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Antimicrobial Activities Of Rauvolfia Vomitoria (ASOFEYEJE) Against Seleced Organisms

Balogun, Olubunmi D.

Akintunde, Suliat Labake

Department of Science Laboratory Technohology,
School of Applied Science, Federal Polytechnic Offa, Kwara State

Abstract: ***Antimicrobial activities of Rauvolfia vomitoria was Investigated against six bacteria strains of clinical origin, Pseudomonas aeruginosa, Salmonella typhi, Escherichia coli, Klebsiella, proteus vulgaris and staphylococcus aureus. Using agar dilution method. Rauvolfia vomitoria Afzel (Apocynacene) is one of the medicinal plants commonly used in folk medicine in the treatment of Psychotic disorder and hypertension. The Phytochemical analysis of Rauvofia vomitoria leaves extract revealed the presence of alkaloid, saponis, glycosides, Phenol, and Tanins. The ethanolic extract of this plant inhibited the grow of Salmonella typhi and Klebsiella pneumonia at 15,000μg/ml concentration. However aqueous extract of the plant showed no antimicrobial activity against any of the test microorganism at 15, 000μg/ml concentration. The minimum inhibitory concentration of the extracts range between 12,000 – 15, 000μg/ml concentration while for the minimum bactericidal concentration no value was obtained. The antimicrobial activities demonstrated by the extracts of this plant therefore offer a scientific basis for the traditional use of these plants in treatment of opportunistic infections that come with psychotic disorder, hypertension, fever and is a potential source for the production of drugs for the treatment of various ailments.***

I. INTRODUCTION

Rauvolfia vomitoria is a shrub or small tree up to 8m tall. The leaves grow in whorls of three and a elliptic and pointed at the end, 5 - 12cm long and 3-6cm wide. Flowers are tiny, sweet-scented, pale greenish-white, and somewhat hairy inside. The orange fruits are shaped like small balls, each containing a single seed (Kutalek and Prinz, 2007).

Railvolfia vomitoria also called serpent wood, serpent snake, root and swizzle stick, as well as, as Asofeyeje in Yoruba, Ira in Hausa, Akata in Bini, Mmoneba and Utoenyin in Efik, is found in the forest of the Southern Part of the Nigeria (Kutalek and Prinz, 2007).

The plant *Rauwolfia vomitoria* belongs to the family, it is mostly found in the forest part of southern Nigeria. The plant is also called serpent wood, serpent snake root and swizzle stick in Hausa, Akata in Bini and Mmoneba and Utoenyin in Efik (Ehiagbonare, 2007).

Africa is a continent endowed with an enormous wealth of plant resources. Over 5,000, different species are known to occur in the forest regions alone, and most of them have been used for several centuries in traditional medicine for the prevention and treatment of diseases. African plant does not consist of dietetics alone, but include many potent herbs (FapoJuwoni and Asinwa, 2013).

The use of traditional botanic knowledge as a promising instrument in bio prospecting of useful plant for human and animal medicine has recently increased. This result in ethnomedical and medical ethnobotanical research methods and techniques which contribute to validation and development of new plant based drugs. These have called for preview of our traditional herb especially *Rauvolfia vomitoria* (Fapojuwomi and Asinwa, 2013).

Rauvolfia vomitoria occurs naturally in forest but is mostly found in forest regrowth where fallow periods are prolonged. *Raulvolfia vomitoria* is associated with, palms, Tremagileenesis and combretum species and is one of the last species to disappear in this particular several stage. It belongs to the family of Apocynaceae, its commmno names are swizzle stick (Fapojuwomi and Asinwa, 2013).

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Rauvolfia vomitoria is a widely planted as ornamental plant, it is used as fire wood for instance is Sierra Leone. The bark yields a good fibre, and yellow dye is obtained from the bark. The seeds are used in making decorative necklaces. The sweet scent of RauvolfiaVomitoria flowers are frequented by bees. It is also used as timber (Fapojuwomi and Asinwa, 2013).

II. MATERIALS AND METHOD

COLLECTION AND IDENTIFICATION OF PLANT MATERIALS

The leaves of *Rauvolfia vomitoria* were obtained from Owode Market, Offa, Kwara State, Nigeria. The plant was the duely authenticated by Mr. Prince Olanipekun. N.O. an - ethno- botanist of the Department of Science Laboratory Technology, Federal Polytechnic Offa, Kwara State, Nigeria.

PREPARATION OF THE PLANT SAMPLES

The plant sample was carefully taken to the laboratory in sterile polythene bag. It was then sun dried for about 5 days after which dried sample was crushed into fine powder with a mortar and pestle.

EXTRACTION OF PLANT SAMPLE

The extraction of the plant material was carried out using ethanol and distilled water as extracting solvent. The cold maceration extraction method of Cowan (1999) was used. One hundred grams (l00g) of the sample (*Rauvolfia vomitoria* leaves) was extracted by soaking in 400ml of the extracting solvents for 24hours and 48hours for distilled water and absolute ethanol respectively. The resulting mixtures were filtered out separately with a Muslin cloth and the filtrates were evaporated to dryness with steam bath. The dried aqueous and ethanolic extracts were stored in sterile containers at 4°c unit required for further use.

SOURCE OF MICROORGANISMS

The microorganisms used for this study were *staphylococcus aureus* (clinical strain), *Salmonella typhi* (clinical strain), *Klebsiella pemtmoniae* (clinical strain), *Escherichia Coli* (clinical strains), *pseudomonas aeruginosa* (clinical strains).These organisms were obtained from Microbiology unit, University of florin Teaching Hospital. Ilorin, Kwara State. Nigeria.

CONFIRMATION AND IDENTIFICATION OF BACTERIAL ISOLATES

The bacterial isolates were cultured on nutrient agar and incubated at 37°C for 24hours and subsequently characterized by examination of colonical morphology by sub-culturing on to differential selective media such as Desoxy cholocate citrate agar, Brilliant green agar, Eosin Methylene blue agar and mac-conked agar. The colonies from various media were grain stained and further characterized by some biochemical test which includes catalase, coagulase, indole, oxidase and citrate utilization tests.

The organisms were finally identified by comparing their characteristics to that of known taxa using scheme of cheesbrough (2004).

STANDARDIZATION OF MICROORGANISMS

0.1ml of 1% Barium chloride was added to 9.9ml of 1% surphuric acid which was later reconstituted into 10ml of sterile distilled water to make 0.5ml MC farland standard solution. The both culture of the test organism was then compared in terms of turbidity to 0.5% MC farland. A loopful of the standardized culture was used for antimicrobial assay.

DETERMINATION OF ANTIMICROBIAL ACTIVITY OF EXTRACTS

To test for antimicrobial activity of the plant extracts, agar dilution method of Babayi et al, (2004) was employed 1g of aqueous extract was reconstituted in 5ml of sterile distilled water and vortexed for homogenecity. 1ml of reconstituted *Rauvolfia vomitoria*, aqueous extract was added to petri-dishes containing 19ml of sterile molten nutrient agar to make a final concentration of 200mg/ml. The plate was prepared in duplicate and allowed to set at room temperature. A loopful of standardized test organism was streaked on the solidified agar with the incorporated extract and incubated for 24hours at 37°C. The ethanolic extract was equally treated. Control agar plates were made in parallel and included OVC (organism viability control), MSC (Medium Sterility Control) and BSC (Extract) Sterility Control)

SCREENING EXTRACTS FOR PHYTOCHEMICAL COMPONENTS

The phytochemical screening was conducted on the plant material *(Rauvolfia vomitoria* leaves ethanolic extract) that showed antimicrobial activity. The screening was conducted to confirm the presence or absence of various secondary metabolic (Alkaloids, Glycosides, Saponins, Tannin etc.) in the plant materials. A modified method of Lajubutu *et al.* (1995) was used.

III. RESULTS

ANTIMICROBIAL ACTIVITY OF AQUEOUS EXTRACTS

The result of antimicrobial activity of aqueous extract of Rauvolfia vomitoria leaves were shown in Table 1. The result revealed that the aqueous extract of Rauvolfia Vomitoria leave displayed no antimicrobial activity against any of the test microorganism at 200mg/ml concentration.

ANTIMICROBIAL ACTIVITY OF ETHANOLIC EXTRACT

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The result of antimicrobial activity of ethanolic extract of Rauvolfia vomitoria leaves were shown in Table 2. The result revealed that the ethanolic extract of Rauvolfia vomitoria leaves inhibited the growth of Salmonella typi and Klebsiella pneumonia while Staphylococcus aureus, E.coli, Psuedomonasueroginosa and Proteus vulgaris were resistant to the same extract at 200mg/ml concentration.

Organisms	Results
Staphylococcus	-
Salmonella typhi	-
KlebsiellaPneumoniae	-
Escherichia Coli.	-
Proteus Vulgaris	-
Pscudomonas aeruginosa	-

Table 1: Antimicrobial activity of aqueous extract of Rauvolfia vomitoria leaves of 200mg/ml concentration

Organisms	Results
Staphylococcus	-
Salmonella typhi	+
Klebsiella Pneumoniae	+
Escherichia Coli.	-
Proteus Vulgaris	-
Pseudomonas aeruginosa	-

Key:
- = No activity
+ = Activity

Table 2: Antimicrobial activity of ethanolic extract of Rauvolfia vomitoria leave of 200mg/ml concentration

MINIMUM INHIBITORY CONCENTRATION OF ETHANOLIC

EXTRACT OF RAUVOLFIA VOMITORIA

The results of Minimum inhibitory concentration of the ethanolic extract of Rauvolfia vomitoria on the test organism that were sensitive to the same extract during, determination of antimicrobial activity is shown in Table 3. The results revealed that Salmonalla typhi and Klebsiella pneumoniae were inhibited by Rauvolfia vomitoria leaf aqueous extract at I20mg/ml concentration.

Extract Test	Concentration of Extract (ug/ml)								
Organisms	200mg/ml	180mg/ml	160mg/ml	140mg/ml	120mg/ml	100mg/ml	80mg/ml	60mg/ml	40mg/ml	20mg/ml
RVE Salmonella typhi	+	+			+	+	+*	-		-
Klebsiella Pneumoniae	+	+	+	+		+*	-	-		

Key:
+=Activity
-= No Activity
** = Minimum Inhibitory Concentration*
RVE= Raitvoljia vomitoria Ethanolic Extract

Table 3: Minimum Inhibitory Concentration of Rauvolfia vomitoria leaves ethanolic extracts (ug/ml).

MINIMUM BACTERIOCIDAL CONCENTRATION OF AQUEOUS AND ETHANOLIC EXTRACT

The result of minimum bacteriocidal concentration of ethanolic extract of *Rauvolfia vomitoria* leaves on the test organisms that were sensitive to the same extract during determination of minimum inhibitory concentration is show in table 4. The result revealed that sensitive test organisms which include *salmonella typhi* and *klebsiella pneumonia* were only inhibited and not killed by the extracts at the concentrations used.

ExtractTest	Concentration of Extract (ug/ml)											
Organisms	200mg/ml,	60mg/ml	140mg/ml,	120mg/ml,	100mg/ml,	80mg/ml,	/ml,	40mg/ml,	20mg/ml
RVE	+	+	+	+	+	+	+	+
Salmonella Typhi		+							
Klebsiella Pneumoniae	+	+	+	+	+	+	+	+	+	+	+	+

Key:
+ = Growth
RVE = Rauvolfui vomitoria Ethanolic Extracts

Table 4: Minimum Bacteriocidal Concentration of Rauvolfia vomitoria leaves Ethanolic Extract (ug/m l)

PHYTOCLIENIICNL SCREENING OF ETHANOLIC EXTRACT OF RAUVOLFIA VOMITORIA LEAVES

The result of phytochemical screening of ethanolic extract of *Rauvolfia vomitoria* leaves was shown in Table 5. The result shows the Tannins, Saponins, Alkaloids, Phenol and Glycoside were found present.

Phytochemical Components	Results
Tannins	+
Saponins	+
Phenol	+
Alkaloids	+
Glycosides	+

Key:-
- = Absent
+ = Present

Table 5: Phytochemical Screening of Ethanolic Extract of Rauvolfia vomitoria leaves

IV. DISCUSSION

The results obtained from the study revealed that ethanolic extract of *Rauvolfia vomitoria* leaves contained bioactive agents that contain antimicrobial properties against bacteria. This work revealed that the ethanolic leaves extract of *Ravolfia vomitoria* at 200mg/ml concentration showed effective antimicrobial activities which also confirmed their inhibitory effect on *Salmonella typhi* and *Klebsiella pneumoniae.* (Jantsch, 2011)

However, this work revealed that aqueous extract of *Rauvolfia vomitoria* leaves have no activity against all the test isolates at 200mg/ml concentration. This may be due to various factors which include: time of collection of the plant materials, geographical location of the plant sample collected concentration of the extract used. It may also be due to the fact that the active component of the plant materials might have been denatured during the cause of extraction or that the extract has been sufficiently diluted to the extent that the active ingredients of the extract have been impaired. The resistance of the clinical strain of all the test isolates to both-

aqueous extract of *Rauvolfia vomitoria* leave could also be traced to the resistance of the test microorganism which could also be as a result of misuse of antibiotics by patient(s) from whom the organism where isolated (Fapojuwomi, 2013)

The minimum inhibitory concentration of the extract was 120mg/ml concentration respectively. The low minimum inhibitory concentration (MIC) value of *Rauvolfia vomitoria* leaf ethanolic extracts at 120mg/ml concentration or-cerved for salmonella typhi and *Klebsiella pnemonia is* good indication of high efficacy against these bacteria. However, no value was obtained of the minimum bacteriacidal concentration for any of the extracts against the test organism. This indicates that the plant extracts is bacteriastatic and not bacteriacidal at the concentration used (20mg/ml – 200mg/ml

The phytochemical screening of the ethanolic extract revealed the presence of Tannins, Saponin, Glycosides, Phenol and Alkaloids. These secondary metabolites are the bases for any bioactivitics display by the plant material against any test isolates.

This study suggest that the extract of *Ranvolfia vomitoria* inhibitory effects againsts some of the test organism 200mg/ml concentration. The successful inhibition of these bacteria is a good development, especially when we consider the records of multi-resistance to various conventional antibiotics by bacteria over the years (Anijibuwo, 2009)

The result of phytochemical screening of chemical constituents of *R.vomitoria* extracts under study on qualitative basis revealed the presence of active compound in the ethanolic extract. As shown in table 5, the ethanol extract indicate the presence of alkaloids, saponins, tannins, glycosides and phenols.

The analysis of the plant extract revealed the presence of phytochemical which are known to exhibit medical and physiological activities. For example, Tannins are plypenolic compounds that bind to proline rich protein that interfere with protein synthesis and has shown to have antibacterial activity.

The activity has been attributed to their ability to form complexes with extracellular and soluble proteins and bacterials cell walls.

Saponins and glycosides have been found to have inhibitory effects on gram positive organisms. Therefore, the phytochemical analysis revealed that the ethanol extract has chemical compounds that have been found to possess antibacterial activities which could contribute to the results obtain from antibacterial analysis. The result of the study as shown in table 2, revealed only the ethanol crude solvent extracts prepared from the leaves of *Rauvolfia vomitoria* showed inhibitory activity against bacteria. Only gram-negative bacteria, *Salmollena* and *Klebsiella* were susceptible to the extract, while neither of the gram. Negative bacterium showed any inhibition a 200mg/ml, the ethanol extract showed antibacterial activity against *Salmonella* and *Kelbsiella* respectively. The resistance of the gram negative bacteria to the aqueous extract could be attributed to its cell wall structure. Gram-negative bacterial have an effective permeability barrier, comprised of a thin lipopolysaccharide. Exterior membrane which could restrict the penetration of the extruding plant extract. It has been reported earlier that Gram-negative bacteria are usually more resistance to the plant-origin antimicrobial and even show no effect compared to Gram-positive bacteria Gram positive bacteria has a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Usman, 2009).

This study provides scientific insight to further determine the antimicrobial principle and investigate other pahamacological properties of *Rauvolfia vomitoria.* On the basis on the present findings, *Rauvolfia vomitoria* leaves possess the capabilities of being a potential source in the search for a natural antimicrobial agent against infection and/or diseases caused by *Salmollena* and *klebsiella.*

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