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PHYTOCHEMICAL BASED STRATEGIES FOR PATHOGEN CONTROL AND ANTIOXIDANT CAPACITIES OF *RAUWOLFIA SERPENTINA* EXTRACTS

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Abstract

Rauwolfia serpentina (Apocynaceae) is used among rural Indian communities to treat arthritis, skin cancer, burns, eczema, psoriasis, digestive problems, high blood pressure, sedative and diabetes, despite very little supporting scientific evidence. Due to increased interest by both the scientific community and industry regarding the medicinal uses of this plant species, we identified, quantified and compared the phytochemical contents and antioxidant capacities of extracts of *Rauwolfia serpentina.* Apart from extensively characterizing this medicinal plant with regards to its organic acid, polyphenols/phenolic acid, alcohol, aldehyde, ketone, alkane, pyrimidine, indole, alkaloid, phytosterol, fatty acid and dicarboxylic acid contents and antioxidant capacities, we describe a modified extraction procedure for the purpose of general phytochemical characterization, and compare this to a aqueous ethanol extraction technique. From the results it is clear that *Rauwolfia serpentina* contains a variety of compounds with confirmed antioxidant capacity and other putative health benefits relating to the prevention or treatment of diabetes, cardiovascular disease, cancer and hypertension. The results also indicate that separate extractions of the Leaf extracts, better serve for general phytochemical characterization purposes, hence justifying its use for biological *in vivo* efficacy studies.

Key Words: *Rauwolfia serpentine*; Phytochemical content; Antioxidant Capacity; Polyphenols; Phytosterols.

Introduction

Rauwolfia serpentina L. Benth. ex. Kurz (family: Apocynaceae) is a woody perennial shrub, commonly known with different names; sarpagandha, snake root plant, chotachand, chandrika, etc. The roots of this plant have been used for centuries in ayurvedic medicines under the name sarpagandha and nakuli for the treatment of mental disorders. It has been stated that the drug is useful in mental disease, epilepsy, sleeplessness and several other ailments (Ojha and Mishra, 1985). Due to its medicinal values, the root of this plant has been popular both in India and Malaya-peninsula, from ancient times as an antidote to the stings of insects and poisonous reptile. It has also been used as febrifuge and stimulant to uterine contraction for insomnia and most of all for insanity (Vakil, 1949).

Accumulated evidences indicate that free radicals involve in the pathophysiology of various diseases such as diabetes, cancer and cardiovascular diseases [Weisburger, J.H., 2002]. The antioxidant compounds can neutralize free radicals, thus playing an important role in the prevention of these diseases [Niki. E et al., 2005]. In recent years, many studies evidenced that plants containing high content of antioxidant phytochemicals can provide protection against various diseases [Urquiaga, I. and F. Leighton, 2000]. The free radical scavenging activity of these phytochemicals is predominantly determined by their structures [Bravo, L., 1998]. In particular, phenolic phytochemicals have antioxidative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory activities [Arts, I.C. et al, 2005 and Scalbert, A et al 2005]. The antioxidant and antimicrobial properties of various plants have been reported by several studies [Shirwaikar, A et al., 2004, Annie, S. et al., 2003, Prashanth kumar, V et al., 2000, Latha, M et al., 2006]. In recent years the popularity of complementary medicine has increased. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India [Warier, P.K., 1995]. The World altitude Health Organization has also recommended the evaluation of the plants effective in conditions were safe modern drugs are lacking

[FAO/WHO, 1980]. Recently an intensive search for novel types of antioxidants has been carried out from the numerous plant materials.

It is hypothesized that knowledge of the phyto constituents of *Rauwolfia serpentina* L. would provide an insight into its biological functions beyond nutrition when consumed. In the present study, extracts of *Rauwolfia serpentina* L. was phytochemical analysed and tested for antioxidant function *in vitro*. The antimicrobial activity of these extracts was also examined.

Materials and Methods

Plant material

Fresh plants of *Rauwolfia serpentina* L. were collected from the Kolli Hills, Tamilnadu, India. They were identified at the Department of Botany. The leaves were carefully removed from the plants were weighed then dried at 40^{o}C for 24 h. The dried leaf samples were weighed and ground into powder prior to extraction.

Preparation of Rauwolfia serpentina extracts

Dried samples of leaf powder (3.5 g each) were separately extracted with 100 ml each of petroleum ether (20 – 80^{o}C) and 80% acetone for 2 h using soxhlet apparatus. The residual solvent was removed by evaporation at 40^{o}C for 24 h *in vacuo* using a rotatory evaporator. The resulting organic extracts were further reconstituted to different concentrations (0 – 100% v/v) with 0.1% Tween-20 in phosphate buffered saline (pH 7.2) followed by storage in sterile capped bottles under refrigeration condition (4^{o}C) prior to use for subsequent assays.

Phytochemical analysis of Rauwolfia serpentina extracts

The phyto constituents present in the organic extracts were determined qualitatively according to Sofowora (1993), Trease and Evans (1989) and Harbone (1973) as well as by thin layer chromatography (TLC). In TLC, the extracts spotted on silica coated plates, were developed using butanol-glacial acetic-water (100 : 10 :10) as the solvent system. The developed plates were then sprayed with with vanillin solution (1% (w/v) in 50% phosphoric acid) for steroid detection, Dragendorff's reagent for alkaloid detection and sodium metaperiodate (0.1%) followed by ethanolic benzidine for glucose detection. Nicotinic acid, cholesterol D-glucose and tannic acid at 1% solution were prepared accordingly and used as standards in the TLC assay. The TLC results were further used to validate the presence of tannins based on positive reaction (brownish green – blue black coloration) with 0.1% FeCl3, alkaloids based on positive reaction (brown coloration) with Dra-gendorff's reagent (Trease and Evans, 1989; Sofowora, 1993), steroids based on positive reactions (violet to blue or green) with acetic anhydride and H2SO4, steroidal glycosides by Keller-Killani test and cynogenic glycoside based red coloration of picrate paper (Harbone, 1973; Trease and Evans, 1993). The observation of persistent frothing in distilled water (2 ml) by 1% standard saponin solution (3 ml) followed by formation of emulsion with olive oil (0.5 ml) was used to indicate the presence of saponin in the extract (Trease and Evans, 1989).

Total phenolic content determination

The total phenolic compounds present in the extracts were determined spectrophotometrically at 750 nm as described by Lee et al. (2001) using Folin-Ciocalteau phenol reagent. Standard solutions of gallic acid (20 – 200 mg/ml) were used to prepare a standard curve. The total phenolic contents of the extracts are expressed in milligrams per ml of gallic equivalents (GAE) of triplicate measure-ments.

Antioxidant activity determination

The antioxidant activity of the *Rauwolfia serpentina* L. extracts was determined via the 2,2 – diphenylpicrylhydrazyl radical neutralization assay as described by Brand-Williams et al. (1995). Extracts were also tested as antioxidants using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS) assay (Cano et al., 1998). In both assays, 50 µl of each extract was added to with 950 µl of the free radical methanolic solution and stirred continuously for 15 min for DPPH or 10 min for ABTS. Decreases in absorbance were respectively measured at 414 and 515 nm for ABTS and DPPH systems, respectively, and compared to that of infusion made from green tea a well-known antioxidant food (Cao et al., 1996). The infusion was prepared by boiling 1 g green tea bag with 100 ml of deionised water for 5 min. Antioxidant activity of the each extract was expressed as vitamin C equivalent antioxidant capacity (VCEAC) in millimolar (mM) by extrapolation from the standard curve. Two – fold serial dilutions of Lascorbic acid (Aldrich, Germany) in deionised water to a final concentration range of 0.75 – 6.0 mM were used to prepare the calibration curve.

Microorganisms

The microorganisms to which the antimicrobial properties of the organic extracts of *Rauwolfia serpentina* L. were tested were obtained from Biogeneic Research Laboratory, Namakkal, India. The bacterial isolates were multidrug resistant determined by disc diffusion assay

according to Iwalokun et al. (2004) (Table 1). They were recovered as viable isolates by subculturing their stock cultures in 16% glycerol- trypticase soy (TSB) or brain heart infusion broth (BHI) at –20°C or malt extraxt medium. The lactobacilli were recovered by growth in Mann Rogossa Sharpe (MRS) medium under anaerobic conditions (De Mann et al., 1960). Other bacterial isolates were recovered by growth on trypticase soy agar or blood agar under aerobic conditions at 37°C for 24 h. The recovered microbes were gram stained to confirm their gram reaction status and speciated according to Cowan and Steel (1974). The gram-negative organisms include *Escherichia coli, Salmonella typhi, Pseusomonas aeruginosa, Streptococcus mutans*, while the gram-positive isolates were *Bacillus licheniformis, Bacillus subtilis, Staphylococcus aureus,* and *Lactobacillus acidophilus*

Antimicrobial testing of Rauwolfia serpentina L. extracts

Rauwolfia serpentina L. extracts were tested for antimicrobial activity by agar well diffusion technique (Akpata and Akinrimisi, 1977) with a little modification. An overnight culture of each microbial isolate was emulsified with nutrient broth to a turbidity that was equivalent to 0.5 McFarland (108 cfu/ml). 100 µl of each standard inoculum was then streaked on nutrient agar per spot to attain a confluent growth (Bauer et al., 1966). Wells were made on the agar using a sterile cork borer and filled with 100 µL *Rauwolfia serpentina* L. organic extract. The plates were incubated accordingly as described previously. Standard strain of *E. coli* ATCC 25922 was used as control organism for bacterial assays. Bacteria control wells contained 100 µl of ciprofloxacin and fluconazole, respectively, at 5 µg per well. A well containing 100 µl of Tween-20-PBS solution (pH 7.2) was generally used as a positive control well in every assay. Growth inhibition was measured as diameters of inhibitory zones in the nearest 0.1 mm.

Table 1. Bacterial isolates and antibiotic resistance profiles.

Isolates	N	Antibiotic resistance
Salmonella typhi	5	Cot, Col, Tet, Str, Chl, Amp
Staphylococcus aureus Staphylococcus epidermidis Staphylococcus aureus	8	Str, Amp, Pen, Amo, Aug
Escherichia coli	4	Str, Amp, Amo, Pen
Streptococcus mutans	2	Str, Amp, Amo, Aug, Pen, Met
Proteus spp	10	Cot, Col, Chl, Str, Tet
Pseudomonas aeruginosa	4	Ery, Str, Cot, Amo, Amp, Tet
Klebsiella pneumoniae	5	Amp, Tet, Cot, Col, Str, Cot
Lactobacillus acidophilus	6	Str, Ery, Tet, Kan, Col, Cot, Amo
	4	Amp, Tet, Str, TMP-STX
	8	Tet, Col

Isolates were grouped based on their display of 100% resistance to antibiotics by disk diffusion assay following identification according to Cowan and Steel (1974). N = number of isolates tested; Str = streptomycin; Ery = erythromycin; Aug = augmentin; Cef = cefotaxime; Cro = ceftriaxone; Tet = tetracycline; Amp = ampicillin; Amo = amoxycillin; Kan = kanamycin; Gen = gentamicin; Chl = chloramphenicol; Col = colistin sulphate; cot = cotrimoxazole; and TMP-STX = Trimetoprim – sulphamethoxazole. The antibiotic resistance patterns of the isolates except *L. acidophilus* were given as previously described (Iwalokun et al, 2004).

Minimum inhibitory concentration (MIC)

The MICs of the organic extracts were determined by broth dilution method according to Alade and Irobi (1993) with a little modification. The extract was serially diluted with normal saline (0.85 g/dl) to 5 –50 mg/ml preparations dispensed (1.0 ml) into test tubes containing 1.0 ml of nutrient or potato dextrose broth. Each sensitive bacterial or fungal isolate (100 µl of 108cfu/ml) was inoculated into the test tubes. The tubes were mixed, covered with cotton wool and incubated appropriately as previously described. Thereafter, the tubes were then examined for microbial growth. The MIC was defined as the minimum concentration of the extract that did not allow any visible growth or turbidity of the organism in broth. MIC50 refers to concentration (%) of *Rauwolfia serpentina* L. required to prevent the growth of 50% of organisms tested. While MIC90 oil extract concentration, which inhibits the growth of 90% of the organisms tested.

Results

The present study has revealed the antibacterial and antifungal activity of petroleum ether (PE) and acetone (AE) extracts of *Rauwolfia serpentina* L. against multi drug resistant bacterial pathogens presented in Table 1. Data presented in Table 2 showed the fruiting body wet and dry weights per *Rauwolfia serpentina* L. strain analyzed ranged from 1.18 – 1.37 g (mean weight 1.27 ± 0.038 g) and 0.126 – 0.174 g (mean weight 0.152 ± 0.0025 g), respectively. The petroleum and acetone extract yields ranged from 3.32 – 3.40 mg / g dry *Rauwolfia serpentina* L. weight yielding an average of 3.36 ± 0.01 mg/g dry weight.

Table 2. Oil yield and phytochemical analysis of extracts of *Rauwolfia serpentina*.

Parameter	Amount	
Fruiting body wet weight (g) / strain	1.18 – 1.37 (1.27 ± 0.038)	
Fruiting body dry weight (g) / strain	0.126 – 0.174 (0.152 ± 0.0025)	
Extract yield (mg/g dry weight)	3.32 – 3.40 (3.36±0.01)	
Phytoconstituents		
	PE	AE
Terpenoids	±±	±
Tanins	±	±
Steroidal glycosides	±	±
Carbohydrates	±	±
Cyanogenic glycosides	-	-

Phytochemical analysis revealed the presence of terpenoids, tannins, steroidal glycosides and carbohydrates in both extracts but with higher terpenoids contents in the PE fraction. Cyanogenic glycosides, alkaloids, flavonoids and anthraquinones were not

detected in both extracts. Total phenol contents of 325.7 and 352.8 mg/L GAE were elicited by PE and AE extracts, respectively, and were further found to be significantly ($P < 0.05$) lower than 1005.3 mg/L GAE ($P < 0.05$) in the green tea infusion used as standard (Figure 1). Preliminary antimicrobial testing of petroleum ether extract of *Rauwolfia serpentina* L. by agar-well diffusion produced zones of growth inhibition of 3.0 – 7.8 ± (0.1 – 0.9) mm for the gram positive bacteria, 5.0 – 8.2 ± (0.1 – 0.7) mm for the gram negative bacteria and 8.1 – 10.8 ± (0.1 – 1.6) mm for the fungal isolates tested (Table 3). Among the susceptible gram-negative bacteria tested, inhibitory zones due to petroleum extract were higher than those of the acetone extract.

Table 3. Preliminary antimicrobial testing of *Rauwolfia serpentina* extracts. Inhibition zone diameter (mm ± SD)*

Isolates	*Rauwolfia serpentina*		Standard drugs	0.1% (v/v) Tween-20
	PE	AE		
Gram - positive bacteria				
Staphylococcus aureus ATCC 25923	7.5 ± 0.1	7.6 ± 0.3	27.5 ± 0.7	G
Staphylococcus aureus (8)	7.2 ± 0.9	7.1 ± 0.7	27.7 ± 0.9	G
Staphylococcus aureus (2)	7.0 ± 0.8	7.0 ± 0.6	28.1 ± 0.4	G
Staphylococcus epidermidis (4)	7.1 ± 0.8	7.0 ± 0.7	29.1 ± 0.5	G
Bacillus subtilis ATCC 6633	7.1 ± 0.1	7.2 ± 0.2	27.8 ± 0.3	G
Bacillus subtilis (5)	7.8 ± 0.2	7.6 ± 0.8	28.4 ± 0.6	G
Bacillus licheniformis (10)	7.7 ± 0.2	7.7 ± 0.4	27.8 ± 0.4	G
Lactobacillus acidophilus (2)	3.0 ± 0.2	3.2 ± 0.1	28.9 ± 0.4	G
Gram –negative bacteria				
Salmonella typhi ATCC 13391	7.5 ± 0.1	7.2 ± 0.4	28.1 ± 0.1	G
Salmonella typhi (5)	7.2 ± 0.3	7.0 ± 0.1	28.4 ± 1.3	G
Escherichia coli ATCC25922	7.0 ± 0.0	7.0 ± 0.0	29.5 ± 0.1	G
Escherichia coli (10)	8.2 ± 0.7	7.6 ± 0.8	28.5 ± 1.0	G
Proteus sp. (3)	6.9 ± 0.5	6.5 ± 0.6	27.8 ± 0.7	G
Klebsiella pneumoniae (4)	7.1 ± 0.2	7.0 ± 0.1	28.0 ± 0.1	G
Pseudomonas aeruginosa (3)	5.6 ± 0.4	5.2 ± 0.2	28.2 ± 0.6	G
Streptococcus mutans (4)	5.5 ± 0.5	5.0 ± 0.7	27.7 ± 0.9	G

Figures in parentheses represent the number of isolates tested; All and two of the tested *L. acidophilus* strains were sensitive to ciprofloxacin and *P. ostreatus*, respectively. Three (3) of the ciprofloxacin sensitive *Pseudomonas aeruginosa* isolates (6 0f 6) were also sensitive to *Rauwolfia serpentina*. G = Bacterial growth around the in phosphate buffered 0.1% Tween-20 control wells; # = growth inhibition zones for ciprofloxacin (5µg per well) and fluconazole (5µg per well) used as standard drugs. *The diameters of zone of inhibition were expressed in millimeter (mm) as mean ± standard deviation. @Seventy-nine (89.8%) of the 88 isolates showed sensitivity to *Rauwolfia serpentina*. PE = Petroleum ether extract; AE = Acetone extract.

Among the gram-positive bacteria tested, local isolates of *L. acidophilus* (2 of 8) and *B. subtilis* (5 of 5) were observed to be the least [3.0-3.2 ± (0.1 - 0.2) mm] and most [7.6 - 7.8 ± (0.2-0.8) mm] sensitive strains in both organic extracts. In gram-negative bacteria, local isolates of *E. coli* and *Streptococcus mutans* exhibited the highest [7.6 – 8.2 ± (0.7 –0.8) mm] and lowest [5.0 - 5.5 ± (0.5-0.7) mm] susceptibilities, respectively. Furthermore, highest susceptibility [10.5 - 10.8 ± (1.2-1.6) mm] by *S. cerevisiae* strains (4 of 4) and lowest susceptibility [8.0-8.1 ± (0.1-0.3) mm] by *C. albicans* ATCC1880 were observed among the fungal isolates. On the whole, 79 (89.8%) of the 88 isolates tested showed sensitivity to acetone and petroleum ether extracts of *Rauwolfia serpentina* L. (Table 3). The petroleum ether extract of *Rauwolfia serpentina* L. further showed stronger inhibition of these organisms in broth compared to the acetone extract (results not shown) with MICs of 78.3 – 100.0 ± (0 – 4.1)% extract for gram positive bacteria with *Staphylococcus* sp. having mean MIC50 and MIC90 of 71.7 ± 2.9 and 79.2 ± 8.8%, respectively.

Table 4. Minimum inhibitory concentrations determinations of *Rauwolfia serpentina* petroleum ether extract against multi drug resistant bacteria.

Isolates	MIC
Gram - positive bacteria	
Staphylococcus aureus (15) *Bacillus sp.* (16)	80.3 ± 4.1a
Lactobacillus acidophilus (2)	78.3 ± 3.8 ab
	100.0 ± 0.0
Gram –negative bacteria	
Salmonella typhi (10)	75.0 ± 5.1a
Escherichia coli (15)	71.3 ± 7.5
Proteus sp.	92.6 ± 4.4a
Pseudomonas aeruginosa (3) *Klebsiella*	100.0 ± 0.0a
pneumoniae (4) *Streptococcus mutans* (4)	88.6 ± 2.6 ab
	100.0 ± 0.0a

Data are presented as mean ± SD of experiments in duplicates. Figures in parenthneses indicate the number of isolates tested in duplicates. Disparity between mean values was analyzed by student's t- test. aP < 0.05 (MIC50 vs. MIC90 or MIC); bP< 0.05 (MIC90 vs. MIC); P < 0.05 = Significant).

The two sensitive *L. acidophilus* were inhibited with MIC90 of 100% extract, equivalent to the MIC (Table 4). The inhibition of gram-negative bacteria: *S. typhi* and *E. coli* by PE was observed at lower MICs (72.5 – 75.0%), MIC50 (66.3 – 67.5%) and MIC90 (71.3 – 72.5%) (Table 4). Antioxidant activty of the extracts by DPPH and ABTS methods revealed a disparate VCEACs of 3.6 – 3.8 mM for PE and 4.1 – 4.4 mM for AE ($P > 0.05$) compared to 6.2 – 6.4 mM in the green tea infuson ($P < 0.05$) (Figure 2).

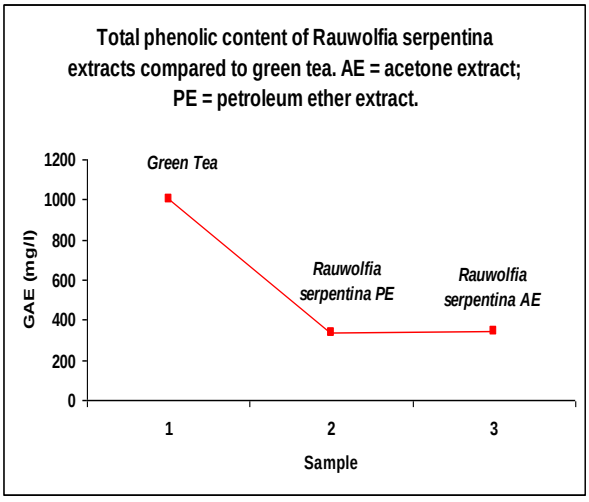

Figure 1. Total phenolic content of *Rauwolfia serpentina* extracts compared to green tea. AE = acetone extract; PE = petroleum ether extract.

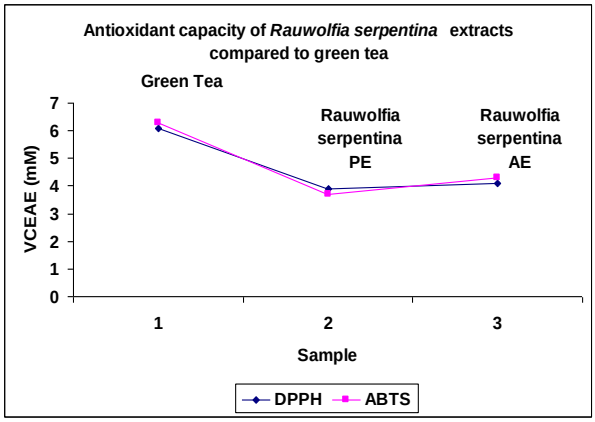

Figure 2. Antioxidant capacity of *Rauwolfia serpentina* extracts compared to green tea. Data are mean ± SEM of 3 determinations; AE and PE = Acetone and petroleum ether extract, respectively. aP< 0.05 (Green tea vs *P. ostreatus*) bP> 0.05 (*P. ostreatus* – AE vs PE).

DISCUSSION

Rauwolfia serpentina is a medicinally famous herb used for various medicinal purposes (Salma *et al.*, 2008). The present study has further revealed the antimicrobial potency of the oil of the *Rauwolfia serpentina* extracted with petroleum ether and acetone. Both extracts were observed to inhibit gram positive and gram-negative bacteria tested *in vitro* to suggest that *Rauwolfia serpentina* has a broad-spectrum antibacterial and antifungal activity. Similar antimicrobial potentials have been observed in the culture extracts of *Irpex lacteus* (Rosa et al., 2003) *Agrocybe* sp. (Kavanagh et al., 1950; Mavoungou et al., 1987), and juice of *L. edodes* (Kuznetsov et al., 2005). Antimicrobial potencies of the essential oils from mushrooms such as *Cuminum cyminum, carum carvi, Coriandum sativum* and *Foeniculum vulgare* have also been reported (Iacobellis et al., 2005) with activity against bacterial pathogens such as *Pseudomonas, Klebsiella, Salmonella and E. coli* as observed for *Rauwolfia serpentina* in this study.

The observed disparity in the susceptibilities of gram-ve bacteria tested with petroleum ether extract eliciting greater effect provides an indication that the organic solvents used have varyning abilities to extract bioactive substances from *Rauwolfia serpentina.* This is further evidenced by the different levels of terpenoids and phenolics observed in the two organic extracts. A clue that the petroleum extract may have greater antibacterial potential was provided by the observed MIC50 and MIC90 inhibitory concentrations of the extract against *S. aureus*, *S. typhi* isolates tested. Similar antibacterial characteristics have been observed in fluoroquinolones such as ciprofloxacin (Iwalokun et al., 2001) and medicinal plants such as *Ocimum gratissimum* (Iwalokun et al., 2003). Meanwhile, terpenoids have been implicated as the phytoconsitituents responsible for the antibacterial activity of mushrooms including *Cuminum cyminum* and *Carum carvi* (Iacobellis et al., 2005). The observed phenolic and tannin conctituents of *Rauwolfia serpentina* may also elicit antibacterial activity as found in many medicinal plants with mechanisms of action characterized by cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and micriobial adhensins (Cowan, 1999).

A recent study by Kuznetzov et al. (2005) also revealed the insensitivity of Bifidobacteria and Lactobacilli to the antibacterial action of *L. edodes* juice. The present study also observed *Rauwolfia serpentina* to elicit antioxidant capacity using the DPPH and ABTS methods thereby expanding its nutraceutical values. However, the observed higher phenolic contents and antioxidant capacity in the acetone extract of *Rauwolfia serpentina* compared to the petroleum extract provides an indication for a superiority of acetone over petroleum ether in the exudation of antioxidant substances from *Rauwolfia serpentina*. Flavonoids, which are also phenolic compounds with antioxidant activity (Mau et al., 2002; Gil et al., 2000) were not detected in *Rauwolfia serpentina*. Our observation agrees with findings of Mattila et al. (2001). The absence of flavonoids may be of biological advantage in their various ecological niches since these bioactive compounds inhibit enzyme activities (e.g. tyrosinase) involved in their pigmentation, growth and development (Xie et al., 2003). The low and varied phenolic contents of *Rauwolfia serpentina*, could also emanate from the differences in the leaf wet and wet weights of the tested in this study.

This may be attributed to the variations in their growth conditions since they were not cultivated in the same location. Therefore, appreciable amount of extract and thus phenolic compounds and terpenoids can be recovered from *Rauwolfia serpentina* if its optimum growth condition is known. This may further enhance its medicinal and market values. Based on the results of this study, it can be concluded *Rauwolfia serpentina,* has antioxidant potentials and possess a broad-spectrum antibacterial activity, optimisable by multiple organic leaf extraction. Further investigations that would identify the active phenolic and terpenoid compounds and determine the optimal plant growth conditions of *Rauwolfia serpentina* are necessary.

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