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Comparative Study on Phytochemical Quantification and Antimicrobial Activity of *Raufolvia Vomitoria* Leaves, Seeds and Root Extracts

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ABSTRACT

One of the world medical problems is the challenge of bacteria and fungi resistance to commercial antibiotics. Recently some organisms such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterococcus faecalis* to mention but a few have developed drug resistance. This study aimed to quantitatively determine the phytochemical constituents of *Raufolvia vomitoria* seed, leaf and root. It further aimed to evaluate the antimicrobial effect of the ethanolic extract of seed, leaf and root against some selected bacteria (*Bacillus subtilis, Bacillus cereus, Pseudomonas syringine, Pseudomonas aeurginosa, Escherichia coli, Xanthomonas axonopodis and Staphylococcus aurus*) and fungi (*Cyrtomium falcutum, Rhizoctonia* solani, *Colletotrihum lindematianum, Trichophyton rubrum*). Phytochemical analysis was determined using standard procedures and antimicrobial activity was performed by agar well diffusion. The result revealed that the root had the highest alkaloids, phenols,phytates and saponins contents of 15.72 %, 1.24%, 1.66% and 5.99% respectively. The seed had the highest flavonoid contents of 9.38 and the leaf recorded the highest oxalates content of 0.17 %. The result revealed that all the ethanolic extracts are potent against the test microbe. Inhibition zones of the extract against bacteria ranged from 1.20 mm to 10.00 mm while the mycelia inhibition against fungi ranged from 4.76 mm to 26.98 mm. The research demonstrates that extracts from the seed, leaf and root of *Raufolvia vomitoria* could be used for production of broad pharmaceutical and medicinal based products.

Keywords: *Rauvolfia vomitoria*, phytochemical, bacteria, fungi, Extract and Medicinal

INTRODUCTION

One of the world medical challenges is the current trend of multi drug resistance by bacteria and fungi. Recently some organisms such as *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli*, and *Enterococcus faecalis* to mention but a few have developed drug resistance WHO, (2004) in Njau et al., (2014). Antibiotic resistance has not only increased human mortality rate but also contributed to increase in poverty level, because huge amount of money is being deployed into disease prevention and cure (Njau et al., 2014).

Natural drugs are the early source medicine for human being and extracts from plant's parts (seed, root, flower, stem and bark) have been used for curing various ailments in the time past. Although, the advent of modern medicine has affected the development of local herbal medicine, notwithstanding plant materials are still being used to greater extent for combatting illnesses and various diseases, and researches for novel plant extracts and isolates for the development of new medicine is the focus of many researchers (Ozusaglam and Karakoca, 2013).

Ethnobotanical medicine provides alternative sources of new drugs, an example is *Rauvolfia* species most found in sub-Sharan Africa and it is an important medicinal plant. They have been effective in the treatment of malaria, diabetes, and both parasitic and microbial infections (Amole et al., 1998; Campbell et al., 2006; Pesewu et al., 2008; Zirihi et al., 2005). In particular, *Rauvolfia vomitoria* has been used in the treatment of coughs, gastrointestinal disturbances, skin infections, hypertension, impotence, insomnia, diarrhea, dysentery, scabies, worm infections and malaria (Odugbami, 2008; Oyedeji, 2007). The chemical components of this plant include indole alkaloids (Amer and Court, 1980; Katič et al., 1980) . R. *vomitoria* root contained high concentration of alkaloids; majorly reserpine and ajmaline. These alkaloid's pharmacological abilities include antimalarial, antitumor and antidiabetes (Dewick, 2002; Katič et al., 1980). Reserpine and ajmaline have good antioxidant activities, thus this plant is capable of reducing risks of certain type of degenerative diseases (Erasto et al., 2007; Mazza et al., 1999).

To the best of our knowledge there is no research on the quantitative determination of the root, leave and seed of *Raufolvia vomitoria*, neither is there any reported comparative antimicrobial effect of their ethanolic extracts. The study is therefore aimed at comparing the quantitative phytochemical and antibacterial activity of ethanolic extracts leaves, seeds and root of *Raufolvia vomitoria against* some bacteria and fungi.

MATERIALS AND METHODS

Plant sample preparation

The seeds, leaves and roots of *R. vomitoria* were obtained from Agbani farm land in Agbado-Ekiti, Gbonyin Local Government Area, Ekiti State, Nigeria. All the samples were authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Ondo State, Nigeria. All samples were cleaned to remove dust, sand, dirt and contaminations. The materials were then dried at (27±1^{o}C) room temperature. The samples were pulverized and sieve range from 212-249µm was used.

Preparation of extract from seed, root and leaves of *R. vomitoria*

About 50 g plant sample was extracted using 95 % analytical grade ethanol (500 mL) in soxhlet extractor for 8 h. After 8 h the extract was concentrated by exposing the solvent to the air for drying.

Quantitative phytochemical analysis

The dried powdered samples of the root, seed and leaf of the plant were subjected to quantitative phytochemical analysis using existing methods and the following were determined; alkaloid (Sathya et al., 2013), total phenol (Hussain et al., 2011), flavonoid (Boham and Kocipai, 1994), phytate (Olajide et al., 2011), oxalate (Oladele et al., 2009) and saponin (Obadoni and Ochuko, 2002).

Antibacterial activities of the extracts

The antimicrobial activity of the extracts was determined using the agar-well diffusion method of (Murray et al., 2006). 20 mL of nutrient agar was dispersed into sterile petri dish and inoculated. The plates were allowed to set and well of 6mm was properly bored using sterilized cork bore. The wells were filled with the extract of 0.01 mg/ mL. The plates were incubated at 37^{o}C for 24h. The zones of inhibition were measured with Vernier calipers in mm and recorded appropriately. The experiment was done in triplicate and mean values reported. Streptomycin sulphate and ciprofloxacin were used as positive control.

Antifungal activities of the extracts

Poisoned food techniques (Mishra et al., 2012) was the method of choice for this investigation, 0.01mg/ml concentration of the samples were prepared and thoroughly mixed separately with 20 ml of potatoes Dextrose agar (PDA) in a sterile sample bottle before pouring in a plate. For the control sets, distill water was used instead of the sample as negative control and 0.01mg/ml ketoconazole was used as positive control. The poured plated were inoculated aseptically with a 6mm fungal disc of 72hrs old of pure culture of the test isolates and incubated between 25-27^{o}C for 72hr for 5days. The observations were recorded at the end of the

incubation period. Percentage mycelial growth inhibition was calculated by following formula;

Percentage growth inhibition = $\frac{NTR-TR}{NTR} X\ 100$

Where, NTR= average diameter of fungal colony in negative control set (without treatments)

TR= average diameter of fungal colony in treatment sets

The experiments were carried out in triplicate and the average values recorded was as shown in the table below

Statistical Analysis

The data obtained in triplicate were analysis by Probit Analysis using Duncan's Multiple Range Test (DMRT) and Analysis of Variance (ANOVA)

RESULTS AND DISCUSSION

Preliminary phytochemical screening of the leaf, stem and root of *Rauwolfia vomitoria* according to (Adebayo et al., 2014) revealed the presence of alkaloid, phenols and flavonoids, The results of the quantitative phytochemical analysis of the root, leaf and seed of *Rauwolfia vomitoria* are presented in Table 1. The analysis reveals the presence of alkaloids, flavonoids, phenols, phytates, oxalates and saponins to different degree. The high alkaloids content (15.72 %) in the root was in accordance with findings of others, that had found that the root has high alkaloid content (Dewick, 2002; Katič et al., 1980). The seed has 14.90 % and leaf has 3.16 % alkaloids content. The high alkaloids content of the root and seed might be because those parts serve have storage faculty of the plant (Benbott et al., 2012).

whereas (Ojo et al., 2012), presented the alkaloid content (2.21, 2.23 and 3.39 %), flavonoids (0.37, 0.39 and 2.18 %), oxalates (3.69, 3.78 and 3.06 mg /g) and polyphenols (2.10, 1.91 and 1.35) respectively in aqueous, ethanol and methanol extracts of the back of *Rauwolfia vomitoria*. The results of these authors confirmed the presence of various phytochemicals determined in this study, although different results were obtained. This was expected because of the plant materials were not obtained at the same place, seasonal variation could also affect the result likewise some laboratory experimental errors.

In our study the flavonoids contents are 9.38 % (seed), 8.60 % (leaf) 7.56 % (root), showing that the seed has highest content and this may be attributed to the reddish color of the seed. The root had the highest phenols 1.24 %, while 0.32 % and 0.08 % were recorded for the leaf and seed. The high phenolic content of the root might be because they act as pest and disease resistance agent for the effective growth of the plant (Li et al., 2010) .

Phytates salts of phytic acid, have been implicated to reduced absorption of dietary minerals both in human and animal (Konietzny and Greiner, 2003; Lopez et al., 2002). Consumption of phytates rich food, however has greater health benefit than the negative effect of reduced dietary mineral absorption. Dietary phytates has been reported to have medicinal value to prevent and protect kidney stone formation (Grases et al., 2000), atheriosclerosis and coronary heart disease (Jariwalla et al., 1990) and a variety of cancers (Vucenik and Shamsuddin, 2003) . Therefore, the moderate phytates recorded in this research 1.66 %, 1.52 % and 0.48 % respectively for the root, leaf and seed would be of health benefit to both human and animals for phytates supplement. The percentage oxalates recorded the lowest value of the phytochemical. The seed had 0.00 %, root has 0.08 % and the leaf 0.17 %. The results seem encouraging knowing fully well that the plant is being used for various medicinal purposes and the low oxalates content would reduce the risk of calcium oxalate urinary stone formation. The root contained the highest percentage (5.99 %) of saponins, followed by the leaf (4.80 %) and then the seed (4.56 %).

The antimicrobial results of the crude ethanolic extracts of the root, seed and leaf *R. vomitoria* are as presented in Table 2 and 3 at 0.01 mg/mL extract and control concentration. As presented in Table 1 the determined chemical components of the plant suggest presupposes antibacterial activities of the plant parts. The result indicated that those extracts were active against the entire microbes with zones of inhibition that varied from 4.76 to 26.56 mm for fungi. The antimicrobial results proof the potency of the extracts with broad-antibacterial activities as

they are active against both Gram-positive and Gram-negative bacteria. Their extracts are active against pathogenic bacteria such as *E. coli, Bacillus cereus, B. subtilis, S.aureus, X. axonopodis, P.syringine* and *P.aeuru* with zone of inhibition ranging from 1.50 to 10.00 mm. Although, all the control used were much more active than the crude plant extracts, the result shows that the fungi were more susceptible to the plant extracts than the bacteria

Table 1- Quantitative Phytochemical of *Raufolvia vomitoria*

Phytochemical Compounds	Seed	Leaf	Root
Alkaloids (%)	$14.90^{b} \pm 1.02$	$3.16^{a} \pm 0.28$	$15.72^{b} \pm 0.68$
Flavonoids (%)	$9.38^{c} \pm 0.06$	$8.60^{b} \pm 0.02$	$7.56^{a} \pm 0.37$
Phenols (%)	$0.08^{a} \pm 0.38$	$0.32^{a} \pm 0.16$	$1.24^{b} \pm 0.21$
Phytates (%)	$0.48^{a} \pm 0.01$	$1.52^{b} \pm 0.04$	$1.66^{c} \pm 0.02$
Oxalates (%)	$0.00^{a} \pm 0.02$	$0.17^{b} \pm 0.03$	$0.08^{c} \pm 0.01$
Saponins (%)	$4.56^{a} \pm 0.84$	$4.80^{a,b} \pm 08$	$5.99^{c} \pm 0.80$

Values are means of three replicate ± standard deviation.Column means followed by different letters are significantly different at $P< 0.05$

Table 2- Zones of inhibition (mm) of the extracts obtained from root, seed and leaf of *R. vomitoria* at 0.01 mg/mL

Sample	*B.subtilis*	*X.axonopodis*	*B.cereus*	*P.syringine*	*P.aeruginosa*	*E.coli*	*S. aureus*
RVS	3.00	4.00	6.00	4.00	5.00	3.00	7.00
RVL	2.00	7.00	5.50	10.00	3.25	7.50	8.00
RVR	1.50	3.00	3.50	3.00	1.45	1.20	4.00
Streptomycin	26.00	11.50	15.00	14.50	15.00	15.00	16.00
Ciprofloxacin	17.00	12.00	15.00	28.00	20.00	26.00	24.00

Where, RVS is *Raufolvia vomitoria* seed, RVL *Raufolvia vomitoria* leaf and RVR is *Raufolvia vomitoria* root

Table 3- Percentage growth inhibition of extracts obtained from root, seed and leaf of *R. vomitoria at* 0.01 mg/mL

Sample	*C.falcutum*	*R. solani*	*C. linalimutianum*	*T. rubrum*
RVS	4.76	17.95	20.15	18.75
RVL	26.98	9.62	34.33	21.56
RVR	25.40	17.30	13.73	26.56
Ketoco	60.00	85.00	53.73	88.88

Where, RVS is *Raufolvia vomitoria* seed, RVL *Raufolvia vomitoria* leaf and RVR is *Raufolvia vomitoria* root

Ketoco= Ketoconazole (a standard antifungal agent)

The high percentages of alkaloids, saponins, and flavonoids in the root, seed and leaf of R. *vomitoria and* moderate presence of phytates, phenols and oxalates could make the plant a good source of ethnobotanical medicine. According to Vignesh and Chandra, (2015), alkaloid in *Rauvolfia canescens* leaf was attributed to its antibacterial potency, while Sonibare et al., (2011) revealed that alkaloids and saponins in *Rauvolfia vomitoria* leaf

conferred on it antimicrobial activities. Also, Mert-Türk, (2006) stated that phytates, phenols saponins and alkaloids and some other secondary metabolites in plants play important role in plant defense against pathogens. The natural protection role of these phytochemicals against plant pathogens could be harnessed for medicinal and pharmaceutical benefit of human being. The results obtained in this study were in agreement with antimicrobial potency of plant materials using *Rauvolfia vomitoria* leaf (Sonibare et al., 2011), *Rauvolfia canescens* leaf (Vignesh and Chandra, 2015) and using stem, leaf and root extracts of *Rauvolfia vomitoria* against some pathogens, although with the use of different pathogen (Adebayo et al., 2014).

CONCLUSION

The broad antifungal and antibacterial activities of the plant could be linked to the presence of phytochemicals in the plant. Therefore, in the face of microbes resistances to modern drugs, isolation, purification and chemical functionalization of phytochemical presence in this plant could be a good solution to the failure witnessed in some drugs during diseases treatment.

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