ANTIBACTERIAL EFFECT OF AWOLOWO WEED (*Chromolena odorata*) EXTRACT ON *Salmonella typhii* ISOLATED FROM TYPHOID PATIENTS

ABSTRACT

This study aimed to determine the phytochemical constituents and antibacterial activity ofChromolenaOdorata leaves against *Salmonella* species isolated from typhoid patients. The phytochemical screening of the leaf extracts were carried out the antibacterial assay of the extracts against *Salmonella Typhi.*Chromolenaodorata samples were extracted using methanol, ethanol and water, the phytochemical screening of the extracts were carried out using a standard method. The antibacterial assay of the extracts against *Salmonella Typhi* using the agar well diffusion method at concentrations of 50, 100, 200, 400 and 500 mg/mL.Resultsshowed seven plant secondary metabolites including saponins, tannins, flavonoids, terpenoids, alkaloids, cardiac glycoside and steroid. The ethanol and aqueous extracts showed antibacterial activities against Salmonellatyphi at concentration of 50 mg/mL with the zone diameter of inhibition of 14 mm and 15 mm respectively. The ethanol extracts also showed zone diameter of inhibition of 23 mm at 250 mg/mL and 27 mm each at 500 mg/mL. Three extracts gave the highest zone diameter of inhibition at 500 mg/mL while 100 mg/mL gave the least zone diameter of inhibition for ethanol extract and 50 mg/mL for the aqueous extract. The methanol, ethanol and aqueous extracts displayed antibacterial activities against *Salmonella. typhi* with a statistical significance difference at (P≤.05).. The minimum inhibitory concentration of the extracts was 250, 200 and 100 mg/mL for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively.Thus Chromolenaodorata can be harnessed for further investigations and plant extracts combine with other medicinal plant for synergic effects.

Keywords: Antibacterial, Effects, Chromolenaodorata, Leaves extract, Salmonella typhii

1. INTRODUCTION

Infectious diseases (IDs) are among the top ten deadly diseases globally, and the menace of growing antimicrobial resistance has challenged and reduced the effectiveness of most clinical antibiotic (Thang, *et al.,*2021). Irrational use of antibiotics causes an increased rate of Anti-Microbial resistance, resulting in high Anti-Microbial resistance andrelated deaths globally (Zachariades, *et al.,*2021). *Salmonella* make up a large genus of gram-negative bacilli within the family *Enterobacteriaceae*and it constitute a genus of more than 2300 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease (Blackmore, 2018) and also be associated with localized infections and/or bacteremia (Blackmore, 2018).

Salmonella spp. causes an intestinal infection in humans known as Salmonellosis, members of the genus *Salmonella* are ubiquitous pathogens found in humans and livestock, wild animals, reptiles, birds, insects and can multiply under various environmental conditions outside the living hosts.*Salmonella* infection appears to be one of the most common examples of an enteric disease that is transmitted from animals to humans. The transmission occurs both through food products, such as meat, dairy products, and eggs, and by direct contact between animals and humans through the fecal-oral route (Irving. *et al.,* 2006).*Salmonella* contamination of food products can significantly reduce consumer demand and affect producer profits (Brooks, *et al.,*2017). A multi-target drug approach could be a sustainable strategy to overcome Anti-Microbial Resistance problems, because bacterial resistance to single-target antibiotics develops too rapidly to be sustainable, and resistance to new drugs often develops even before they reach the market (Katzung, *et al.,*2014).

Naturally, plant-derived molecules possess the ability to multi-target as they fight against predators like bacteria, fungi, viruses, insects, and herbivores, from their germination to maturity stages, and undergo challenging defense mechanisms for survival (Vorobyova*et al.,*2014). So, instead of developing new antibiotics, exploring potential antimicrobial agents from plant sources is convenient and cost-effective.). Synthetic drugs are not only expensive and inadequate but also often had issues with adulterations and side effects. Customers are more concerned about the pathogenicity and the high mortality rate of the product they used. Therefore, with the advancement of the technology, scientists are challenged to come out with new ideas of alternative and novel drugs to overcome the usage of microbial resistant drugs (Phan*et al.,*2021.).

Chromolaenaodorata (L) or *Euphatoriumodoratum* (L.), belongs to the family Asteraceae. *Chromolaenaodorata* is a perennial weed that belongs to the family Asteraceae and is the most notorious perennial scrambling weed that has proven to be a significant economic and ecological burden to many tropical and subtropical-regions of the World (Lakin's, 2018).The weed is also known as Awolowoweed and is one of the worst invading alien plant species in the humid and semi-humid tropics of the World (Parsons and Cuthbertson, 2001). Characteristically, it is a plant of secondary succession that invades fallows or newly cleared land, and is often shaded out when forest trees and shrubs are fully established.

Studies conducted by Aliyu (2020)revealed that in areas where Awolowo weed grows, the growth of other plants is always hampered. It is a traditional medicinal plant that is widely used for its wound healing property. In particular, the several parts of this herb have been used to treat wounds, burns, and skin infections. Furthermore, it has also been shown to possess anticancer, antidiabetic, anti-hepatotoxic, anti-inflammatory, antimicrobial, and antioxidant properties. Its phytochemical components are alkaloids, flavonoids, flavanone, essential oils, phenolics, saponins, tannins, and terpenoids. Other important constituents of this plant are Eupolin, chromomoric acid, quercetagetin, and quercetin, all of which contribute to its remedial properties (Iwu, 2013).The dried leaf of *C. odorata* contained ash (11%), crude fat (11%), fiber (15%), moisture (15%), crude protein (18%), and carbohydrate (31%) (Chakraborty, *et al.,* 2011). Its active phytochemical substances are as follows: flavonoid aglycones (flavanones, flavonols, flavones) including acacetin, chalcones, eupatilin, luteolin, naringenin, kaempferol, quercetin, quercetagetin, and sinensetin, terpenes and terpenoids; essential oils; alkaloids including pyrrolizidine; saponins and tannins; phenolic acids including ferulic acid, protocatechuic acid; phytoprostane compound including chromomoric acid (Abraham and Pradeep, 2015).

Basic phytochemical screening consists of performing simple chemical test to detect the presence of alkaloids, tannins, saponins, flavonoids, digitalis glycosides etc in a plant extract. Synthetic drugs are not only expensive and inadequate but also often had issues with adulterations and side effects. Customers are more concerned about the pathogenicity and the high mortality rate of the product they used. Therefore, with the advancement of the technology, scientists are challenged to come out with new ideas of alternative and novel drugs to overcome the usage of microbial resistant drugs (Phan*et al.,*2021.). The significance of the study is to make appropriate recommendations for further development and effective application of *Chromolaenaodorata*The success of this work will stimulate the formulation of many new drugs of various antimicrobial efficacies. The scope of the study is

to determine through phytochemical screening the costituents of *Chromolaenaodorata*extract against *salmonella spps* isolated from typhoid patient which entails collection of 500g of *Chromolaenaodorata,*preparation and collection of extract using ethanol, and water and determination of the antimicrobial activities against *salmonella spp* isolated from typhoid patient .

2. MATERIAL AND METHODS

2.1 Sample Collection

chromolenaodorata samples were collected from Awka, Anambra state.where the fresh leaves were dried for three days under room tempreture and without direct sunlight after drying the leaves were cut into smaller sizes using knife and tray, the sliced leaves were further grinded into powder using electronic grinder. the powdered sample were weighed using a digital weighing balance to determine the weight. Laboratory and other facilities used in the practical work where obtained from Alpha research laboratory Awka, Anambra state.

2.2 Extraction methods

Hexane extract of *ChromolenaOdorata*The plant material collected (100g) was air dried, pulverized, and macerated with N-hexane. It was allowed to stand for 48 h and then filtered. The filtrate was evaporated under reduced pressure and was dried using a rotary evaporator at 55°C.

Ethyl acetate extract of *ChromolenaOdorata*

The plant residue (100g) from *ChromolenaOdorata*was dried and further used for ethanol extraction. It was re-soaked with 5 L of ethanol, allowed to stand for 48 h and then filtered. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

Ethanol extract of *ChromolenaOdorata*

Fresh ground *ChromolenaOdorata*(100g) was soaked with 2.5 L of ethanol, and was allowed to stand for 48 h and then filtered. The filtrate was also evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

2.3 Preliminary Phytochemical Screening

The extracts will be subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents by the following methods according to (AOAC, 2010).

Test for Alkaloids

The extracts were treated with dilute (10%) hydrochloric acid and filtered. The filtrates were treated with various alkaloidal reagents.

Mayer's test: The extracts were treated with Mayer's reagent (Potassium mercuric iodide). Appearance of cream colour indicates the presence of alkaloids in all extracts.

Dragendroff's test: The extracts were treated with the Dragendorff's reagent (Potassium bismuth iodide), the appearance of reddish brown precipitate indicates the presence of alkaloid in all extracts.

Hager's test: The extracts were treated with the Hager's reagent (Picric acid), the appearance of yellow colour precipitate indicates the presence of alkaloids in all extracts.

Wagner's test : The extracts were treated with the Wagner's reagent (Iodine solution) the appearance of brown colour precipitate indicates the presence of alkaloids in all extracts.

2.4 Test for Cardiac Glycosides

Keller-Killani test : When a pinch of the extracts were dissolved in the Glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated Sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides in all extracts.

2.5 Test for Flavonoids

Shinoda's test: The extracts were dissolved in alcohol, to that one piece of magnesium followed by conc. hydrochloric acid were added drop wise and heated. Appearance of magenta color shows the presence of flavonoids in all extracts.

Ferric Chloride test: To the extracts, few drops of neutral ferric chloride were added. Blackish red colour was observed in all extracts.

2.6 Test for Saponins

Foam test: The extracts were diluted to 20 ml with distilled water and shaken well in a graduated cylinder for 15 minutes. The formation of foam in the upper part of the test tube indicates the presence of saponins in all extracts

2.7 Test for Steroids

Salkowski reaction: To 2 ml of extract, added 2ml chloroform and 2 ml conc. H_2SO_4. Shaked well. Chloroform layer showed red color and acid layer showed greenish yellow fluorescence.

Liebermann-Burchard test: When the extracts were treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, absence of green colour indicates the absence of steroids in all extracts.

2.8 Test for Tannins

Lead acetate solution: When the extracts were treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins in all extracts.

2.9 Test for phenol

Ferric chloride test: 2 drops of neutral ferric chloride solution was added to 1ml of diluted aqueous solution of the test sample. A greenish purple color indicates the presence of phenolic compounds.

Sample collection

A single blood culture was taken from patients with diagnosed cases of typhoid. A total of 10 blood samples were collected. 2ml of venous blood were collected aseptically and inoculated onto 18ml of Brain Heart Infusion Broth Agar (Oxoid,England), which was incubated at 37^{o}C for 3days (Naidoo, *et al.,* 2011).

Identification

Identification of Salmonella species was done biochemically using Kliger Iron Agar Agar (Oxoid,England), Simmon Iron Medium Agar (Oxoid,England),Ureabaseagar (Lab M, England) and Simmons citrate agar (LabM,England), were used to screen the isolates before serologic testing was performed (Cheesbrough, 2020).

Serological testing

Serologic identification of Salmonella species were performed using Well colexcolour Salmonella test kit. One or two suspected Salmonella colonies from the culture plate were carefully emulsified in approximately 200µl of sterile saline in a suspension tube.Holding the bottle vertically, resuspended latex reagent 1 and 2 were dropped in to a separate circle on a flat reaction card after shaking vigorously for few seconds. About 40µl of bacterial suspension was transferred to two of the reaction circles containing latex reagent 1 and 2 respectively and mixed. The card was placed on a suitable flat bed rotator and run at 150±5rpm for 2minutes then switch off and observed for agglutination without removing the card rotator. Positive controls with the positive control reagents (green, blue and red control) were carried out along side with the latex reagent 1 and 2 respectively without the inoculums. Results were interpreted according to the manufacturer guidelines for usage of the kit (Cheesbrough, 2020).

Preparation of Disc

Disc of diameter 6mm were perforated from the whatman filter paper using a perforator. The discs were sterilized using a bijou bottle at 160^{0} C for 2hrs in a hot air oven. It was brought out and allowed to cool before further use according to (Cheesbrough, 2020).

Preparation of Antibiotic Disc

250mg each of amoxillin were separately dissolved in 2mls of water.1 ml of the various stock solutions was diluted in 1ml of water and 1 ml of this stock was added to 10 paper discs in glass petri dish and allowed to dry in the oven 3hrs at 40^{0}C, so that the drug would stick to the discs (Cheesbrough, 2020).

Disc Preparation for Ethanolic Extract

1 gram of ethanol extract of leaves was mixed with 2mls of water. The mixture was properly done in a test tube and 1ml of the mixture was poured into a glass petri dish containing 10 paper discs. The disc was put in the oven to dry so as to allow the plant extract stick to the paper disc for further use (Cheesbrough, 2020).

Susceptibility Test

Using Plant Extract SDA and nutrient agar plated were inoculated with respective test organisms using syringe and needle followed by spreading using glass spreader for each test organism. Plates were in triplicate for each test organism in aqueous and ethanol

extract. The plates were allowed to dry for 15 mins in an incubator. The dried water and ethanol discs as mentioned above were transferred using flamed but cooled forceps into the surface of the inoculated agar plates. They were sufficiently spaced to prevent the resulting zone of inhibition from overlapping. The plates were incubated at 37 0C for 18-24hrs to observe the zone of growth inhibition produced by the extract (Cheesbrough, 2020).

Susceptibility Test Using Antiboitics

Antibiotic sensitivity test was carried out on all isolates using Kirby Bauers paper discs diffusion techniques. *Amoxicillin* used After the inoculation of test organisms into various agar plates. The plates were allowed to dry in an incubator for 15mins.The antibiotic discs were placed on the agar using sterile forceps. Each disc was placed far from each other to prevent their zones of inhibition from overlapping. The plate with the antibiotic disc were then incubated at 37 ^{0}C for 2hours to observe the zone of inhibition produced by the antibiotics (Cheesbrough, 2020).

3. RESULTS

Phytochemical screening of extracts

The result of the phytochemical screening revealed the following metabolites as present in the methanol, ethanol and aqueous extracts.The phytochemical studies of the ethanol extract revealed the presence of four (4) out of the seven (7) phytochemicals with presence of alkaloids, saponins, phenol, tannins, compounds while flavonoids, steroid and glycoside was absent. The ethyl acetate extract showed the presence of five phytochemicals (glycoside, steroid, tannin, saponin and phenol) while alkaloid and flavonoid were absent. Finally the result also showed that methanol extract reveled the presence of five (5) out of the seven (7) phytochemicals with presence of glycosides, saponins, phenol, tannins, steroid compounds while flavonoids, and alkaloids, were absent.

Table 1:Qualitative phytochemical composition of different extracts of *C. pentandra*

Metabolites	Methanol	Ethanol	Aqueous
Saponins	+	+	+
Tannins	+	+	+
Reducing sugars	+	-	-
Carbonyl or aldehyde	+	+	-
Phlobatannins	+	-	+
Steroids	-	-	-
Flavonoids	+	-	+
Terpenoids (Salkowski method)	+	+	+
Alkaloids	+	+	+
Cardiac glycoside	+	+	-
Anthroquinones	+	+	-

Key
+++ = Present in high concentration
++ = Present in moderate concentration
+ = Slightly or sparingly present
- = Absent.

Isolation of Salmonella typhii
The growth pattern of the blood Brain Heart Infusion Broth Agar (Oxoid,England), which was incubated at 37^{o}C for 3days are shown below.

Table 2: Bacteria growth pattern and bacteria count of isolate

SAMPLE	NATURE OF GROWTH	Total bacterial Count (Cfu/ml)
Sample 1	Moderate	0.91 x10^4
Sample 2	Moderate	0.75x10^4
Sample 3	Heavy	3.25 x10^4
Sample 4	Scanty	1.65 x10^4
Sample 5	Moderate	3.25 x10^4
Sample 6	Moderate	0.96 x10^4
Sample 7	Moderate	1.27 x10^4
Sample 8	Moderate	0.91 x10^4
Sample 9	Scanty	0.75x10^4
Sample 10	Moderate	1.27 x10^4

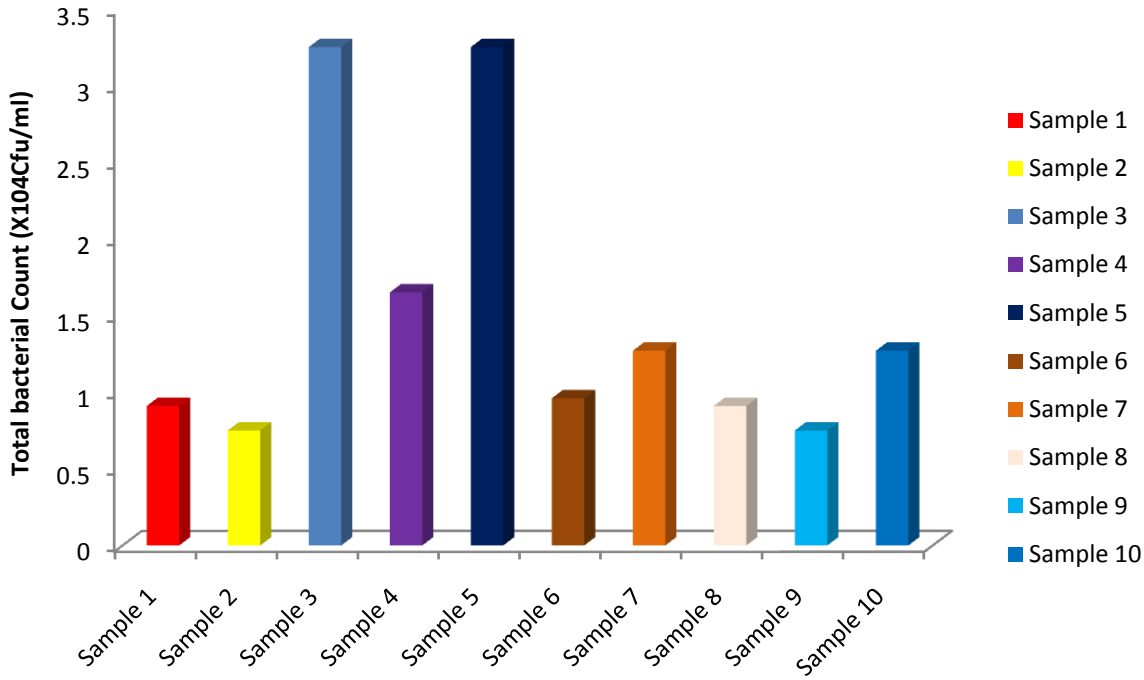

Fig 1: Bacteria growth pattern and bacteria count of isolate

Table 3: Morphological Characteristics

Isolate	Colour	Form	Elevation	Cell arrangement	Gram reaction	Suspected organism
A	Grennish	Irregular	Flat	Small rods	Positive	*Citrobacterspp*
B	Yellow	Circular	Flat	Large rods	Negative	*Proteus spp*
C	Cream	Circular	Flat	Short rods	Negative	*Klebsiellaspp*
D	pink	Irregular	flat	Short rods	Negative	*Esherichia Coli*
E	Cream	Irregular	Flat	Coccus	Positive	*Shigellaspp*
F	Greenish	Circular	Flat	Large rods	Negative	*salmonella sp*

Table 4: Biochemical Characteristics

ISOLATE	MOTILITY	COAGULASE	CATALASE	PROBABLE ORGANISM
A	-VE	-VE	+VE	*Citrobacterspp*
B	+VE	-VE	+VE	*Proteus spp*
C	-VE	-VE	-VE	*Klebsiellaspp*
D	-VE	+VE	-VE	*salmonella sp*
E	+VE	-VE	-VE	*Shigellaspp*
F	+VE	-VE	-VE	*Salmonella spp*

Antibacterial activity

The zone of inhibition for the various extracts at different concentrations are shown in table 4. The highest zone of inhibition of the extracts against *Salmonella* Typhi (*S*. Typhi) were 26 mm, 27 mm and 27 mm at 500 mg/mL for methanol, ethanol and aqueous extracts respectively and 29 mm for the control (amoxicillin). This was followed by the zone of inhibition of 24 mm, 23 mm and 25 mm at 250 mg/mL for methanol, ethanol and aqueous extracts respectively and 25 mm for the control at 250 mg/mL.

Table 5: Zone of inhibition of the different concentrations of extracts against *S. Typhi* (mm)

Extract	Concentration (mg/mL)			
	100.00mg/ml	200.00mg/ml	250.00mg/ml	500.00mg/ml
Methanol	10.0 ± 1.0^{a}	15.0 ± 2.0^{a}	24.0 ± 1.0^{a}	24.0 ± 2.0^{b}
Ethanol	14.0 ± 1.0^{a}	17.0 ± 1.5^{a}	23.0 ± 1.0^{b}	24.0 ± 1.0^{a}
Aqueous	15.0 ± 2.0^{b}	21.0 ± 1.0^{a}	25.0 ± 1.5^{a}	27.0 ± 1.0^{a}
Amoxicillin	20.0 ± 1.5^{a}	23.0 ± 1.0^{a}	25.0 ± 1.0^{b}	28.0 ± 2.0^{b}

Values are expressed as mean±SD. Values with the same alphabets are significantly different ($P \leq .05$)

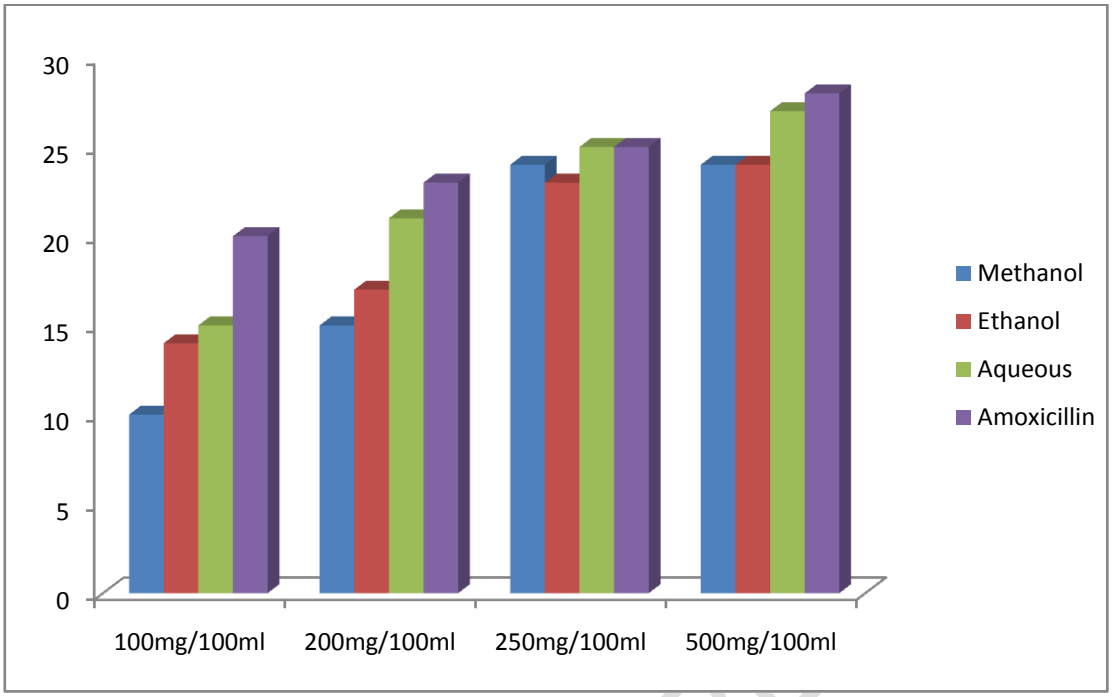

Fig 2: Zone of inhibition of the different concentrations of extracts against *S. Typhi*

Minimum inhibitory concentration (MIC)
The results of the MIC of the different extracts against *Salmonella.typhi* L for methanol, ethanol, aqueous extracts and amoxicillin (control) respectively.

Table 6: Minimum inhibitory concentration (MIC)

Microorganisms	Ethyl acetate	Ethanol extract	Methanol extract	Amoxicillin
S. Typhi (mg/mL)	200.00	200.00	250.00	100.00

Table 7: Qualitative Phytochemical

Metabolites	Methanol	Ethanol	Aqueous
Saponins	+	+	+
Tannins	+	+	+
Reducing sugars	+	-	-
Phlobatannins	+	-	+
Steroids	-	-	-
Flavonoids	+	-	+
Terpenoids	+	+	+
Alkaloids	+	+	+
Cardiac glycoside	+	+	-
Anthroquinones	+	+	-

Key

+++= Present in high concentration
++ = Present in moderate concentration
+ = Slightly or sparingly present
- = Absent.

Table 8: QUANTITATIVE PHYTOCHEMICAL COMPOSITION

PHYTOCHEMICALS	MEAN VALUE(mg/100g)
ALKALOID	8.8200
FLAVONOID	1.0567
PHENOL	100.5267
SAPONIN	34.1667
TANNIN	18.3667
STEROID	.1300
TERPENOID	3.3000

***Values are mean scores ± Standard deviation of three (3) replicates**

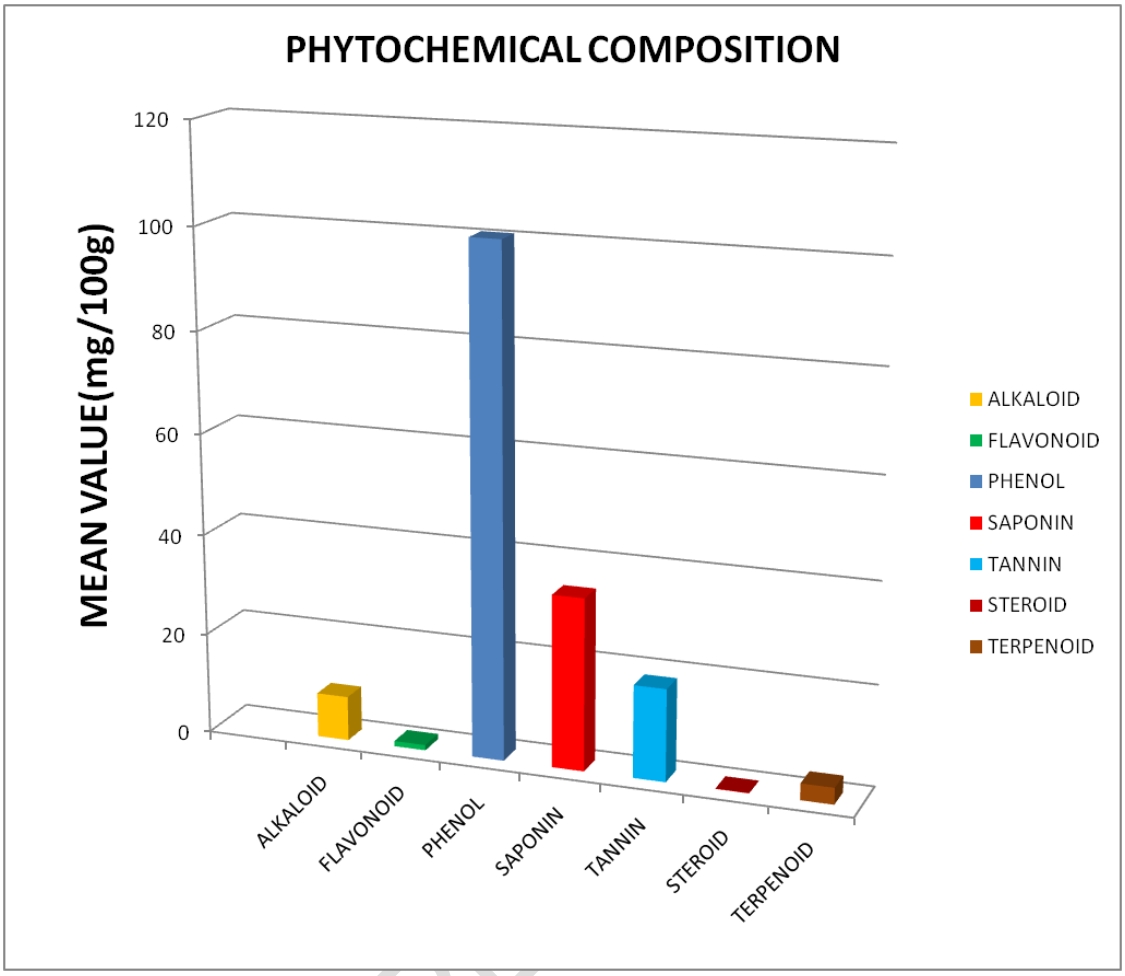

Figure 3: Mean values ofPhytochemical Composition

DISCUSSION

5.1 Discussion

The result of the Phytochemical screening of *Chromolaenaodorata* (Awolowo) Leaves as showed in table 1 indicated that the sample is rich in phytonutrients as they all contained phenols, glycosides, steroids, tannin and saponins. Phytochemicals are bioactive, non-nutrient, naturally occurring plant compounds found in vegetables, fruits and spices (Echo and Ikenebomeh, 2018). The phytochemical studies of the ethanol extract revealed the presence of four (4) out of the seven (7) phytochemicals with presence of alkaloids, saponins, phenol, tannins, compounds while flavonoids, steroid and glycoside was absent. The ethyl acetate extract showed the presence of five phytochemicals (glycoside, steroid, tannin, saponin and phenol) while alkaloid and flavonoid were absent. Finally the result also showed that methanol extract reveled the presence of five (5) out of the seven (7) phytochemicals with presence of glycosides, saponins, phenol, tannins, steroid compounds while flavonoids, and alkaloids, were absent.

Presence of tannins as shown in the result suggests the ability of this plant to play a major role as anti-diarrhea and antihaemorrhagic agent (Asquith and Butler, 1986). Steroidal compounds are of importance in pharmaceuticals because of their relationship with compounds used as sex hormones (Akinmoladun, *et al.,* 2017). The presence of tannins in leaves of fertility tree might have accounted for its sharp taste and have been reported to hasten the healing of wounds and inflamed mucous membranes (Wise *et al.,*2017).

Cardiac glycosides are known to lower the blood pressure according to many reports and functional for heart diseases. The results obtained in this study thus suggest that spices are

an increasingly. valuable reservoir of bioactive compounds of potential substantial socioeconomic importance (Henneberg and Stohmann, 2014).

Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. Several alkaloid containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God.

Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Iwu, 2013).

Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Okwu, 2011).

The result of the antibacterial activities of the three extracts of ChromolaenaOdorata (Awolowo) Leaves showed that at low concentrations (10-200 mg/mL), the ZDI of the control [(≥20.0±1.5) mm] is significantly higher ($P \leq 0.05$) than the ZDI of the extracts [(10.0±1.5) mm]. However, at higher concentrations, the ZDI of control did not differ from those of the extracts ($P \geq 0.05$). According to Baker and Silverton(2016), an organism is considered sensitive to a chemical agent only when the ZDI is either equal to the control, more than or not more than 3 mm smaller than the control. The highest ZDI of the extracts against *S*. Typhi are 26 mm, 27 mm and 27 mm at 500 mg/mL for methanol, ethanol and aqueous extracts respectively and 29 mm for the control (amoxicillin). This is followed by the ZDI of 24 mm, 23 mm and 25 mm at 250 mg/mL for methanol, ethanol and aqueous extracts respectively and 25 mm for the control. These data are above the reference range of the Clinical Laboratory Standard Institute as such are considered sensitive in this work (Chakraborty, *et al.,*2011).

This study highlighted the antimicrobial effects of Chromolaenaodorata on some known pathogens. Some antibiotics have been obsolete because of the problem of drug resistance (Ghani, and Uheshaja, 2020). Thus improvement of health using herbs as raw materials should be reconsidered.*Chromolaenaodorata*is a known invasiveweed in Nigeria and readily spreads with ease inhabiting any available space. The ability of *Chromolaenaodorata* exhibiting antimicrobial activities in the current research work indicates a potential for alternative use of the weed as raw materials for the production of medicine that can be used in diseases caused by *Staphyloccocusspp, Escherichia coli* and *Candida albicans* (Paterson, &Zachariades, 2013).

This study shows that Awolowo leaves are rich in nutrients and that their utilization should be strongly recommended for good health. Awolowo leaves are reservoirs for free radical scavenging molecules such as vitamins, alkaloids, tannins, terpenoids, phenolic acids, flavonoids and other metabolites, which are basically rich in antioxidant activities. It is quite interesting to inform that these plant metabolites can be genetically manipulated to increase their yield. This implies that it might be unnecessary to go over the counter medicine stores to buy synthetic drugs to this respect (Okon and Amalu, 2013). The antibacterial properties of *ChromolaenaOdorata* (Awolowo) Leaves extract can be harnessed for the production of new antibiotics or the enhancement of already existing antibiotics that are fast developing resistance to combat the problem of multi-drug resistance of *S. Typhi*.

5.2 Conclusion

The results of phytochemical analysis revealed the presence of alkaloid, saponin, tannin, anthraquinone, Flavonoid, phenols, terpenoid and glycoside which are the active components of the plant responsible for the medicinal values of the plant. The extracts exerted antibacterial activity against S. typhi. Each of the extracts used showed a large zone of inhibition against the organisms used confirming the use of the *ChromolaenaOdorata* (Awolowo) Leaves in the treatment of diseases like typhoid fever, diarrhoea and dysentery. The demonstration of antibacterial activity by *ChromolaenaOdorata*(Awolowo) Leaves extracts against S. typhi has provided a scientific basis for its local usage as a medicinal plant in the treatment of typhoid fever.

The fact that antibacterial activity of the extracts increased after purification using polar solvents is evidence that the bioactive compounds in the plant S. Awolowoae are promising source of a potent antityphoid drug that will be effective, cheap and accessible to all. Therefore, further research to refine, detect and characterize bioactive compounds and its effect on S. typhi, including toxicological studies, needs to be carried out.

It is recommended that further studies or investigations on toxicity test on the bioactive ingredients should be carried

Few research papers are available on the wound healing properties of Awolowo leaf, more work is needed on the seed and it's characterization.

More work is also needed on the bacteriological examination of it's herbal extract.

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