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ANTIBACTERIAL ACTIVITY OF *Crassocephalum crepidioides* (FIREWEED) AND *Chromolaena odorata* (SIAM WEED) HOT AQUEOUS LEAF EXTRACT

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ABSTRACT

The present study evaluates the antibacterial activity of hot aqueous extract of Crassocephalum crepidioide sand Chromolaena odorata against three bacterial isolates namely Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli. The antibacterial activity was tested using disc diffusion method while MIC was determined using broth dilution technique. Preliminary phytochemical analysis was also conducted to substantiate their medicinal and antibacterial activities. The result of phytochemical screening showed the presence of alkaloids, tannins, flavonoids, saponins, steroids and phenols in extracts of both plants while anthraquinones and phlobatannins were absent. Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli were sensitive to the hot aqueous extract of Crassocephalum crepidioides and Chromolaena odorata however, Klebsiella pneumonia was most sensitive to Crassocephalum crepidioides with MIC of 15mg/mL while Staphylococcusaureus was most resistance with MIC of 45mg/mL in both extract. The result obtained confirmed the antibacterial potential of both Crassocephalum crepidioides and Chromolaena odorata against test organisms.

KEY WORDS

Antibacterial, Chromolaenaodorata, Crassocephalum crepidioides

INTRODUCTION

Vegetables are important components of a healthy diet owing to their nutritional and phytochemical constituents, thus, can act as nutraceuticals. They represent a natural reservoir of nutritional supplements to prevent nutritional disorders and phytochemicals which naturally proffer protection to plants but to humans' therapeutic purpose. Extracts from plants and their products are utilized in herbal medicine for the treatment of infections and diseases from time immemorial [1]. Recently, scientific researchers have been motivated to validate the therapeutic properties of medicinal plants claimed to possess antibacterial properties and elucidate the structure of new natural products

with antibacterial properties due to development of resistance to antibiotics used in current clinical practices and increase in incidence of new emerging infections and diseases [2].

Chromolaena odorata or Siam weed is called 'ewe Akintola' or 'ewe Awolowo', and 'Obuinenawa' by the Yoruba and Igbo tribe of Nigeria respectively. This weed is a perennial shrub belonging to the Aster family which flourish in most soils and is found abundantly along road sides, on waste land and areas where waste water float [3]. Medicinally, *Chromolaena odorata* has been used in the treatment of malaria [4], for wound healing [5], prevention of blood loss from wound due to astringent property of the leaves [6], diarrhea, tooth ache, skin diseases, colitis, and dysentery [7]. It is used as antibacterial, antifungal, antiprotozoan, antihypertensive and hepatoprotective therapy[8, 9, 10].*Crassocephalum crepidioides* (Fire weed), called 'ebolo' or 'gbolo', and 'Obuinenawa' by the Yoruba and Edo tribe of Nigeria respectively grows widely and covered up in tree crop plantation [11]. They are eaten raw or cooked as vegetables and have been reported to be of high nutritional value by Adjatin et al.[12]. The plants' parts have been used in treating fever, liver disorders such as hepatitis [13], indigestion and used as purgative and laxative [14, 15]. It has been shown to possess antioxidant, chemo preventive and anti-inflammatory properties [16]. Few reports exists on the antibacterial activity of aqueous extract of these leaves but none considered the hot aqueous extract, thus, we aimed to investigate the antibacterial activity of hot extract of these plants.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of the plants used were collected in Ikorodu Lagos State, Nigeria and identified in Biology unit of Science Laboratory Technology, Lagos State Polytechnic Nigeria by comparing with herbarium specimens using morphological features.

Extraction of plant material

The leaves of *C. crepidioides* and *C. odorata* were picked, washed under running water to remove soil debris and dried under shade at room temperature until constant weight was obtained. The leaves were then ground in a mortar and pestle into fine powder before extraction. 50g of the powdered samples were extracted with distilled water boiled at 100^0C for 4 hours before filtration. The mixture was filtered with whatman No 1 filter paper under sterile conditions. The resulting extracts were evaporated in a water bath to form a paste. The resulting extract was transferred into a sterile labeled bottle and stored at 4^oC for future use.

Phytochemical Analysis

Phytochemical constituents of the aqueous leaf extract of *C. crepidioides* and *C. odorata* were

determined according to the methods of Harborne[17] and Sofowora[18].

Test microorganisms

Escherichia coli, Klebsiellapneumoni and *Staphylococcus aureus* were obtained from Microbiology laboratory of Lagos State Polytechnic, Ikorodu, Lagos. The microorganisms were sub-cultured and the pure cultures re-sub cultured on nutrient agar slants and thereafter stored at 4ºC until required for study. Previously inoculated overnight broth culture nutrients both were used for antibacterial activity of the water extract.

Antibacterial activity of the water extract

The antibacterial activity of the aqueous leaf extract of *C. crepidioides* and *C. odorata* were determined using agar well diffusion method. Selected bacterial strains (*Staphylococcus aureus, Escherichia coli, and klebsiella pneumoniae*) were inoculated into 10mL sterile nutrient broth and incubated for 18 hours at 37^{o}C. Using a sterilized cork borer with 6mm diameter, wells were made in the inoculated agar before 0.1mL of the plant extracts with concentration of 50, 100 and 150mg/mL to wells in different plates. The plates were incubated at 37^{o}C for 24h and diameters of inhibition zone were measured in millimeters.

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the extracts was determined using the method of Vollekova *et al.*[19] modified by Usman *et al.,*[20].

RESULTS AND DISCUSSION

Plants remain a natural reservoir of phytochemicals with various physiological and therapeutic properties. Phytochemicals obtained from different plant species and families have been explored for their medicinal values including antibacterial activities. Hot water extract utilized in this study produced significant antibacterial activities for *Chromolaena odorata* and *Crassocephalum Crepidioides* against the bacterial strains utilized in this study. **Table 1** showed the zone of inhibition of aqueous extract of *Chromolaena odorata* and *Crassocephalum Crepidioides* against the test organism (*Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli*) at various concentrations, the plant extract showed appreciable zone of inhibition against all the test organism in a dose dependent manner, though, *Klebsiella pneumonia* was most sensitive to the two extracts while E. coli showed the least sensitive with lowest zone of inhibition. Anyasor et al., [21] reported the inability of cold aqueous and ethanol extract of *Chromolaena odorata* to inhibit *Klebsiella pneumonia* while the ethanol extract was only able to inhibit Escherichia *coli* at a concentration of 150-200mg/mL. Kigigha et al. [22] also reported resistance of E. coli to cold aqueous extract of *Chromolaena odorata* but *Staphylococcus aureus* and *Klebsiella pneumoniae* were sensitive. This is in total disagreement with result obtained in our study which was in accord

with the reports of Nurul et al., [23] who reported sensitivity of gram positive bacteria to extracts of *Chromolena odorata* while inhibition growth of *Staphylococcus aureus, Klebsiella pneumoniae* growth by methanolic extracts of *Chromolaena odorata* was exposed by Nwinuka et al.,[24]. The variation in these reports can be attributed to the solvents used in extracting phytochemicals which influences the type of compounds extracted [25] and the organisms utilized in the study. Hot water will extract hydrophilic and heat stable compounds such as anthocyanins, tanins, saponis, terpenoids, lectins and starches [26]. The greater activity observed in this report when compared with the works of Anyasor et al., [21] is in conformity with the reports of [28] which showed greater antibacterial activity of hot aqueous extract than cold aqueous extracts of *Tetrapleura Tetraptera* Fruits.

Table 2 and 3 exposed the MIC of aqueous extract of *Crassocephalum crepidioides* and *Chromolaena odorata* against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Escherichia coli*. The MIC of *Chromolaena odorata* against *Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli* is 45, 30 and 30mg/mL respectively while that of *Crassocephalum crepidioides* was 45, 15 and 30mg/mL respectively.

From the results obtained in this study, *Klebsiella pneumonia* was most susceptible to *Crassocephalum crepidioides* while *Staphylococcus aureus* was most resistance producing a MIC of 45mg/mL, thus, high dosage of the antibacterial agent present in the plants extracts will be required to cure diseases caused by this microorganism.

The preliminary phytochemical analysis of *Chromolaena odorata* and *Crassocephalum crepidioides* hot aqueous extracts in **Table 1** showed the presence of flavonoids, tannins, saponins, terpenoids, phenols, steroids and alkaloids while anthraquinone and phlobatannins are absent. Result obtained from the phytochemical analysis of *Chromolena odorata* is in accord with the work of Kavitha et al. [29] and Akinmoladun and Akinloye [8]. Tannins are abundant in leafy vegetable and have been shown to possess antimicrobial [30] and antiviral activity [31] by inhibiting cell wall synthesis resulting to death [32]. Saponins are synthesized by plants to proffer protection against microbial attacks which translates to their proven therapeutic use as antimicrobials [33]. Akaloids are natural products widely utilized in the treatment of vast diseases and have been reported to possess antimicrobial activities [34, 35, 36]. Thus, the detection of tannins, alkaloids and saponnins in these extracts may be responsible for their antibacterial activity.

Table 1: Zone Of Inhibition (mm) of *Crassocephalum crepidioides* and *Chromolaena odorata*

Test Organism	*Crassocephalum crepidioides* (mg/mL)			*Chromolaena odorata* (mg/mL)		
	50	**100**	**150**	**50**	**100**	**150**
Staphylococcus aureus	13	16	18	14	16	18
Klebsiella pneumonia	16	18	21	15	18	20
Escherichia coli	10	12	16	12	14	16

Values are expressed as mean of three determinants in milliliter (mm)

Table 2: Minimum Inhibitory Concentration (MIC) values hot aqueous extract of *Crassocephalum crepidioides* against test organisms.

Test Organism	Extract concentration (mg/mL)								
	2.5	**5.0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**
Staphylococcus aureus	-	-	-	-	+	+	+	+	+
Klebsiella pneumonia	-	-	+	+	+	+	+	+	+
Escherichia coli	-	-	-	+	+	+	+	+	+

Key: + = No growth - = Turbidity/ growth of bacteria

Table 3: Minimum inhibitory concentration (MIC) values for hot aqueous extract of *Chromolaena odorata* against test organisms.

Test Organism	Extract concentration (mg/mL)								
	2.5	**5.0**	**15**	**30**	**45**	**60**	**90**	**120**	**150**
Staphylococcus aureus	-	-	-	-	+	+	+	+	+
Klebsiella pneumonia	-	-	-	+	+	+	+	+	+
Escherichia coli	-	-	-	+	+	+	+	+	+

Key: + = No growth - = Turbidity/ growth of bacteria

Table 4: Phytochemical screenings of hot aqueous extract of *Chromolaena odorata* and *Crassocephalum crepidioides*

Phytochemicals	*Crassocephalum crepidioides*	*Chromolaena odorata*
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Anthraquinones	-	-
Saponins	+	+
Phlobatannins	-	-
Terpenoids	+	+
Steroids	+	+
phenols	+	+

Key:+ = Present;- = Absent

CONCLUSION:

With respect to the results obtained, hot aqueous extract of Chromolaena *odorata* and *Crassocephalum crepidioides* leaves possess broad spectrum antibacterial activity resulting from its ability to inhibit both gram positive and gram negative bacteria utilized in the study.

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