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Evaluation of the Nutrient Composition and Invitro Assessment of the Biocidal Attributes of Siam Weed (Chromolaena Odorata) on Echerichia Coli and Geotrichum Candidum

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Abstract

A research was carried out to evaluate the nutrient composition and in-vitro assessment of the bactericidal and fungicidal attributes of the Siam weed *(Chromolaena odorata*) leaf extracts on *Escherichia coli* (bacterium) and *Geotrichum candidum* (fungus) in the Plant Pathology Laboratory in Rivers State University of Science and Technology, Port Harcourt. Results showed that *C.odorata* contain essential nutrient elements such as moisture, ash, fat, carbohydrate, protein and fibre. Essential vitamins (A, B and C) and minerals like phosphorus and potassium were also present in reasonable quantity. Other anti-nutrient elements such as tannins, saponins, phytates and oxalates were also identified in relatively large amount. *E coli* are gram negative rod shaped bacteria that are none spore bearing found in the lower abdomen of warm blooded animals and highly facultative. *Geotrichum candidum* is spore bearing fungus that is widely associated with the rot of fresh fruits and vegetables resulting into soft rots. Results obtained from this work showed that *E. coli* strains were resistant to the crude extracts of Siam weed while *G. candidum* were inhibited by the same extracts. The reason could be that Siam weed extracts were basically inhibitors of cell wall synthesis present in fungi and not in bacteria. *E. coli* possesses a thin layer of peptidoglycan, much complex lipopolysaccharides, large irregular capsules, occasionally slime layer. However, since the chemical component of Siam weed extract are cell wall disruptors, these may have presented very little or no tag on the cell wall to act upon as against the fungus which was not protected with such components.

Keywords: Nutrient composition, in-vitro assessment, Biocidal, Siam weed, Escherichia coli, Geotrichum candidum

Introduction

Chromolaena odorata L R. King and H. Robinson is a species of flowering shrub in the sun flower family, *Asteraceae* . It is native to North America, from Florida and Texas to Mexico and the Caribbean and has been introduced to tropical Asia, West Africa and parts of Australia. (Purseglove, 1977).It is considered invasive weed of field crops and has been reported to be the most problematic invasive species within protected rain forest in Africa (Shruhsaker et al, 2005). It is termed invasive weed because it grows from pre-existing plants. Its occurrence is said to be limited to latitude 30^0 N and 30^0C and in altitudes up to 1000m in locations where rainfall amounts to 2000 mm (Muniappan and Marutani, 1988). It was introduced to Nigeria in the 1940s and by the late 1960s.*C. odorata* has become an important weed in Ghana, Nigeria, and Cameroun(Cruttwel, 1988).*C. odorata* can grow on agricultural area, natural forest, planted forests, grass lands and shrub lands (Shruhsaker*et.*

al, 2009). Despite the fact that *C. odorata* is an invasive weed affecting the growth and productivity of crops, it also has medicinal values although its mode of action is not well understood by botanists. Leaf extracts of Siam weed have been reported to possess anti-bacterial activities against some bacteria such as *Bacillus subtilis, Staphylococcus aureaus and Samonella typhi* (Struhsaker*,et. al, 2005)*

Escherichia.Coli a gram negative, rod-shaped bacterium is found commonly in the lower abdomen of warm blooded organisms. Most *E. coli* strains are harmless, but some stereotypes can cause serious food poisoning in humans and are occasionally responsible for product recalls. The harmless strains are parts of the normal flora of the gut, and can benefit their host by producing vitamins $K_2$and preventing the establishment of pathogenic bacteria within the intestine(Hudault*et. al, 2001) E. coli* bacteria constitute about 0.1 % of gut flora, and faecal oral transmission in the major route through which pathogenic stains of the bacterium causing disease cells are able to survive outside the body for a limited amount of time which make them ideal indicator organisms to test environmental samples for faecal contamination (Hudault*et al*, 2001). The bacterium can also be given easily and inexpensively in a laboratory setting, and has been intensively investigated and an important species in the field of biotechnology and microbiology where it has served as the host organism for the majority of works with recombination DNA.

Geotrichum candidum is one of the most important fungi that is frequently associated with the deterioration of most fruits and vegetables in the tropics causing quality and quantity loses (Chuku, *et al*, 2010). It belongs to the fungi family *Deuteromycetes* with septate hypha that are dichotomously branched. Conidia are mostly aerial, erect or recumbent, cylindrical or barrel shaped or ellipsoidal and mostly formed by the breaking up fertile hypha chains.(onuegbu, 2002, Chuku *et al* 2010). Its role as a pathogenic fungus hindering the germination of cassava stems has been reported (Chuku, *et a*l, 2008). This work therefore assessed the efficacy of the ethanol and distilled water extracts of*Chromolaena odorata* as a bactericidal agent on *E. coli* and a fungicidal agent against *G. candidum*. Other assessments on the proximate composition of *C. odorata* were also considered. The knowledge of which will create the awareness on the effective utilization of this shrub which is found everywhere in the tropics.

Materialsand methods

Collection of leaves of C. odorata and preparation of leaf extracts.

Leaves of *C. odorata* were harvested from the Agricultural Teaching and Research Farm in the Rivers State University of Science and Technology, Port Harcourt. The farm is located at 18m above sea level in the humid tropical zone and has a bimodal rainfall pattern (FAO,1994). One kilogram of the fresh leaves was weighed with a sensitive balance and shared into two equal parts washed and ground in a sterile grinder. Each part of the ground samples was transferred into two different 500ml conical flasks and one part was added one litre of ethanol and the second half was added one litre of distilled water. The ground samples were filtered with two layered cheese cloth and stored in the refrigerator for bactericidal and fungicidal studies.

Determination of nutrient composition of C. Odorata

Fresh leaves of Siam weed were harvested from the University Research and Teaching Farm and taken to the Food Science and Technology Laboratory for nutrient composition analysis. The parameters estimated were the moisture, ash, fat, carbohydrate, protein, fibre, vitamins A, B and C, phosphorus, potassium, tannins, saponins, phytates and oxalates. These parameters were estimated using the Association of the Official Analytical Chemists methods of analysis (AOAC, 2005).

Bactericidal and Fungicidal Assessment

Preparation ofMacConkey and Nutrient Agar for bacterial growth

MacConkeyAgar was prepared according to the manufacturer's instructions. 52 grams of MacConkey Agar powder was weighed into a conical flask and 1 litre of distilled water was added to allow it soak for 10 minutes. The conical flask and its content were swirled to allow for proper mixing and then sterilized and autoclaved for 15 minutes at 121^0C. Cooled to 47^0C and poured into sterilized Petri Dishes and allowed to solidify. Nutrient Agar on the other hand was prepared following also the manufacturer's guideline. 28grams of nutrient Agar powder was weighed into a conical flask and 1 litre of distilled water also added and allowed to soak for 10 minutes. The conical flask and its content were swirled to allow for proper mixing and then sterilized and autoclaved for 15 minutes at 121^0C. Cooled to 45^0C and poured into sterilized Petri Dishes and allowed to solidify. Stock culture of *E. coli* that has been identified through oxidation fermentation test technique was transferred into the prepared culture and maintained in test tubes for further studies.

Preparation of Sabouraud Dextrose Agar for the growth of G. candidum

Sabouraud dextrose Agar the medium used for the growth of the test fungus was prepared according to the manufacturer's instructions. The mouth of the flask was plugged with none absorbent cotton wool and wrapped with Aluminium Foil. The conical flask with its contents was autoclaved at 121^0C and a pressure of $1.1 kgcm^{-3}$ for 15 minutes. The molten agar was allowed to cool for 40^0C and then dispensed into Petri Dishes at 15mls per plate and allowed to cool and solidify. Stock culture of *G. candidum* from rice was inoculated unto Sabouraud agar in Petri dishes and incubated for growth.

Assessment of bactericidal efficacy of Siam weed leaf extracts on E. coli

The disc were impregnated with What man's paper and were soaked at different concentrations (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128) of test tubes containing extracts of Siam weed and ethanol and allowed to stay for 30 minutes and thereafter were brought out to dry and sterilized. The *E. coli* that were identified by oxidation and fermentation technique from the stock culture were further sub cultured to get fresh pure cultures on freshly prepared MacConkey agar after incubating overnight. The technique of inoculation was aseptically done with a flamed wire loop. The pure

cultures obtained were introduced into solidified nutrient agar plates via flamed wire loop. The colonies were spread over the plates by streaking. Furthermore, the impregnated disc s were placed into the nutrient agar where the *E. coli* colonies were inoculated and the entire contents of the nutrient agar were incubated for 24 hrs at 37^0C to obtain a sensitivity pattern.

Assessment of fungicidal efficacy of Siam weed leaf extracts on G. Candidum

Colonies of *G. candidum* from five day old stock culture were used for this study. Similarly, different concentrations of the Siam weed leaf extracts were prepared (0, 20, 40, 60, 80, 100). These concentrations were prepared by varying the ratios of the Siam weed extracts and distilled water. Blotter papers were also soaked in the various concentrations for 30 minutes and thereafter brought out and dried and sterilized. Pure cultures of *G.candidum* obtained after series of inoculations into sabouraud dextrose agar were introduced onto the blotter papers and placed on sabouraud dextrose agar in Petri dishes. The entire set up were labelled accordingly and incubated and the extent of fungal inhibition at different concentrations was monitored for the extent of inhibition.

Results

Nutrient composition of Siam weed (C. odorata)

Result of the nutrient composition of Siam weed is presented in Tables 1a and 1b.The result revealed the presence of ash, fat, carbohydrate, protein, and fibre with moisture constituting the highest proportion of the entire leaf composition. In addition, the leaf of *C. odorata* contains essential vitamins such as vitamins C, A_1, and B_1. However, some mineral and anti-nutrient elements such as phosphorus, potassium, tannins, saponins, phytates and oxalates were also found in the leaf of *C. odorata.*

Table 1a: Proximate composition of *C. odorata.*

Moisture	(%)	52.5±0.005
Ash	"	2.5±0.006
Fat	"	3.4±0.003
CHO	"	11.75±0.001
Protein	"	17.5±0.003
Fibre	"	2.3±0.002
Vit.C	(mg)	12.5±0.004
Vit. A	(IU)	16.2±0.001
Vit. B_1	(mg)	1.8±0.001

Table 1b Mineral and Anti-nutrient composition of *C. odorata*

Phosphorous (%)	51.2±0.002
Potassium "	35.2±0.005
Tannins (mg/100g)	25.3±0.003

Saponins	"	23.05±0.004
Phytates	"	65.5±0.001
Oxalates	"	8.7±0.002

Bactericidal effect of ethanol and distilled water leaf extract of Siam weed on E.coli

Results of the bactericidal effect of ethanol and distilled water leaf extract of Siam weed are presented in Tables 2a & 2b respectively.

Table 2a.Anti-bactericidal effect of Ethanol leaf extract of *Chromolaena odorata on E. coli*

Organism	Inhibitory zone (mm)						
	Stock	1:2	1:4	1:8	1:16	1:32	1:64
E. coli	R	R	R	R	R	R	R

No inhibitory reaction observed in the negative control (Ethanol).
R= No inhibitory zone, effect or resistance.

Table 2b.Antibactericidal effect of distilled water leaf extract of *Chromolaena odorata on E. coli*

Organism	Inhibitory zone (mm)						
	Stock	1:2	1:4	1:8	1:16	1:32	1:64
E. coli	R	R	R	R	R	R	R

No inhibitory reaction observed in the negative control (distilled water).
R= No inhibitory zone, effect or resistance.

Results of the bactericidal attributes of distilled water and ethanol leaf extracts of Siam weed on *E. coli* showed that Siam weed irrespective of the different concentration and methods of extraction could not inhibit the growth of *E. coli.*

Anti-fungal attributes of ethanol and distilled water leaf extract of Siam weed on G. candidum

Results of antifungal attributes of ethanol and distilled water extracts of *C. odorata-on G. candidum* are presented in Table 3. Distilled water extracts inhibited *G. candidum* at higher concentrations than at lower concentrations. However, ethanol leaf

extracts of *C. odorata* greatly inhibited the growth of the test fungus even at lower concentrations when compared with the control plates. There was generally total inhibition of the test fungus at 80% and 100% concentrations of both the distilled water and ethanol leaf extracts respectively.

Table 3: Antifungal assessment of leaf extracts of *C. odorata*

Distilled water/ Ethanol concentrations of *C. odorata.*	*Rate of inhibition ofG.candidum.(%)*	
	Distilled water extract	Ethanol extract
0	*0±0.001*	
20	*30±0.003*	*40±0.002*
40	*50±0.001*	*60±0.002*
60	*80±0.002*	*100±0.001*
80	*100±0.003*	*100±0.002*
100	*100±0.002*	*100±0.001*
Mean inhibition	*60±0.006*	*80±0.004*

Discussion

The high nutrient compositions of most tropical fruits, herbs and vegetables are well documented (Achinewhu, 1996) Siam weed possessed essential nutrient elements and anti-nutrient components which qualifies it as an important medicinal herb. The presence of tannins, oxalates and saponins which contain certain compounds that deter the consumption of the leaves of this shrub by animals may be responsible for the bactericidal and fungicidal ability of the leaf extracts of Siam weed. The anti-bactericidal property of ethanol and distilled water leaf extracts of Siam weed at different concentrations ranging from stock to 1:64 dilution, using the double serial dilution technique was shown to have no effect on *E. coli*(Tables 2a&2b).This is because Siam weeds leaf extracts are basically inhibitors of cell wall synthesis whereas *E. coli* only possesses a thin layer of peptidoglycan and irregular lipopolysaccharides, much layer and irregular capsules and occasionally slime layers. These factors may have contributed to the resistance of organisms to Siam weed extracts (Erturk *et al*, 2006) The leaf extracts of Siam weed have been shown to have antibacterial effects on some bacteria such as *Bacillus subtle, Staphylococcus aureus* and *Salmonella typhi (* Pierangeli and Wendell; 2009*).* Leaf extract of Siam weed was reported to have controlled bacterial leaf spot caused by *Xanthomonas compestris pv. Vesicatoria* (Opara, 2005) due to the phytochemicals present. The responses of gram positive and gram negative organisms vary greatly. While gram positive organisms are susceptible to leaf extracts of Siam weed, gram negative organisms such as the *E.coli* are highly resistant to Siam weed leaf extracts due to their

cell wall components (Erturk,2006). *E. coli* in addition possesses pili which has made mating easier thereby increasing exponentially the population of these species which accounts also for the resistivity of the organisms. The leaf extracts of *Combretum woodii* have been reported to have high activity against both the gram positive and gram negative bacteria(Eloff *et al*, 2005)The control of *E. coli* using biocides of plant origin becomes very important because of the risk associated with synthetic chemicals. *E. coli* has been reported as one of the most important bacterium in the human gastrointestinal tracks and colonizes an infant's gastrointestinal tract within 40 hours of birth arriving with water and food (Zharxybayera *et al*, 2011). The non-pathogenic E. *coli* strain Nisole 1917 also known as Metaflor is used as probiotic agent in medicine mainly for the treatment of various gastroenterological diseases including inflammatory bowel disease (Grozdanoy *et al*, 2004). Because of its long history of laboratory culture and ease of manipulation, *E.coli* plays a prominent role in modern biological engineering and industrial microbiology (Kamada *et al,* 2005). Other clinical diseases of *E.coli* include urinary tracks infection which can result in bacteraemia with clinical signs of sepsis (Brook et al 2010). However, therapeutic approach has always been used to control diseases associated with *E. coli*. Several reports on the antifungal attributes of Siam weed and other plant extracts are available. The invitro antifungal activities of *Chromolaena odorata and Moringa oleifera* leaf extracts on phytopathogenic fungi of cucumber have been reported (Chiejina and Onaebi, 2013). Siam weed extracts *have been said to have antifungal effects against Candida albican responsible for* opportunistic oral and genital infection in human (Pierangeli and Windell, 2009). Many plant extracts have been known to possess antifungal properties against several pathogenic fungi including *G. candidum*. Crude extracts of *Aloe vera* has been reported to control *Aspergillus niger* invitro (Chuku, 2006), reduced some insect pests and diseases associated wi*th Telfairia occidentalis* (Chuku, *et al*, 2012).The leaf extracts of bitter leaf was reported to have inhibited the growth of *Candida spp, Aspergillus niger, Rhizopus spp and Microsporum canis (Nwaukwu et al, 2013).* Fungi because of their wide host range and their versatile nature of existence dominate their environment infecting both man, animal and plants. Their ability to be transferred from plants to man and animals in form of mycotoxin has posed a great concern to Scientists who are working relentlessly to ensure their control. The use of fungicides have been the best alternative for the control of these microorganisms for a very long time but not without associated environmental and health hazards (Onuegbu, 2002). Ethanol extract of siam weed was found to be more effective in terms of the inhibition of G candidum particularly at higher concentrations and this could be largely attributed to its polarity. The implication of this is that as the concentration of ethanol increases, the positive ions present become very strong to form stable ionic bonds which causes greater inhibition of the organisms (Zeugen and Hartley, 1985). The ability of the crude extracts of *C. odorata* a very common weed in our environment to inhibit the growth of *G. candidum* is an advancement in biotechnological approach of disease control.

Conclusion

The work on the bactericidal and fungicidal attributes of Siam weed has shown that the crude extracts of Siam weed could not inhibit the growth of *E. coli* a

gram negative bacterium but inhibited the growth of *G. candidum* a fungus. The inhibition of *G. candidum* by the leaf extracts of Siam weed appears to be pioneering since literature is not available. The reasons for the resistance of *E. coli* to Siam weed have been highlighted. However Siam weed have been reported to inhibit the growth of other strains of bacteria especially the gram positive bacteria. Further investigations using other plant extracts on *E. coli* and other gram positive bacteria are advocated.

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