Antimcrobial Effect of Independence Leaves (Chromolaena Odorata) Extracts

[1]Okpashi V. E., [2] Bayim P. R. and [3] Obi-Abang M.
[1]Department of Biochemistry, university of Nigeria, Nsukka, Nigeria (vic2reshu@gmail.com)
[2]Department of Chemical Sciences, Cross River University of Technology, Calabar. Bayim22@yahoo.co.uk
[3]Department of Chemical Sciences, Cross River University of Technology, Calabar megabiabeng@yahoo.com

Abstract: This study was carried out to investigate the possibility of using Chromolaena odorata to ascertain whether it has the potential to inhibit bacterial and fungal growths. The antimicrobial effects of independence leaves (Chromolaena Odorata) siam weed obtained from Bumaji in Boki Local Government Area of Crosss River State were studied. An ethanolic extract of the dried and coarse leaves was prepared, serial dilutions of the extracts 100%, 50%, 25%, 12.5%, 6.2%, 3.13% and 1.5% respectively were carried out in water for all the drugs and extracts. They were tested for sensitivity and resistivity on bacterial such as Escherichia coli, Saimonella, Staphylococcus aureus and Bacilus anthracilis, using penicillin, zinacef, ciprofloxacin, ampicillin and ceftriaxone as standard controls. The serial dilutions were also tested on fungi such as candida albicans and tricophyton tonsurans, while clotrimazole and nystatin were used as standard control for antifungals. Chromolaena odorata was observed to exhibit concentration- dependent antibacterial effects similar to control bactericidal agents and antifungal effects similar in pattern to the control drugs used in this research. C. odorata extracts could thus possess antibacterial and antifungal ingredients. A weed capable of combating both bacteria and fungi growths extensively.

Keywords : Chromolaena odorata, Sensitivity, Resistivity, Antimicrobial and Antifungal.

Introduction

Plants are ubiquitous in nature and are essential for human life, though some do have detrimental effects. In Nigeria, there are various indigenous and foreign plants that are well known to heal most of the infectious diseases. *Chromolaena odorata* (independence leaf), this name was adopted in Nigeria when she got her independent and the plant was introduced into Nigeria in 1960 , also called *Osmia odorata* L. or *Euphatorium odoratum* L.), it belongs to the family *Asteraceae*. In taxonomical order of the plant, *Chromolaena* is the genus name while, *Chromolaena odorata* is the species name. Its common names include “Awolowo”, in Igbo language,” Obirato” in Bumaji-Boki, siam weed, triffid weed in Housa, bitter bush or jack in the bush (Okon and Amalu, 2003). Although, native to south and Central America it has spread throughout the tropics, Nigeria inclusive. In traditional medicine, it is used as an antispasmodic, antiprotozoal, antitrypanosomal, antibacterial, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic and hepatotropic agent (Iwu, 1993; Phan *et al.*, 2001; Akinmoladun *et al.*, 2007). The present study is designed to assess the antimicrobial, antifungal effects of *C.odorata*.

Siam weeds (*Chromolaena odorata* L.) King-Robinson) is a large multi-stemmed perennial shrub in the *Asteraceae* family. It was first discovered on mainland Australia in 1994 near the towns of Mission Beach and Tully on the tropical coast of north east (Queensland 2011). Infestations of Siam weed have also been found in other tropical coastal areas of Queensland, (Brooks *et al*, 2012). Some epidemiological evidences suggest that increased fibre consumption may contribute to a reduction in the incidence of certain diseases including colon cancer, coronary heart disease, diabetes, high blood pressure, obesity and various digestive disorders (Walker, 1978; FAO, 1990; Eriyamremu and Adamson, 1994; SACN, 2008; Igboh *et al* 2009).They increase fecal bulk and rate of intestinal transit have prebiotic effects. Plants are indeed essential to mankind and most plants are harmless, but some cause irritant, allergic and phototoxic dermatitis when in contact with human skin. (Sulzberger, 1979).

The incidence of plant extract application to treatment of diseases varies with country and locality. Most herbs inducing plants belong to a limited number of families like *Alliacae, Anacardiaceae, Cactaceae, Compositae, Cruciferae, Hennadiaceae, Lilliaceae, Orchidaceae, Primulaceae, Urticaceae* etc. and most sensitizers are chemicals related to catechols and lactones (Bajaj and Saraswat, 2008). In Nigeria, *C odorata* is the leading cure for bacterial infections and has assumed epidemic proportions, (Pasricha, 1981). Plant efficacy can be occupational and non-occupational, the former being

common in gardeners, farmers, florists and undertakers. *C. odorata* is a shrub which exists ubiquitously in our environment. It is used in traditional medicine as a remedy for chronic skin ulcers.

The use of medicinal plants for treatment of various infections in traditional communities has been an age-long global practice. It has been estimated that 80% of African population use herbal regimen for treatment and control of diseases (Hugo and Russell, 2003). This provides a rationalization for studying medicinal plant extracts as a possible source of alternative therapy against infections. Apart from the expensive costs of some antibiotics, most of the clinically important antibiotics have major setbacks. A good number of conventional antibiotics have being found to be neurotoxic, nephrotoxic and hypertensive, and few others cause severe damage to the liver and bone marrow depression (Chong and Pagano, 1997; Eze *et al*, 2013). The primary benefit of using herbal drugs is that they are relatively safer and cheaper than the synthetic alternatives (Aiyegoro and Okoh, 2009). In addition, herbal medicine is a complex mixture of different phytochemicals acting by different mechanisms, which makes it difficult for pathogens to develop resistance (Daferera *et al.*, 2003). It becomes pertinent to assess the antimicrobial effects of *C. odorata.* The drug so developed would serve as an alternative to currently used antimicrobial agents when most of them are expensive and the later is derived from local raw material.

Methodology

Plant Materials

These plants Independence leaf (*chromolaena odorata*) were collected from the premises of chief Okpashi A. from Bajiki-Bumaji, Boki Local Government Area of Cross River State.

Extraction of Plant Material

The leaves of the plants were plucked, rinsed with water and air-dried at room temperature for 4-7 days. The dried leaves were grinded using a pistle and mortal to obtain fine coarse. The active ingredients were extracted by soxhlex extractor using 95% ethanol. Briefly, 100 g of each leaf coarse was added to 900 ml of 95% ethanol. After which the filtrate was evaporated to dryness under air pressure. The dried crude extracts were stored in the refrigerator (at 4^oC) under aseptic conditions for use.

Sterilization of Materials

All glass wares used in this research were sterilized in the hot air oven at 1150^oc for 15 minutes.

Chemicals/Reagents

All chemicals used for this research were of analytical grade and products from Serva, Heidelberg limited, New York.

The Test Organisms

The test bacterial organisms used for this research *Escherichia Coli,Salmonella typhi, Bacillus anthracis, staphylococcus aureus* and *candida albicans* were transferred into freshly prepared plates of Nutrient Agar and incubated for 37^oc for 24 hours.

Determination of Minimum Inhibitory concentration (MIC) of the Extracts

The MICs of the extracts on the isolates were determined by macro broth dilutions techniques following the recommendations of the Clinical and Laboratory Standard Institute (CLSI, 2006) (formerly called NCCLS). Different concentrations of the extract ranging from100%, 50%, 25%, 12.5%, 6.2%, 3.13% and 1.5% respectively were prepared in tubes of 1ml Mueller-Hinton broth by serial dilutions.

Then 1ml of an overnight nutrient broth culture of the test isolates (adjusted to 106 CFU/ml) was added to each tube of the 1ml Mueller Hinton broth containing the extract. Each tube was mixed and incubated at 37^oC for 24 h. The experiment was conducted using the extract and standard control drugs.

Statistical analysis

All investigations were carried out in triplicate and results obtained were presented as sensitization and resistance using descriptive analysis.

Results

The plant extracts exhibited different levels of antibacterial and antifungal activities and appeared to be one of the effective resistant and sensitized therapies for bacteria and fungi at different dilution concentration of 100%, 50%, 25%, 12.5%, 6.2%, 3.13% and 1.5% respectively in the agar well diffusion experiments.

Table 1, 2, 3, 4 and 5showed the inhibitory concentrations (ICs) of plant extracts on different organisms obtained using macro broth dilution method. table 1 showed the resistivity and sensitivity of the antibacterial activity of the extract *C. Odorata* in the agar well diffusion test at different percentage dilution on *E. Coli*, compared with other antibacterial and antifungal drugs, such as penicillin, zinacef, ciprofloxacin, ampicillin and ceftriaxone and clotrimazole and nystatin used as antifungal drugs.

Inhibition zone diameters that ranged from 15 mm to 21 mm. The mean MICs of the extract varied from 100% to 25% as shown in table 1. The susceptibilities of the test organisms to the extract (at 100 %) can be ranked statistically (R) as follows: *Escherichia Coli* > (*Salmonella typhi = Bacillus anthracis = staphylococcus aureus and candida albicans)* > *P.* The antibacterial activity of the extract of *C. odorata* can be found in table 1, 2, 3, 4, and 5 respectively.

Table 1.Result of *E. Coli*, control drugs and extracts

Organism	*E. Coli* 100%	*E. Coli 50%*	*E. Coli* 25%	*E. Coli 12.5*	*E. Coli 6.25%*	*E. Coli 3.12%*	*E. Coli 1.57%*
Penicillin	S	S	S	S	R	R	R
Chromolaena odorata	R	R	R	R	S	R	R
Clotrimazol	R	S	R	R	R	R	R
Nystatin	R	R	R	R	R	R	R
Ceftriaxone	R	R	R	R	R	R	R
Zinacef	S	S	S	S	S	S	S
Ampicillin	R	R	R	R	R	R	R
Ciprofloxacin	R	R	R	S	R	R	R

The result showed that at variable concentration of the extracts and the respective standard drugs, the organism response was dose-dependent.

Where R= Resistance

S= Sensitivity

Table 2.Result of *Saimonella typhi* control drugs and extracts

organism	*Saimonella typhi* 100%	*Saimonella typhi 50%*	*Saimonella typhi* 25%	*Saimonella typhi 12.5*	*Saimonella typhi 6.25%*	*Saimonella typhi 3.12%*	*Saimonella typhi 1.57%*
Penicillin	S	S	S	S	S	S	S
Chromolaena odorata	S	S	R	R	R	R	R
Clotrimazol	R	R	R	R	R	R	R
Nystatin	S	S	S	S	S	S	S
Ceftriaxone	S	S	S	S	S	S	S
Zinacef	R	R	R	R	R	R	R
Ampicillin	R	R	R	R	R	R	R
Ciprofloxacin	R	R	R	S	S	S	S

The result showed that at variable concentration of the extracts and the respective standard drugs, the organism response was dose-dependent.

Where R= Resistance

S= Sensitivity

Table 3.Result of *Baccilus Anthracilis* control drugs and extracts

Organism	*Baccilus Anthracilis* 100%	*Baccilus Anthracilis 50%*	*Baccilus Anthracilis* 25%	*Baccilus Anthracilis 12.5*	*Baccilus Anthracilis 6.25%*	*Baccilus Anthracilis 3.12%*	*Baccilus Anthracilis 1.57%*
Penicillin	R	R	R	R	R	R	R
Chromolaena odorata	S	S	R	R	R	R	R
Clotrimazol	R	R	R	R	S	S	S
Nystatin	S	S	S	S	S	S	S
Ceftriaxone	S	S	S	S	S	S	S
Zinacef	S	S	S	S	S	S	S
Ampicillin	R	R	R	R	R	R	R
Ciprofloxacin	S	S	S	R	R	R	R

The result showed that at variable concentration of the extracts and the respective standard drugs, the organism response was dose-dependent.

Where R= Resistance

S= Sensitivity

Table 4 .Result of *Candida Albicans* control drugs and extracts

Organism	*Candida Albicans is* 100%	*Candida Albicans 50%*	*Candida Albicans* 25%	*Candida Albicans 12.5%*	*Candida Albicans 6.25%*	*Candida Albicans 3.12%*	*Candida Albicans 1.57%*
Penicillin	S	S	S	S	S	S	S
Chromolaena odorata	S	S	S	S	R	R	R
Clotrimazol	R	R	R	R	R	R	R
Nystatin	S	S	S	S	S	S	S
Ceftriaxone	S	S	S	S	S	S	S
Zinacef	R	R	R	S	R	R	R
Ampicillin	R	R	S	S	S	S	S
Ciprofloxacin	R	R	R	S	S	S	S

The result showed that at variable concentration of the extracts and the respective standard drugs, the organism response was dose-dependent.

Where R= Resistance

S= Sensitivity

Table 5. Result of *Staphylococcus* control drugs and extracts

Organism	*Staphylococcus* 100%	*Staphylococcus 50%*	*Staphylococcus* 25%	*Staphylococcus 12.5%*	*Staphylococcus 6.25%*	*Staphylococcus 3.12%*	*Staphylococcus 1.57%*
Penicillin	R	R	R	R	R	R	R
Chromolaena odorata	S	S	S	S	S	R	R
Clotrimazol	S	S	S	S	S	S	S

Nystatin	R	R	R	R	R	R	R
Ceftriaxone	S	S	S	S	S	S	S
Zinacef	R	R	R	R	R	R	R
Ampicillin	S	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S	S

The result showed that at variable concentration of the extracts and the respective standard drugs, the organism response was dose-dependent.

Where R= Resistance

S= Sensitivity

Discussion

Chromolaena odorata is one of the world's worst tropical weeds. It is a member of the tribe *Eupatorieae* in the sunflower family *Asteraceae*. The weed goes by Many common names including Siam weed, devil weed, French weed, communist weed, hagonoy, cohoy (Aweng *et al*, 2012) etc. *Chromolaena* is being used traditionally for its many medicinal properties, especially for external uses as in wounds, skin infections, inflammation etc. Studies have demonstrated that the leaf extract has antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties (Vaisakh and Anima, 2012).*Chromolaena odorata* was selected as an antimicrobial material in this study because it has several advantages for example, easy to found and collected.

Its leaves have a soft, spongy with high penetrating properties and easy to growth (Aweng *et al*, 2012). This prospective study evaluated the experimental activities of microorganisms such as *Escherichia Coli, Salmonella typhi, Bacillus anthracis, staphylococcus aureus* and *candida albicans* presenting with plant extracts of *chromolaena odorata* and some frequently used drugs such as penicillin, zinacef, ciprofloxacin, ampicillin, ceftriaxone, while clotrimazole and nystatin which were used as antifungal drugs to test for sensitivity and resistivity. The alternate or slow response of some bacteria on some notable antibacterial drugs was not established. The study also evaluated test results of the particular organism with high resistance and low sensitivity. *C odorata* showed resistance for *Escherichia Coli* at 100% to12.5%, but showed sensitivity at 6.25%, see table 1. Furthermore, at 3.13% and 1.57%, the plant showed resistance to *Escherichia Coli*. Compare with penicillin to showed sensitivity at 100% to 12.15% but resistivity at 3.13% and 1.57%. We can say from this result that ampicillin, *c. odorata* and nystatin produced similar effect on *Escherichia Coli*.

The influence of *Salmonella typhi* in this study was striking with ampicillin, clotrimazol and zinacef showing resistance at all percentage concentration of 1.57% to 100%, while ceftriaxone and nystin showed sensitivity at same percentage concentration 1.57% to 100% respectively. At high dose of 100% and 50%, the plant extracts showed sensitivity, but develops resistance at 25% to 1.57%. This un-assumed behavior of *Salmonella typhi* on the extract is not understood why *Salmonella typhi* was resisted by the plant extract at low dose concentration, as indicated in table 2. Whereas, other drugs such as penicillin, ceftriaxone and nystatin, showed absolute sensitivity despite the dosage or concentration. However, *chromolaena odorata* compete favorably on *Salmonella typhi* with zinacef, clotrimazol ampicillin and ciprofloxacin (Frain-Bell and Johnson , 1979). Avoidance of the causative organism is impractical in most set-ups; therefore prevention and early treatment plays an important role in reducing the attack of infectious bacteria and fungus on human. Working habits, hygienic measures and photoprotection are of paramount importance in preventing opportunistic infections. In case of *Chromolaena odorata*, it showed sensitivity at 100% and 50% on *Bacillus*, but showed resistance at 1.57% to 25% respectively. While conventional drugs such as penicillin, ampicillin and clotrimazol showed resistance at 1.57% to 100%. Zinacef, ceftriaxone and nystatin indicate absolute sensitivity in all the dose concentration as illustrated in table 3. Clotrimazol showed resistance between 100% to 12.5%, while ciprofloxacin showed sensitivity at same percentage dosage.

Also, table 4 and 5 showed that the reaction of the various bacteria culture and fungal culture to the respective antibacterial and antifungal drugs, *chromolaena odorata* also possess effective photochemical that can combat those microorganisms. The preliminary findings from this study demonstrate that *Chromolaena odorata* exhibited convincingly the ability to function as bactericidal and fungicidal agents and can compete favorably with other notable conventional antibacterial and antifungal drugs.

Conclusion

The preliminary findings from this study demonstrate that *Chromolaena odorata* exhibited convincingly the ability to combat bacteria and fungi infectious diseases in same pattern like other bactericidal and fungicidal agents with respect dosage, and further revealed that a combination of *Chromolaena odorata* with some other bactericidal and or fungicidal agent will be an effective therapy. Further investigation will be made to find out some new bioactive compounds which may be useful for drug development especially as bactericidal and fungicidal agents.

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