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Antimicrobial Effect of Chromolaena Odorata (L.) and Vernonia Amygdalina Del Leaf Extract on Clinical Isolates from Infected Wound

Ekaiko, M .U[1*], Ajuga, M.U[1], Okeke, I. M[1] Arinze A.G[1] Ndulaka J.C[2]
[1,2]*Department of Microbiology/Abia State Polytechnic, Nigeria*

Abstract: *Herbal medicines has been widely used and forms an integral parts of primary health care in most developing countries today due to its effectiveness, availability and low cost. In the present study, the antimicrobial effect of the leaf extract of Chromolaena odorata (L.) King and Robinson and Vernonia amygdalina Del., (African bitter leaf) was investigated on clinical isolates from infected wound samples. The result revealed that the plant were effective in inhibiting the growth of the test organism (Staphylococcus, E. coli, Pseudomonas and Candida) from the wound samples. The plant extract showed varying zones of inhibitions ranging from 18.9 and 16.2mm for Chromolaena odorata (L.) and Vernonia amygdalina Del. Respectively for Staphylococcus spp, 14.2 and 12.8mm, for E. coli, 20.1 and 16.3mm for Pseudomonas spp and 12.9 and 10.6mm for Candida spp. respectively at 100 percent concentration. Both plant showed an MIC at 25 % except for Candida spp. which showed an MIC at 50 %. respectively for both plant. Research has shown that these plants contains various phytochemical constituents at various concentrations which may be responsible for their antimicrobial properties. The present study therefore reveals the medicinal importance of these plants as used traditionally for the treatment of various ailments.*

Keywords: *Chromolaena odorata, Vernonia amygdalina, Antimicrobial, Microbiological analysis, Minimum inhibitory concentration*

I. Introduction

The clinical success of medicinal extracts from plants and plant parts have rekindled the interest in medicinal plants as potential sources of novel drugs. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Herbal medicine has been widely used and forms an integral part of primary health care in China, Ethiopia and Argentina. A significant proportion of pharmaceutical products in current use are designed from plants. *Vernonia amygdalina* Del., (African bitter leaf) and *Chromolaena odorata* (L.) King and Robinson are primarily indigenous to Nigeria/Africa and used extensively in the management and treatment of a number of ailments like diabetes mellitus.

Vernonia amygdalina commonly known as bitter leaf (English), Oriwo(Edo), Ewuro (Yoruba), Shuwaka (hausa), and Olubu (igbo), is a tropical shrub that grows up to 3 meters high in the African tropics and other parts of Africa particularly Nigeria, Cameroon, and Zimbabwe. The leaves are dark green coloured with a characteristics odour and a bitter taste. It is reputed to have several health benefits. It is effective against amoebic dysentery, gastrointestinal disorder and has antimicrobial and anti parasitic activity [1]. *Vernonia amygdalina* is a perennial herb belonging to the Asteraceae family. The species is indigenous to tropical Africa and is found wild or cultivated all over sub Saharan African [2]. The leaves are eaten after crushing and washing thoroughly to remove the bitterness [3].

Siam weeds (*Chromolaena odorata* (L.) King and Robinson) is a perennial scandent or emiwoody shrub in the Asteraceae family. It has been used for a variety of ailments in many tropical countries for a long time, especially to stop bleeding. Numerous studies have demonstrated that Siam weed extract (SWE) accelerates hemostasis and wound healing [4]. However, the molecular targets of SWE in wound healing activity have not been identified.

Various types of wounds including injuries, cuts, pressure, diabetic, gastric and duodenal ulcers continue to exert tremendous impact on the cost of health care delivery systems all over the world and to patients and their dependents, especially in the developed countries with increasing aging population. A wound may include a breakdown in the protective function of the skin, the loss of continuity of epithelium with or without loss of underlying connective tissue following injury to the skin [5]. Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers of the skin or other organs of the body after injury. The wound healing process involves a complex series of interaction between different cell types, cytokine mediators and the extracellular matrix. The dynamic process of wound healing consist of four continuous, overlapping, and precisely programmed Phases, namely; haemostasis, inflammation, proliferation

and tissue remodeling [6]. The event of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or non-healing chronic wound. [7]

The present work aimed at evaluating the antimicrobial effect of the leaf extracts of *Vernonia amygdalina* Del., (African bitter leaf) and *Chromolaena odorata* (L.) King and Robinson on microorganism isolated from wound samples.

II. Materials and Methods

Collection of Leaf Materials/Identification

The leaves of *Vernonia amygdalina* was collected from Abia State in Abia State Polytechnic, botanical garden. The leaves of *Chromolaena odorata* (L.) R.M. King and H. Robinson was collected from Obingwa Local Government Area in Abia State.

Extraction Procedure

The extraction was carried out by using whatman No.1 filter paper method of extraction. The crude extract was filtered using sterile whatman No.1 filter paper, and the respective solvent was evaporated at 40^{o}c with the help of a heating mantle or water bath. The filtered extracts were then stored in the refrigerator at 4^{o}c.

Microbiological Analysis of Wound

Swabs of infected wound samples were collected from hospitals within Aba North Local Goverment Area of Abia State. After seeking for approval from the hospital authority, swabs of different wound of visiting patients were collected using a sterile swab stick and was transported to the laboratory of Biology/ Microbiology, Abia state polytechnic, Aba were it was analysed. Swabs of the wound were cultured in presterilized molten nutrient media, which included, Nutrient agar, MacConkey Agar, Manitol salt agar, Potatoe dextrose Agar, Eosine Methylene Blue Agar, and Salmonella/ Shigella Agar. The plates were incubated for 24hrs for bacteria and 4-5 days for fungi after which pure cultures of the respective colonies were gotten in stock bottles for further identification using the methods as described by Cheesbrouth [8].

Evaluation and Sensitivity Test, Using Agar Well Diffusion Method.

The antibacterial activity test of the crude extracts was determined in accordance with the agar-well diffusion, method described by Irobi *et al.*, (1994). The bacterial isolates were first grown in a nutrient broth for 18hours before use and standardized to 0.5 mc farland standards (10^{5}cfu/ml). Two hundred microliter of the standardized cell suspensions were spread on a Mueller Hinton agar (oxoid). Wells were then bored into agar using a sterile 6mm diameter cork borer. Approximately, 100ul of the plant extracts were dispensed into the wells and allowed to stand at room temperature for about 2hours and then incubated at 37^{0}C. Controls were set up in parallel using the solvents that were used to reconstitute the extracts. The plates were observed for zones of inhibition after 24hours. The effects were compared with those of ciprofloxacin at a concentration of 200mg/mls.

Determination of Minimum Inhibitory Concentration

The estimation of minimum inhibitory concentration was carried out using the method of Akinpelu and Kolawole [9]. Two fold dilutions of the crude extracts was prepared and 2ml aliquots of different concentrations of the solution were added to 18ml of pre-sterilized molten nutrient agar and 5dA for bacteria is 40^{0}C. The medium was then poured into sterile Petri dishes and allowed to dry under laminar flow before streaking with 18h old bacteria cultures. The plates were later incubated at 37^{0}C for 24hours and at 25^{0}C for up to 72hours for fungi, after which they were examined for the presence, or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test micrograms and also the mean value of each extract; synergy and controls drugs were observed and recorded in millimetres.

III. Result

Table 1: Mean zones of inhibitions for the effect of the ethanol extract of *Chromonella odorata* and *Vermmia amyldalina*in clinical isolate from wound sample.

Isolate	Concentrate (%)	C. *Odorata*	V. *Amyldalina*	Control
Staphylococcus	100	18.9	16.2	12.4
	50	15.1	12.2	
	25	11.3	10.1	
E. coli	100	14.2	12.8	10.4
	50	10.2	9.2	
	25	8.3	6.1	
Pseudomonas	100	20.1	16.3	17.1
	50	17.1	13.1	

	25	14.3	10.4	
Candida	100	12.9	10.6	13.0
	50	7.4	6.5	
	25	Nil	Nil	

Table 2: Mean zones of inhibitions for the effect of the aqueous extract of *Chromonella odorata* and *Vermmia amyldalina* in clinical isolate from wound sample.

Isolate	**Concentrate (%)**	**C. *Odonata***	**V. *Amyldalina***
Staphylococcus	100	12.2	10.4
	50	9.1	8.2
	25	6.2	Nil
E. Coli	100	9.3	8.4
	50	6.3	Nil
	25	Nil	Nil
Pseudomonal	100	15.5	13.3
	50	10.3	9.1
	25	8.4	6.4
Candida	100	9.0	7.2
	50	Nil	Nil
	25	Nil	Nil

Table 4: Minimum inhibitory concentration of inhibitions for the effect of the ethanol extract of *Chromonella odorata* and *Vermmia amyldalina* in clinical isolate from wound sample.

Isolate	**MIC % / Plant Extract**	
	V.amyldalina	**C. *Odonata***
Staphylococcus	25	25
E. Coli	25	25
Pseudomonal	25	25
Candida	50	50

Table 5: minimum inhibitory concentration of inhibitions for the effect of the aqueous extract of *Chromonella odorata* and *Vermmia amyldalina* in clinical isolate from wound sample.

Isolate	**MIC % / Plant Extract**	
	V. amyldalina	**C. *Odorata***
Staphylococcus	25	50
E. *Coli*	50	100
Pseudomonas	25	25
Candida	50	100

IV. Discussion

The clinical success of medicinal extracts from plants and plant parts have rekindled the interest in medicinal plants as potential sources of novel drugs. The use of medicinal plants all over the world predates the introduction of antibiotics and other modern drugs into the African continent. Herbal medicine has been widely used and forms an integral part of primary health care in China, Ethiopia and Argentina. [10] In the present the effect of *Chromonella odorata* and *Vermomia amyldalina* leaf extract on clinical isolates from wound sample was investigated, the result revealed that most wound samples harboured some pathogenic microorganism which included, *Staphylococcus, E. Coli, Pseudomonas,* and *Candida* species these findings is in agreement with the report of Mbajiuka *et al* (2014) [11] who also reported similar organism from wound sample. The occurrence of these organisms on wound surface is of public health concern as it may lead to other infections. The presence of these organisms could be as a result of improper personal hygiene among infected individual and improper care of the affected area.

The result of the antimicrobial effect of both plant extract on the isolate showed that the plants exhibited significant effect on the test isolates, showing varying zones of inhibitions. This findings can be attested to the report by other authors [11,12,1,13] who also in their study reported similar finding.

The antimicrobial activity of the extracts may be attributed to the presence of terpenoids and other secondary metabolites. Terpenoids are known to have this activity due to the possible effect on the nonmevalonate pathway. Phytochemical constituents present in the extracts may play a major role in wound healing. Flavonoids and tannins are known to promote wound healing process due to their astringent and antimicrobial activities which are responsible for the increased wound contraction and faster reepithelization. They prevent oxidative damage in wound site that could arise from production of free radicals during the inflammatory phase of the healing process and also inhibit the growth of possible microbial contaminants that may cause infection in wounds.

However, Parehk and Chanda (2007) [14] reported that tannins are known to react with protein to provide the typical tannins effect which is important for the treatment of ulcer [15]. Tannins have been found to

form irreversible complex with proline-rich protein [16] resulting in the inhibition of cell protein synthesis. Herbs that have tannins as their component are stringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery [17]. This observation therefore supports the use of *Vernonia amygdalina* (bitter leaves) and *chromolaena odorata* (L) king and robinson in herbal cure remedies.

V. Conclusion

This study showed that *vernonia amygdalina* (bitter leaves) and *chromolaena odorata* (L) king and robinson extract exerted significant antimicrobial activities against the tested clinical isolates and might be source of active antimicrobial agents for the development of drugs caused by these pathogens. However, this antimicrobial properties observed in this study could be attributed to the bioactive compounds present in the plant.

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