolowo weed in Nigeria is a member of the family *Compositae* (*Asteraceae*), which is a very large cosmopolitan family. It is represented by 13 tribes, 84 genera and over 240 species[19]. It is used in Nigerian traditional medicinal practices to treat various ailments of microbial

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origin. This study therefore investigated the phytochemical composition of this plant, and applied aqueous and ethanol crude extracts of the leaf, stem and root in *in vitro* antimicrobial bioassay in order to verify claims on efficacy in this regard.

## MATERIALS AND METHODS

*Chemicals*

All reagents (SIGMA-ALDRICH Laboratories, USA) used were of analytical standard: ethanol.

*Collection and Authentication of Plant Material*

Plant samples were collected along Edem road, Army Barracks in Nsukka in the month of May 2014. The whole plant was collected and presented for authentication by Mr. A.O. Ozioko, a consultant taxonomist with the International Center for Ethnomedicine and Drug Development (InterCEDD) Nsukka. A voucher specimen number was assigned viz. INTERCEDD0514.

*Preparation of Crude Plant Material*

The plant parts (leaves, stems and roots) were separated and washed under running tap water to remove extraneous materials. These were air-dried under shade for two weeks[20] and pulverized using mortar and pestle. The respective powders were stored as stock in air-tight containers.

*Extraction (Ethanol and Aqueous)*

Dried powder (500 g each) of the leaf, stem and root of *C. odorata* were separately soaked in 2.5 L of ethanol until the powder was well saturated. These were separately distributed into conical flasks (250 ml) and shaken for 6 hr on a mechanical shaker (STUART, Great Britain) and left to stand overnight. The content was filtered using a non-absorbent cotton wool on a Buchner funnel/flask using a vacuum pump. The residue was rinsed repeatedly with fresh solvents and filtered to attain some level of exhaustive maceration (extraction) as judged by loss of colour of the filtrate[21]. The collective filtrate was concentrated to dryness at 40 – 50 ºC using a rotary evaporator attached to a vacuum pump to obtain the crude extracts (130 g, 98 g and 55 g respectively). The various extracts were transferred into labeled, sterile sample containers and preserved in a refridgerator at a temperature of 4ºC.

The same procedure was repeated for aqueous extraction using distilled water.

*Preliminary Phytochemical Screening*

The following tests were carried out on the respective crude drugs to determine the presence or absence of various basic phytochemicals as below:

*Test for alkaloids*

The presence of alkaloids in each sample was investigated using the method described by Harborne[15].

An ethanolic extract was used and obtained by dispensing 2 g of the powdered sample in 10 ml of ethanol. The mixture was thoroughly shaken before filtering using Whatman No (40) filter paper. About 2 ml of the various filtrates were added into separate test tubes and 3 drops of picric acid was mixed with each. The formation of light green colouration indicates presence of alkaloid.

*Test for tannins*

The presence of tannins in the test samples was carried out using the ferric chloride test described by Harborne[15].

A 2 g each of the powdered samples was added to 10 ml of distilled water in three test tubes. Each mixture was shaken for 30 min with a mechanical shaker (STUART, Great Britain) and filtered. The filtrate was used as aqueous extract. 2 ml of the aqueous extract was added into a test tube, 2 drops of dilute ferric chloride (FeCl) was added to the mixture. The formation of a blue-black or blue-green precipitate indicated the presence of tannins.

*Test for flavonoids*

The presence of flavonoids in the sample was carried out using the acid-alkaline test described by Harborne[15].

About 2 ml of the aqueous extract was added into a test tube (for each plant component) and a few drops of Bench Concentrated ammonia ($NH_4$) were added. The formation of a yellow colouration indicated the presence flavonoids. Confirmatory test was carried out by adding few drops of concentrated hydrochloric (HCl) into the yellow solution which turned colourless.

*Test for saponins*

The presence of saponins in the test samples was investigated as described by Harborne[15].

Two tests were involved in the investigation: the froth test and emulsion test.

In the froth test, 2 ml of the aqueous extract was mixed with 6 ml of distilled water in a test tube. The mixture was shaken well and the formation of froth indicated the presence of saponins.

For the emulsion, 3 drops of vegetable oil was added into the test tube containing an aqueous extract of the test sample. The mixture was shaken well and observed for the presence of stable emulsion. The formation of a stable emulsion indicated the presence of saponins.

*Test for terpenoids*

The presence of terpenoids was carried out using Salkowski test[15].

About 2 ml of the extract was mixed in 2 ml of chloroform in a test tube (for each plant component) and 3 ml of concentrated sulphuric acid ($H_2SO_4$) was carefully added to form a lower layer. A reddish brown colour interface was indicative of the presence of terpenoids.

*Test for phenols*

About 1.0 ml each of the extracts was added with 1.0 ml of 10% ferric chloride in separate test tubes. The formation of a greenish brown or black precipitate or colour indicated the presence of phenol nucleus.

*Test for cardiac glycosides*

About 1 ml of concentrated $H_2SO_4$ was prepared in test tube and 5 ml of aqueous extract from each plant sample is mixed with 2 ml of glacial acetic acid ($CH_3CO_2H$), containing 1 drop of Iron Chloride ($FeCl_3$). The above mixture was carefully added to 1 ml of concentrated hydrochloric acid (HCl) so that it was underneath the mixture. If cardiac glycosides were present in any sample, a brown ring was observed indicating the presence of the cardiac glycoside constituent.

*Test for sterols*

Figure 1: Habitat of *Chromolaena odorata* showing the leaves and flowers.

Figure 2: The leaves and stem of *Chromolaena odorata*.

Figure 3: The stem and root of *Chromolaena odorata.*

The presence of the plant steroid in the test sample was carried out using the method described by Harborne[15].

About 2 ml of acetic anhydride was added to 5 ml of ethanol extract of each sample with 2 ml sulphuric acid ($H_2SO_4$). The colour changed from violet to blue or green which indicated the presence of sterols.

*Quantitative Phytochemical Analysis*

This analysis was carried to determine the amount or concentration of the phytochemical constituents present in the plant and screened for above.

*Determination of Alkaloids*

The determination of the concentration of alkaloids in the each of the three samples was carried out using the alkaline precipitation gravimetric method described by Harborne[15].

A 5 g of the powdered sample was soaked in 20 ml of 10 % ethanol acetic acid. The mixture was stored for four hours at room temperature and thereafter was filtered through Whatman filter paper (No. 42). The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9 % ammonia solution and dried in the oven at 60 $^oC$ for 30 min, cooled in a dessicator and reweighed. The weight of alkaloid was determined by the weight differences and expressed as a percentage of weight of sample analyzed.

*Determination of tannins*

The Folin-Denis spectrophotometric method was used; the method was described by Pearson[22].

About 1.0 g of the sample was dispersed in 10 ml distilled water and agitated. This was left to stand for 30 min at room temperature and shaken every 5 min. After 30 min, it was centrifuged and the extract gotten. About 2.5 ml of the supernatant extract was dispensed into a separate 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid was dispensed into a separate 50 ml flask. The absorbance was measured at 250 nm.

*Determination of flavonoids*

The flavonoids content of the samples was determined by the gravimetric method described by Harborne[15].

A 5 g of each powdered sample was placed into a conical flask and 50 ml of water and 2 ml of ethyl acetate solution were added. The solution was allowed to boil for 30 min. The boiled mixture was allowed to cool and filtered through Whatman filter paper (No. 42). About 10 ml of ethyl acetate extract which contained flavonoids was recovered, while the aqueous layer was discarded. A pre-weighed Whatman filter paper was used to filter the second (ethyl acetate) layer. The residue was then placed in an oven to dry at 60 $^oC$. It was cooled in a dessicator and weighed.

*Determination of saponins*

The saponins content of the samples was determined by double extraction gravimetric method[15].

A 5 g of each powdered sample was mixed with 50 ml of 20 % aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 min at 55 $^oC$. It was then filtered through Whatman filter paper

(No. 42). The residue was extracted with 50 ml of 20 % ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90 $^{o}$C and transferred to a separating funnel where 40 ml of diethyl ester was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer became clear in colour. The saponins were extracted with 60 ml of n-butanol; the combined extracts were washed with 5 % aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60 $^{o}$C in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. Saponins content was determined by difference and calculated as a percentage of the original sample.

*Determination of Phenols*

The concentration of phenols in the samples was determined using the Folin-cio Caltean colorimetric method described by Pearson[22].

A 0.2 g of each powdered sample was added into a test tube and 10 ml of methanol was added to it and shaken thoroughly. The mixture was left to stand for 15 min and filtered using Whatman filter paper (No. 42). About 1 ml of the extract was placed in a test-tube and 1 ml Folin-cio Caltean reagent in 5 ml of distilled water was added and colour was allowed to develop for 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wavelength. The process was repeated two times and the average taken.

*Determination of cardiac glycosides*

A 1 g of the sample was weighed into a conical flask. About 2.5 ml of 15 % lead acetate was added and the mixture was filtered. Chloroform (2 ml) was added to the filtrate and the mixture was shaken vigorously. The lower layer was collected and evaporated to dryness. About 3 ml of glacial acetic acid was added and 0.5 ml of 5 % ferric chloride and 0.05 ml of concentrated sulphuric acid ($H_2SO_4$) were added. The mixture was then left to stand in the dark for 2 hours after which the absorbance was measured at 530 nm wavelength.

*Antimicrobial Studies*

The plant extracts were reconstituted in normal saline to obtain concentrations (mg/ml) of 150, 100, 75 and 50. Pure cultures of clinical isolates of *Staphylococcus aureus, Salmonella typhi, Escherichia coli*, *Aspergillus niger* and *Candida albicans* were obtained from the Pathology Department of National Root Crops Research Institute, Umudike, Abia State.

These were sub-cultured in Nutrient Agar (NA) or Saboraud's Dextrose Agar (SDA) and re-identified to ensure the purity of the isolates. A sensititre Nephelometer was used to dilute the microorganisms to 0.5 McFarland turbidity standards in accord with the Clinical and Laboratory Standards Institute, CLSI[23]. Using a sterile glass rod, 1.0 ml of each standardizes suspension was spread evenly on 25 ml sterilized NA (Oxoid) for bacteria or SDA (Oxoid) for fungi. An 8 mm diameter cork borer was used to bore wells through the solidified culture plates. The reconstituted crude plant extracts were appropriately placed into labeled agar wells in duplicate. The plates were left on the work bench for 1 hr, to allow for pre-diffusion of the extracts[24]. Bacterial and Candida cultures were incubated at 37 $^{o}$C for 24 hr while *A. niger* culture was incubated at 25 $^{o}$C for 72 hr. The Inhibition Zone Diameter (IZD) was measured to the nearest mm, individual zones (of three) for each plant concentration against each microorganism and the average was calculated and presented in tables 3 – 5.

*Statistical Analysis*

The results of the experiment were analyzed statistically with student's T-test and ANOVA[25] using the software: IBM SPSS Statistics 21.

## RESULTS

*Phytochemical Result of Chromolaena odorata*

Data from this study indicated the presence of alkaloids, tannins, flavonoids, saponins, terpenoids, phenols, cardiac glycosides and sterols in both aqueous and ethanol extracts as shown (Tables 1 and 2) for qualitative and quantitative results respectively.

The result for quantitative analysis expressed the concentration of phytochemicals as a percentage as shown: The ethanol extract of *C. odorata* leaf contain higher values/ percentage of the phytochemicals present except flavonoids (Table 2). While the percentage of phytochemicals in ethanol and aqueous stem and root extract vary inconsistently.

*Antimicrobial study result of Chromolaena odorata*

Values are the average of two inhibition zone diameters.

The exhibited antimicrobial effect was concentration-dependent and proportional. At 150 mg/ml, both the aqueous and ethanol crude extracts of the leaf exhibited greatest IZD of 19.58 and 18.72 mm respectively against *C. albicans* and *A. niger*, and lowest IZD of the aqueous extract was 11.78 mm against *S. typhi* while the ethanol crude extract had 11.75 mm inhibition against *E. coli*.

Similar with the leaf, the antimicrobial activity of both aqueous and ethanol crude extracts of the stem exhibited a concentration-dependent and proportional activity. *A. niger* and *C. albicans* were most inhibited while *S. typhi* was least affected both the aqueous and ethanol crude extracts (Table 4).

The same observed antimicrobial effect on the leaf and stem was obtained for the root. *C. albicans* and *A. niger* were most inhibited by both aqueous and ethanol crude extracts of the root while *E. coli* and *S. typhi* were least inhibited by the ethanol and aqueous crude extracts respectively.

## DISCUSSION

The healing properties of medicinal plants are usually linked with the presence of phytochemicals otherwise called secondary metabolites and these differ (in type and concentration) from one plant to another, accounting in part for the difference in pharmacological effects of medicinal plants. Therefore, the presence of various phytochemicals in *C. odorata* (Tables 1 & 2) suggests that *C. odorata* possess therapeutic importance[3].

Table 1: Result of qualitative phytochemical analysis of aqueous and ethanolic extracts of *Chromolaena odorata.*

| Phytochemicals | Components | Plant parts/ Observations | | |
|---|---|---|---|---|
| | | A | B | C |
| Alkaloids | Aqueous | ++ | + | + |
| | Ethanol | ++ | + | + |
| Tannins | Aqueous | + | + | + |
| | Ethanol | + | + | + |
| Flavonoids | Aqueous | ++ | + | + |
| | Ethanol | ++ | + | + |
| Saponins | Aqueous | + | ++ | + |
| | Ethanol | + | ++ | + |
| Terpenoids | Aqueous | + | + | + |
| | Ethanol | + | + | + |
| Phenols | Aqueous | + | + | + |
| | Ethanol | + | + | + |
| Cardiac glycosides | Aqueous | + | + | + |
| | Ethanol | + | + | + |
| Sterols | Aqueous | + | + | + |
| | Ethanol | + | + | + |

Key: A = Leaf; B = Stem; C = Root; – = absent; + = present; ++ = more present

Table 2: Quantitative Phytochemical Composition of the Leaf, Stem and Root of *Chromolaena odorata* in Aqueous and Ethanol Solvents.

| Phytochemicals | Aqueous Extract* | | | Ethanol Extract* | | | ANOVA Results | | |
|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Stem | Root | Leaf | Stem | Root | Part of plant | Solvent | Interaction |
| Alkaloids | 1.45± 0.000$^{c}$ | 1.55± 0.014$^{d}$ | 0.77± 0.014$^{b}$ | 1.61± 0.021$^{e}$ | 1.48± 0.000$^{c}$ | 0.73± 0.014$^{a}$ | ** | ns | ** |
| Tannins | 0.69± 0.000$^{e}$ | 0.54± 0.028$^{c}$ | 0.28± 0.000$^{b}$ | 0.74± 0.000$^{f}$ | 0.60± 0.000$^{d}$ | 0.25± 0.000$^{a}$ | ** | ** | ** |
| Flavonoids | 0.94± 0.000$^{e}$ | 0.68± 0.000$^{c}$ | 0.54± 0.014$^{a}$ | 0.92± 0.000$^{e}$ | 0.74± 0.014$^{d}$ | 0.62± 0.021$^{b}$ | ** | ** | ** |
| Saponins | 1.84± 0.014$^{d}$ | 1.35± 0.000$^{b}$ | 0.78± 0.000$^{a}$ | 1.75± 0.014$^{c}$ | 1.32± 0.035$^{b}$ | 0.77± 0.021$^{a}$ | ** | ** | Ns |
| Terpernoids | 0.75± 0.014$^{c}$ | 0.64± 0.014$^{b}$ | 0.47± 0.028$^{a}$ | | 0.62± 0.000$^{b}$ | 0.44± 0.014$^{a}$ | ** | ns | ** |
| Phenols | 0.17± 0.014$^{d}$ | 0.12± 0.000$^{b}$ | 0.09± 0.000$^{a}$ | 0.22± 0.014$^{e}$ | 0.15± 0.000$^{c}$ | 0.13± 0.000$^{b}$ | ** | ** | Ns |
| Cardiac Glycosides | 0.25± 0.028$^{c}$ | 0.18± 0.021$^{b}$ | 0.12± 0.000$^{a}$ | 0.25± 0.000$^{c}$ | 0.19± 0.000$^{b}$ | 0.19± 0.021$^{b}$ | ** | ** | Ns |
| Sterols | 0.37± 0.014$^{d}$ | 0.19± 0.000$^{b}$ | 0.14± 0.021$^{a}$ | 0.41± 0.014$^{e}$ | 0.25± 0.014$^{c}$ | 0.17± 0.021$^{ab}$ | ** | ** | Ns |

N = 3; Values expressed as mean± standard deviation

*row with the same (or sharing similar) superscript are not significantly different

**there is significant difference ($p<0.05$), ns: not significant ($p>0.05$)

Figure 4 depicts that the ethanol crude extracts gave higher yield of alkaloids, flavonoids, tannins, sterols, phenols, cardiac glycosides and terpenoids, while the aqueous crude extracts gave higher yield of only Cyanogenic glycosides and saponins.

Figure 5 depicts that the leaf extracts gave the highest yield of all the phytochemicals assayed. Tannins were highest in the ethanol crude extract of the leaf (0.74±0.000) and lowest in the ethanol extract of the root (0.25±0.000).

The flavonoids composition (mg/100g) is highest in the aqueous extract of the leaf (0.94±0.000) and lowest in the aqueous extract of the root (0.54±0.014). The analysis of variance shows a significant difference ($p<0.05$) in the flavonoids composition between the root, stem and leaf, and between the yield of aqueous and ethanol solvents. There is also interaction ($p<0.05$) between the plant parts and solvents on the yield of flavonoids. Saponins were highest in the aqueous extract of the leaf (1.84±0.014) and lowest in the ethanol extract of the root (0.77±0.021). The analysis of variance shows a significant difference saponins composition of the root, stem and leaf, and between the yield of aqueous and ethanol solvents ($p<0.05$). There is no interaction between the plant parts and solvent in the yield of saponins ($p>s0.05$).

The terpenoids composition (mg/100g) was highest in the ethanol extract of the leaf (0.82±0.028) and lowest in the ethanol extract of the root (0.44±0.014). The analysis of variance shows a significant difference in the terpenoids

Table 3: Zone of Inhibitory Activity of different Concentrations of Aqueous and Ethanol extracts of *Chromolaena odorata* Leaf against Clinical Microorganisms.

| Extract Concentration (mg/ml) | Solvent | Test microorganism/Inhibition Zone diameter (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* | *S. typhi* | *E. coli* | *C. albicans* | *A. niger* |
| 50 | Aqueous | 4.95 | 4.83 | 3.94 | 9.45 | 9.45 |
| | Ethanol | 5.74 | 4.23 | – | 9.30 | 9.30 |
| | Control | 8.78 | 7.63 | 7.56 | 13.49 | 13.49 |
| 75 | Aqueous | 8.45 | 6.72 | 5.72 | 11.40 | 11.40 |
| | Ethanol | 8.60 | 7.50 | 6.94 | 12.77 | 12.77 |
| | Control | 11.83 | 10.48 | 10.67 | 15.66 | 15.66 |
| 100 | Aqueous | 10.68 | 9.68 | 10.38 | 16.33 | 16.33 |
| | Ethanol | 11.77 | 10.58 | 9.80 | 15.87 | 15.87 |
| | Control | 15.35 | 14.80 | 13.87 | 19.82 | 19.82 |
| 150 | Aqueous | 12.78 | 11.78 | 12.43 | 19.58 | 19.58 |
| | Ethanol | 13.50 | 12.65 | 11.75 | 18.72 | 18.72 |
| | Control | 19.12 | 17.53 | 16.55 | 22.53 | 22.53 |

Table 4: Zone of Inhibitory Activity of different Concentrations of Aqueous and Ethanol extracts of *Chromolaena odorata* Stem against Clinical Microorganisms.

| Extract Concentration (mg/ml) | Solvent | Test microorganisms/Inhibition Zone diameter (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* | *S. typhi* | *E. coli* | *C. albicans* | *A. niger* |
| 50 | Aqueous | 5.16 | – | – | 6.92 | 7.91 |
| | Ethanol | 4.80 | – | – | 7.87 | 8.70 |
| | Control | 8.78 | 7.63 | 7.56 | 12.75 | 13.49 |
| 75 | Aqueous | 7.56 | 6.83 | 6.71 | 9.85 | 10.48 |
| | Ethanol | 8.52 | 6.72 | 7.32 | 10.66 | 11.64 |
| | Control | 11.83 | 10.48 | 10.67 | 15.43 | 15.66 |
| 100 | Aqueous | 11.43 | 9.63 | 9.55 | 13.65 | 13.83 |
| | Ethanol | 10.7 | 8.83 | 9.43 | 12.38 | 13.78 |
| | Control | 15.35 | 14.80 | 13.87 | 18.55 | 19.82 |
| 150 | Aqueous | 15.43 | 12.78 | 13.50 | 15.55 | 16.72 |
| | Ethanol | 12.55 | 10.38 | 11.77 | 14.80 | 15.63 |
| | Control | 19.12 | 17.53 | 16.55 | 21.55 | 22.53 |

composition between the root, stem and leaf ($p<0.05$) but no significant difference between the yield of aqueous and ethanol solvents ($p>0.05$). There is however interaction between part of plant and solvent in the yield of terpenoids ($p<0.05$).

The alkaloids composition (mg/100g) is highest in the ethanol extract of the leaf (1.61±0.021) and lowest in the ethanol extract of the root (0.73±0.014). The analysis of variance shows a significant difference ($p<0.05$) in the alkaloids composition between the root, stem and leaf but no significant difference ($p<0.05$) between the yield of aqueous and ethanol solvents. There is interaction ($p<0.05$) between part of plant and solvent on the yield of alkaloid.

Antimicrobial investigation further showed that both water and ethanol extracts of *C. odorata* were active against *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Candida albicans and Aspergillus niger.* The ethanol extracts of the three plant parts exerted greater antibacterial activity than corresponding water extract (Tables 3 – 5) at the same concentrations. These observations may be attributed to two reasons; firstly, the nature of biological active components (saponins, tannins, alkaloids). It has been documented that tannins, saponins and alkaloids are well known for antimicrobial activity[17]. Secondly, the stronger extraction capacity of ethanol could have produced greater number of active constituents responsible for antibacterial activity.

## CONCLUSION

The results of this research offer a scientific basis for the traditional use of both water (aqueous) and ethanol (dry gin) extracts of *Chromolaena odorata.* The leaves of *Chromolaena odorata* can serve as a source of useful drug substance. The high concentration of phytochemicals such as flavonoids, phenols, saponins, sterols and tannins in the leaf of *Chromolaena odorata* would imply that only a little quantity of the plant material would be sufficient for therapeutic use. It is therefore economical to use, moreso, it grows abundantly and is cosmopolitan. From the result, it can be extrapolated that *C. odorata* can be very potent in treating infections caused by *C. albicans* and *A. niger.* Its acclaimed use to remedy skin complaints could be justified. The plant was also effective against the bacteria

Table 5: Zone of Inhibitory Activity of different Concentrations of Aqueous and Ethanol extracts of *Chromolaena odorata* Root against Clinical Microorganisms.

| Extract Concentration (mg/ml) | Solvent | Test microorganisms/Inhibition Zone diameter (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *S. aureus* | *S. typhi* | *E. coli* | *C. albicans* | *A. niger* |
| | Aqueous | 5.56 | – | 3.78 | 5.92 | 6.45 |
| 50 | Ethanol | 4.50 | – | 3.87 | 7.41 | 6.30 |
| | Control | 8.78 | 7.63 | 7.56 | 12.75 | 13.49 |
| | Aqueous | 8.55 | 6.45 | 6.87 | 8.92 | 9.43 |
| 75 | Ethanol | 7.61 | 6.56 | 6.93 | 10.61 | 9.45 |
| | Control | 11.83 | 10.48 | 10.67 | 15.43 | 15.66 |
| | Aqueous | 9.58 | 7.71 | 7.82 | 9.25 | 10.69 |
| 100 | Ethanol | 8.58 | 7.83 | 7.61 | 11.56 | 10.30 |
| | Control | 15.35 | 14.80 | 13.87 | 18.55 | 19.82 |
| | Aqueous | 11.53 | 10.38 | 10.78 | 14.87 | 15.7 |
| 150 | Ethanol | 10.65 | 9.82 | 9.56 | 13.74 | 12.65 |
| | Control | 19.12 | 17.53 | 16.55 | 21.55 | 22.53 |

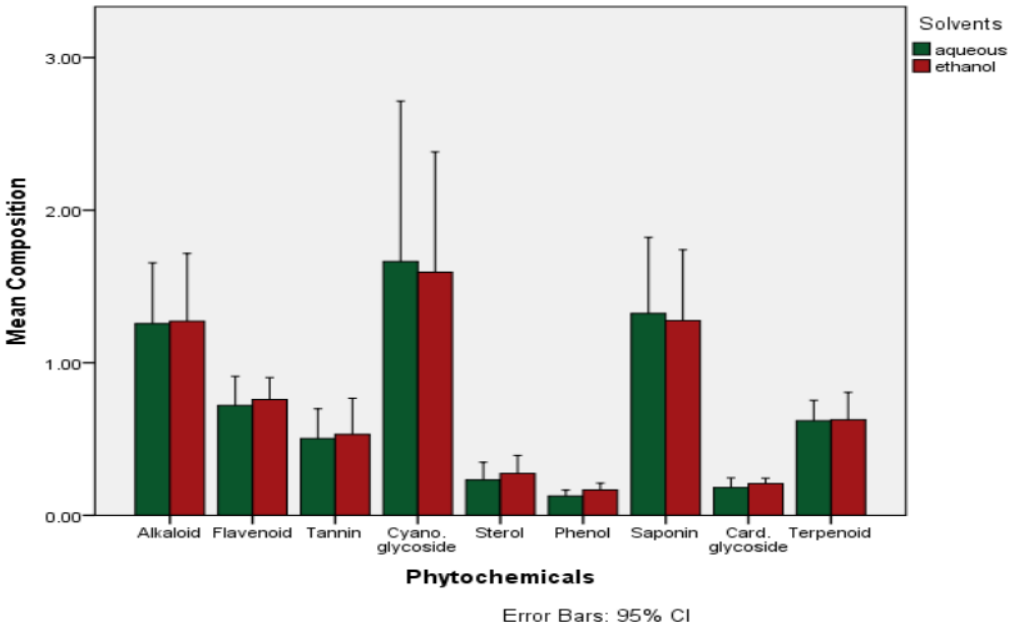


Figure 4: Showing the yield of phytochemicals in root, stem and leaf of *C. odorata*.

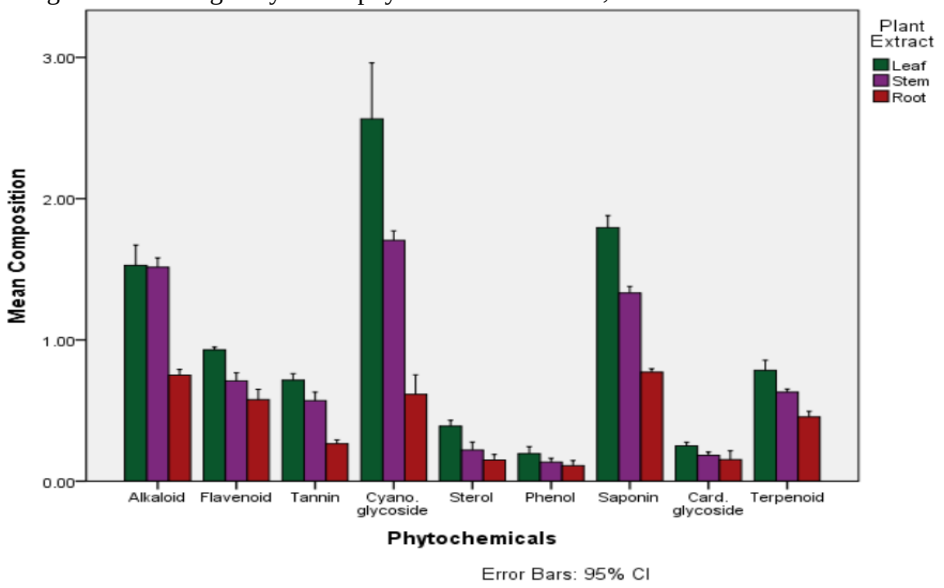


Figure 5: Showing the phytochemical composition of root, stem and leaf of *C. odorata*.

(gram positive and –negative), although not so effective on *E. coli* and *S. typhi*. It could then be regarded as a broad spectrum potential antibiotic.

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