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Phytochemical Compounds and Antimicrobial Activity of Extracts of Rhoicissus Plant *(Rhoicissus revoilli)* (Planch)

P.S. Arwa, J.C. Onyango and R.O. Nyunja
Department of Botany and Horticulture, Faculty of Science,
Maseno University, P.O.Box 333 Maseno, Kenya

Abstract: Phytochemical compounds and anti-microbial properties of the methanol extracts of *Rhoicissus revoilli* (Vitaceae) were studied between January and December 2007 at Maseno University, Kenya. The bacterial used for the antimicrobial analysis consisted of clinical strains of *Streptococcus pryogenes* (gram positive) and S*almonella typhi* (gram negative) bacteria and one strain of fungi (*Aspergillus niger*). The methanol extract was active against the 3 microorganisms. Root extracts showed greater microbial growth inhibition in comparison to leaf extracts. The phytochemical screening for the plant leaves and roots of *R. revoilli* revealed the presence of active compounds of flavonoids, alkaloids, saponin, steroids and antharaquinones. The results from the present study have shown that the species of *Rhioicissus revoilli* have considerable activity against the gram negative bacteria *Salmonella typhi*, gram positive bacteria *Streptococcus pyrogenes* and one fungal strain *Aspergillus niger*. *Streptococus pyrogenes* experienced lesser growth inhibition with root extracts compared to the other 2 microbes. It is possible that the growth inhibition observed in the study occurred due to the presence of different chemical compounds. Absence of tannins in roots but not leaves could explain the greater growth inhibition of *S. typhi* and *A. niger*. Pit method was a better method for testing antimicrobial activity than disc method in the study.

Key words: Clinical strains, *Rhoicissus revoilli,* growth inhibition

INTRODUCTION

Plants naturally synthesize several carbon compounds, basically for physiologic functions or for use as chemical weapons against disease organisms, insects and predators (Fatope, 1995). The investigation of plants for bioactive secondary metabolites is an area which most plant scientists have recently focused with the aim of discovering new clinically useful and commercially important plant products (Dewick, 1997). It is estimated that 70-80% of people all over the world largely depend on traditional herbal medicine to meet their primary health care needs (Hamayun *et al*., 2006). The global demand for herbal medicine is growing (Omino and Kokwaro, 1991; Muregi *et al*., 2003; Zowai *et al*., 2003). While, plant species in traditional medicines continue to be reliable sources for discovery of useful compounds screening plants growing under various environmental conditions could provide another source for compounds with antimicrobial activities) (Njoroge and Newton, 1994; Rajakaruna *et al*., 2002; Muregi *et al*., 2003; Zowai *et al*., 2003; Adebooye and Opabode, 2004; Duraipandiyan *et al*., 2006). Biological and pharmacological compounds take into account different parameters and factors such as species, ecological factors and environmental conditions. Thus, each plant species will present a profile, which will express differently among these factors. Phenological age of plant, percent humidity of the harvested material and method of extraction are possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Rajakaruna *et al*., 2002). There is a wide variation in the susceptibility of organisms to toxic compounds It is probable that a large number of plants with biological activities remain untested.

Rhoicissus plant (*R. revoilli*) is ideally spread in the south, south west of Kenya. Due to diversity of ecological conditions the chemical compositions of the plants is known to vary and due to this reason plants have been used to treat different diseases in different places (Njoroge and Newton, 1994; Agnew and Agnew, 1994; Masinde, 1996).

Corresponding Author: P.S. Arwa, Department of Botany and Horticulture, Faculty of Science, Maseno University, P.O.Box 333 Maseno, Kenya

The plant belongs to the family of the Vitaceae and genus Rhoicissus. Literature search indicate that this plant has been used traditionally by many African communities to treat several diseases (Kokwaro, 1993) such as the root decoction is normally given to breastfeeding mothers and cows to increase milk production. Pounded leaves are rubbed to the skin and freshly cut wounds for faster healing of wounds and ringworms. Pounded leaves decotion is drunk in order to trace intestinal worms including hookworms (Omino and Kokwaro, 1991). Among the Luo community of Kenya pounded leaves decoction is applied to the boils to ensure faster healing via ripening (Arwa personal communication). Some phytochemical constituents of this plant have been reported to have medicinal or antimalarial properties (Kokwaro, 1993). However, there are no reports of antimicrobial activity of this plant in the literature. This study aimed at isolating, identifying and evaluating the antimicrobial properties of the compounds from *Rhoicissus revoilli* (Planch) wild species known to treat diseases in rural communities in Kenya.

MATERIALS AND METHODS

Field collection and extraction: Plant roots and the leaves were collected from areas surrounding Maseno University between January and December 2007. The materials were cleaned off adhered soil or dust in the field by shaking and were placed inside vasculum and taken to Maseno University laboratory where the tissue specimens were washed with distilled water and allowed to dry under shade for 30 days. The voucher specimens in duplicate were deposited in the herbarium of Maseno University. Leaves and roots of *Rhoicissus revoilli* (Planch) wild species were chopped using surgical blades air dried at room temperature for 2 weeks. The roots and leaves were subsequently ground into fine powder using hand and motor driven grinding mill. Yielding 350 and 300 g, respectively and cold extracted with methanol then filtered under vacuum using a 0.2 mm Nalgene filter unit. The resulting extracts were concentrated to remove the methanol using the rotary evaporator at 40°C 250 reumint. The roots and leaves extracts were dried and subsequently weighed to yield a crude extract of 21.8 and 20 g, respectively. Extracts were stored in refrigerator at 4°C until needed for analysis.

Phytochemical screening: The qualitative chemical analysis of the powders was carried out for the determination of the presence of anthraxquinone, saponins, steroids and alkaloids aldehydes and tannins. Phytochemical screening was done according to Kokate *et al.* (2003).

Isolation of pure compounds: The methanol extract (15 g) was chromatographed on silica gel column eluting with cyclohexane: ethyl acetate (CH: EE 9.1) according to Fennel *et al.* (2004) Most of the chemicals used were of analytical grade. Silica gel column (60 g, si 40-63 μm) was from Merck, Germany and commercially prepared TLC plate (MACHEREN-NAGEL) ALLUGRAM SIL G/UV_{254} and POLYGRAM SIL G UV_{254} from MACHEREY NAGEL, Germany. Seven 70 mL fractions were collected from the roots and from leaves. The R_f values of the bioactive fraction in the sample were determined.

Test microorganisms: The clinical isolates were obtained from Kombewa District Hospital, Kenya. Test microorganisms consisted of one gram positive bacteria (*Streptococcus pyrogenes*) and one gram negative bacteria (*Salmonella typhi*) and one fungal straiin (*Aspergillus niger*).

Preparation of the culture media: Nutrient agar pH 7.4 and sabouraud dextrose agar pH5.4, all products of oxid laboratories, England were used in the study. Forty grams of sabourard dextrose agar 28 g of nutrient agar were dissolved in 1 L water heated to dissolve the contents completely. The media, distilled water and petri dishes were sterilized separately by autoclaving at 3°C at 1 bar pressure for 30 min. The nutrient agar and Sabourard dextrose agar petri dishes were prepared by pouring 15 mL of molten media into sterile petri dishes allowed to solidify for 5 min.

Determinination of antimicrobial activity: Extracts from the 2 plant parts (leaves and roots) were diluted in cyclohexane (3 g of the dried filtrate was constituted with 10 mL of 100% cyclohexane to prepare stock solution). Different concentrations of the plant extracts were prepared by diluting the stock extract to cyclohexane according to the ratios 1:9 (10%), 1:18 (5%) and 1:32(2.5%). The control had cyclohexane alone without any extract to nullify the effect of solvent on the test organisms. Two methods were used; this is pit and disc methods according to Murthy and Nagodra (1977) and Rajakurana *et al.* (2002). Susceptibility testing was carried out by measuring the inhibitory zone diameters on the Nutrient Agar (NA) and Sabourard dextrose agar using conventional paper disc method and pit method. The inhibitory zone distances and rounded off to the nearest whole numbers (mm) for analysis.

Pit method: A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the agar media, into which 0.1 mL of the prepared plant extracts of the various concentrations were added. The test

microorganisms were introduced on the media around the pit using applicator sticks bearing cotton wool at the tips. Three replicates for each microbe and plant extract concentration were used. The petri dishes inoculated with bacteria and fungi were kept for incubation for 24 h at 37 and 25°C, respectively. The clear zone of growth inhibition formed around each pit was measured to the nearest millimeter using a transparent ruler.

Disc method: Circular paper discs 8 mm diameter were cut out from Whatman No.1 filter paper using a paper punch and each dipped in a known concentration of the plant extracts for about 2 min, then were gently transferred to the center of the inoculated agar media. Petri dishes inoculated with bacteria and fungi and were kept for incubation for 24 h at 37 and 25°C, respectively. The diameters of growth inhibition zones were measured using a ruler and compared to the cyclohexane control disc to nullify the effect of the solvent on the growth of the test organisms. All work was carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicine (WHO, 2000).

Data analysis: Data collected were subjected to analysis of variance (ANOVA) using SAS statistical package. Means were separated and compared at ($p<0.05$).

RESULTS

Phytochemical screening of the plants parts (Table 1) showed that the plant contains flavonoids, alkaloids steroids and anthraquinones, ketones and tannins. Root extracts lacked adelyhydes unlike the leaves which had both ketones and adelyhydes. Flavonoids were only present in root extracts. There were a lot of sterols in the root extract than in the leaves extract. Higher concentrations of the alkaloids were present in both root and leaves extract. Meta and ortho hydroxyls groups were present in both roots and leaves extracts while para hydroxyl groups were only present in leaves, this confirmed the presence of carbohydrates in the plant extracts. Elution of the column with n-hexane and ethyl acetate led to isolation of fifteen fractions of R_f values of uncharacterized active compounds (Table 2). Seven active compounds were isolated from the leaves and nine active compounds from the roots. There were significant ($p<0.05$) differences between the plant parts, extracts concentration, test microorganisms and bioassay methods used. Pit method had significantly greater growth inhibition (Table 3) compared to disc method. Both plant extracts were found to possess antimicrobial activities. The leaves extracts had higher growth activity compared

Table 1: Phytochemical screening of secondary metabolites present in the plant parts extracts of *Rhoicissus revoilli*: (Planch)

Phytochemical groupings	Leaves	Roots
Flavonoids	-	++++
Anthraquinone	++	+++
Steroid glycosides	++	++++
Alkaloids	++++	++++
Saponins	++	++
Carbonyl compounds		
Ketone	+	+
Tannins	+	-

-: Absent, +: Present, ++ Low concentration, +++: moderate concentration, ++++: High concentration

Table 2: Tn R_f values of active compounds isolated from the methanol plant extracts of *Rhoicissus revoilli* using thin layer chromatography

Plant extract	Spot no.	Distance of solvent from origin (cm)	Distance of spot from origin (cm)	R_f-value
Leaves	1	6.0	2.0	0.30
	2	6.0	1.5	0.25
	3	6.1	2.6	0.42
	4	6.0	3.5	0.58
	5	5.8	3.4	0.59
	6	6.1	2.9	0.48
	7	6.0	4.5	0.75
Roots	1	5.8	1.5	0.26
	2	5.8	2.3	0.40
	3	5.7	3.0	0.52
	4	5.7	2.1	0.37
	5	5.9	3.3	0.56
	6	6.0	1.8	0.30
	7	5.7	2.7	0.48
	8	6.1	1.5	0.25
	9	5.7	4.0	0.61

Table 3: The comparison of the inhibitory effects of the root extracts using the two methods of microbial tests on the test organisms

Microorganism	Pit method (mm)	Disc method (mm)
S. typhi	6.25±0.41	3.75±0.41
S.pyrogenes	2.58±0.41	2.17±0.41
A.niger	5.83±0.41	5.25±0.41

Data values are means of 3 replicates ±SE

Table 4: Analysis of mean data of the antimicrobial activity of *Rhoicissus revoilli* extracts, comparison of two methods of microbial tests and plant part used

Variables	Growth Inhibition diameter (mm)
Microbe	
Salmonella typhi	5.0833a
Streptococcus pyrogenes	4.6875ab
Aspergillus niger	4.2708b
LSD	0.57680
Extract concentration	
10%	9.6111a
5%	5.6389b
2.5%	3.3889c
Control	0.0833d
LSD	0.66610
Plant part	
Leaf	5.0556a
Root	4.3056b
LSD	0.47100
Method	
Pit method	6.0972a
Disc Method	3.2639b
LSD	0.47100

Means followed by different letter(s) down the column are significantly different at $p<0.05$. Data values are means of three replicates

to root extracts (Table 4). The zones of growth diameter inhibition obtained with respect to the test organisms were higher for *A. typhi*, followed by *A. niger* and *S. pyogenes*, respectively.

DISCUSSION

Medicinal plants have been the subject of human curiosity and need (Omino and Kokwaro, 1991; Masinde, 1996; Abulude, 2007; Khalil and Dababneth, 2007). The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds are alkaloids, flavonoids, tannins and phenolics compounds (Weimann and Heinrich, 1997; Atindehou *et al*., 2002; Stuessy, 1990; Edeoga *et al*., 2005). The antimicrobial activity of the methanol extract appear to have broad spectrum of activity, since both gram positive and gram negative bacterial and fungi were sensitive to the extracts. Many alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein and membrane phosphollipids biosynthensis (Shelton, 1991). The zone of inhibition measured for *Salmonella typhi*, a gram negative bacteria using pit method was greater compared to *S. pyrogenes* a gram positive bacteria. Root extracts were more active than those from leaves, probably because they had higher concentration of the active compounds than the roots or it may be due to the absence of tannins in the roots or it may be due absence of compounds than the roots as was evident from the study (Table 1). More interesting, the root extracts had higher fractions of active compounds as determined by thin layer chromatography (Table 2). The relatively greater growth inhibition caused by the leaf extract was as a result of the active constituents in leaves acting in a more synergistic manner. Interesitngly, *S. pyrogenes* experienced lesser inhibition with the root extracts compared to the other 2 microbes (Table 3 and 4). There is a possibility that growth inhibition of microbes was due to different chemical compounds. Absence of adelhydes in roots but not in leaves extracts could explain the greater growth inhibition of *S. typhi* and *A. niger*. The toxic Phytochemical compounds could either damage the DNA or inhibit the synthesis of proteins in these organisms (Fatope, 1995). Cyphostema serpens, a plant in the same family has been used in Luo Community to prevent or treat boils (Kokwaro, 1993). Phytochemical compounds are very heterogenous mixtures of single substances acting in a synergistic or antagonistic manner. Mixtures of active constituents show a broad spectrum of biological and pharmacological activity (Robinson, 1967; Coelho-de-Souza *et al*., 1998; Atindehou *et al*., 2002).

Antimicrobial activities exhibited by the plant could be due to presence of alkaloids, flavonoids and carbohydrates (Meta and Parahydroxyl compounds) present in the plant extracts (Fennel *et al*., 2004). Many alkaloids have pharmacological effects. Alkaloids have been used to treat diseases like malaria, painkillers and managing heart diseases. Alkaloids are derivatives of amino acids and include morphine, atropine quinine cytochalasine, berberine, cocaine and nicotine. Flavonoids (rutin and anthocyanins phenolics, polyphenolic compounds and saponins have biological and pharmacological activities on human health (Hartung, 1990). Fungitoxic phytochemical compounds include the phenolics compounds such as cathinone, muzigadial, chlorogenic acid and scopoletin (Fatope, 1995). Major flavonoid compounds such as rutin and its aglyconequercetin have beneficial biological activity such as antagonizing the increase of capillary fragility associated wth haemorrhagic disease (Griffith *et al*., 1944; Abulude, 2007), reducing hypertension and anticarcinogenic activity (Yang *et al*., 2000). Biological activities of many other flavonoids, polyphenols or phenolics compounds have also been reported (Edenharder and Grünhage, 2003; Ouyang *et al*., 2007). Flavonoids and hydroxystilbenes act by inhibiting enzymes that regulate cell proliferation (Fatope, 1995). The occurrence of hydroxyl groups in methanol extracts could enhance the likelihood of intramolecular reactions such as the formation of cyclic acetals and anhydroethers (Mann, 1994). It is possible that carbohydrates present in the extracts somehow facilitated growth of the microorganisms and hence antagonizing the antibacterial activity of the active compounds in the extracts. The susceptibility of *S. typhi*, *S. pyrogenes* and *A. niger* to the methanol extracts is an indication that the plant is suitable for use as a source of both antibacterial and antifungal compounds. The antimicrobial activities demonstrated by crude extracts of *R. revoilli* may, therefore explain some of the previous claims about this plant for the treatment of diseases like dysentry and venerial infections (Omino and Kokwaro, 1991). The antimicrobial properties exhibited by these plants could be traced to its possession of alkaloids and flavonoids noted for their numerous biological activities such as anti inflammatory, vasoprotective and antithrombitic effects (Mostahar *et al*., 2007). There are no reports of toxic effects of *R. revoilli* used in different African communities including Kenya for various ailments, hence phytochemical studies should be intensified to isolate, characterize and identify the specific active compounds in this plant responsible for the antimicrobial activity.

CONCLUSION

The present study with *R. revoilli* has confirmed antimicrobial activity against gram negative bacteria *S. typhi* and gram positive bacteria *S. pyrogenes*, including the fungal pathogen *A. niger* leaves extracts showed greater inhibition than roots. *Streptococcus pyrogenes* showed lesser growth inhibition with root extracts in comparison with root extracts in comparison with the other 2 organisms, suggesting that the growth inhibition was caused by different active compounds. Roots lacked aldehydes, which were present in leaves extract; therefore adehydes could be the compounds responsible for greater growth inhibition of *S. typhi* and *A. niger*. Antimicrobial activity of the plant could have been caused by saponins, flavonoids, anthraquinones, carbohydrates and alkaloids present in the plant extracts. The results suggest that the extracts of *R. revoilli* could be used as a source of cheaper substitute for conventional drugs. The results are guarantee for further verification using various organic and water compounds, more sensitive and sophisticated bioassay techniques.

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