

Full Length Research Paper

Phytochemical constituents and antimicrobial studies of two South African *Phyllanthus* species

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Many *Phyllanthus* species are indigenous to South Africa but its chemistry or biological activity has not been reported despite its use in ethnomedicine. Leaves extracts of *Phyllanthus parvulus* sord var. *garipensis* and *Phyllanthus burchellii* were screened phytochemically for the presence of secondary metabolites and *in vitro* antibacterial properties. Tannin, alkaloid, saponin, anthraquinone and flavonoid were the secondary metabolites present. A higher percentage of alkaloid, saponin and flavonoid were observed in the quantitative analyses of *P. parvulus* sord var. *garipensis* plant when compared to *P. burchellii*. The acetone, methanol and aqueous solvent extracts of the two plants show good inhibitory effect against most bacteria even at very low concentration of 0.16 µg/ml. However, the aqueous extract of *P. parvulus* var. *garipensis* was most active.

Key words: *Phyllanthus parvulus* var. *garipensis*, *P. burchellii*, quantitative and qualitative phytochemical screening, antibacterial.

INTRODUCTION

The *Phyllanthus* genus belongs to the Euphorbiaceae family. There are over 300 genera with over 5000 species in the Euphorbiaceae worldwide, of which 50 genera and 484 species occur in Southern Africa. The *Phyllanthus* is one of the genera that falls under this enormous family. *Phyllanthus* has about 750 - 800 species, found in tropical and subtropical regions worldwide, with about 22 species native to Southern Africa. Within South Africa, these plants are found in Transvaal, Cape Province and KwaZulu Natal (Archer, 2000; van Coller et al., 1997; Unander et al., 1995). The South African species are herbs, shrubs or trees. Several trees are in the Kruger National Park e.g. *Phyllanthus amapondensis* (red pepper). *Phyllanthus cedrifolius* is found in the forest of Transkei while *Phyllanthus burchellii* and *Phyllanthus parvulus* var. *garipensis* are common habitat of the Ngoye forest in Zululand (Steentoft, 1988). About three species of *Phyllanthus* herbs grows widely within the University of Zululand campus.

A number of the *Phyllanthus* species have been reported to have extensive history in medicine systems. Substantial amount of the genus are used widely in traditional medicine for the treatment of flu, dropsy, diabetes, jaundice, gall and bladder calculus, liver disease (Unander et al., 1995; Calixto et al., 1998; Dhiman and Chawla, 2005).

Most of these species have pharmacological properties e.g. *Phyllanthus niruri* has demonstrated *in vitro* antibacterial actions against *Staphylococcus*, *Micrococcus* and *Pasteurella* bacteria as well as *in vivo* and *in vitro* anti-malaria properties, which validates other traditional uses of the genus (Veeramuthu et al., 2006). Extracts of *Phyllanthus* had been used as antiviral source to treat hepatitis B (Venkateswaran et al., 1987; Thyagarajan et al., 1988; Blumberg et al., 1989; Lam et al., 2006). Powis and Moore (1985) studied the aqueous extracts of *Phyllanthus amarus* and found it to inhibit viral DNA *in vitro*. In addition, they eliminated detectable virus from the sera of woodchucks (*Marmota monax*) acutely or chronically infected with the woodchuck hepatitis virus (WHV). The methanol extracts of five *Phyllanthus* species from India was reported to have strong antioxidant activity (Kumaran and Karunakaran, 2007).

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Table 1. Phytochemical screening of *P. burchellii* and *P. parvulus sond* var. *garipensis*.

Secondary metabolite	<i>P. burchellii</i>	<i>P. parvulus sond</i> var. <i>garipensis</i>
Alkaloids	++	++
Saponins	++	+++
Tannins	+	+++
Flavonoids	+	+++
Phenolic flavonoids	-	++
Antraquinones	-	++
Anthocyanosides	+++	-

+ = Low concentration, ++ = moderate concentration, +++ = high concentration, and - = absence of constituent.

P. niruri has antifungal activity on ringworm, ulcers, scabies and jaundice. Its ethanol extracts have extensive antibacterial and antiviral actions and are antiprotozoal against *Amoeba berghiei* and anthelmintic to *Hymenollepis nana*. Aqueous extracts of the leaves produced an oral hypoglycemic effect comparable to that of toluene butanamide (Unander, 1996). According to Pettit et al. (1990), the root of *Phyllanthus acuminatus* inhibited the growth of murine P-388 lymphocytic leukemia and B-16 melanoma cell lines.

The *Phyllanthus* genus is a source of plant chemicals. Extracts of *Phyllanthus* have secondary compounds like alkaloid, flavonoid, lignin, phenol, tannin and terpene. Many of the "active" constituents are attributed to biologically active lignin, glycosides, flavonoids, alkaloids, ellagilannins and phenyl propanoids that are found in the leaf, stem and roots of the plant. Common lipids such as sterols and flavonols also occur in the plant.

A number of the *Phyllanthus* species have been reported to have extensive history in medicine systems (Unander et al., 1990, 1991). Researches and review on *Phyllanthus* species indigenous to some countries are known for its numerous antimicrobial and antiviral activities. The South African species are known botanically in terms of species, height and leaf arrangement, etc. We are interested in ethnomedicinal and scientific documentation of plants used by traditional healers in the KwaZulu-Natal Province, South Africa. Lack of adequate scientific information prompted the investigation on the chemistry and biological studies of the South African *Phyllanthus* species for medication and possible drug formulation.

MATERIAL AND METHODS

Plant material

Fresh plants were collected around the University of Zululand campus. Voucher specimen were deposited at the University of Zululand Herbarium (voucher No.197 and 198). The plants were identified at Natal herbarium and Pretoria National herbarium as *P.*

burchellii and *P. parvulus sond* var. *garipensis*.

Phytochemical screening

Plant filtrates were prepared by boiling 20 g of the fresh plant in distilled water. The solution was filtered through a vacuum pump. The filtrates were used for the phytochemical screening using the usual procedure as describe by Harbone (1973) and Boham and Kocipal-Abyazan (1974) and Edeoga et al. (2005). Alkaloid was determination using Harborne (1973) method, tannin by Van-Burden and Robinson (1981) method, saponin by Obadoni and Ochuko (2001) method, and flavonoid determination was by the method of Boham and Kocipai-Abyazan (1974).

Solvent extraction

Aqueous extract: 20 g of a fresh plant was boiled in distilled water, the resultant solution was filtered. The filtrate was then concentrated in a rotatory evaporator to get the crude extract of the plant.

Acetone and methanol extract: 20 g of the fresh plant was ground and soaked with methanol/acetone (separately) in a 250 ml conical flask. The flask was covered with cotton wool and aluminium foil to prevent the solvent from escaping. The flask was placed in a shaker for 24 h. The following day the plant filtrate was concentrated in a rotatory evaporator to get the crude plant extracts.

Antimicrobial screening

Agar disc diffusion procedure was used (Chan et al., 1993). Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcesens*, *Bacillus subtilis*, *Microflora conti* and *Klebsiella oxytoca* were collected from Lancet Pathology Laboratory, Department of Microbiology, Durban KwaZulu Natal, Republic of South Africa. An overnight broth culture of 0.1 ml of each bacterium was used to seed sterile nutrient agar medium. 1 ml of plant extract (5 mg/ml) was added to 9 ml of distilled water in a test-tube to make serial dilution of each plant extract. 6 mm sterile paper disk were impregnated with each plant extract by soaking in plant extracts for 10 s. The impregnated disks were then placed at the centre of each plate and incubated for 24 h at 37°C. Antimicrobial studies were done in triplicates and inhibition zones were measured in mm. Blanks solvents of acetone, water and methanol were used as control.

RESULTS AND DISCUSSION

The phytochemical screening of the two *Phyllanthus* species revealed the secondary metabolites which are of medicinal interest as presented in Table 1. Both species are rich in alkaloids and saponins. However, in the *P. parvulus sond* var. *garipensis* extracts tannin, flavonoids and antraquinones were in appreciable amounts (Figure 1). Anthocyanosides was absent in this species. The variance in the quantitative composition of the secondary metabolite establishes the fact that both plant are not the same and are not likely to have the same medicinal potential. These secondary metabolites are known to exhibit medicinal activity as well as physiological activity (Sofowara, 1993).

Table 2. Antimicrobial potentials of *P. burchellii* extracts.

Conc,	Extract	<i>E. coli</i>	<i>S. marcescens</i>	<i>P. mirabilis</i>	<i>P. aerogenosa</i>	<i>K. oxytoca</i>	<i>M. conti</i>	<i>B. subtilis</i>
Control	Acetone	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-
5 mg/ml	Acetone	****	***	****	***	****	****	****
	Methanol	****	**	***	-	-	**	**
	Water	****	**	*	-	*	**	**
2.5 mg/ml	Acetone	***	**	**	**	**	**	****
	Methanol	**	**	**	-	-	-	-
	Water	-	*	*	-	-	-	*
1.25 mg/ml	Acetone	**	**	-	*	*	*	*
	Methanol	*	*	**	-	-	-	-
	Water	-	*	*	-	-	-	-
0.63 mg/ml	Acetone	*	*	-	*	-	*	-
	Methanol	*	*	*	-	-	-	-
	Water	-	*	*	-	-	-	-
0.31 mg/ml	Acetone	-	*	-	*	-	-	-
	Methanol	*	*	*	-	-	-	-
	Water	-	*	*	-	-	-	-
0.16 mg/ml	Acetone	-	*	-	*	-	-	-
	Methanol	*	*	*	-	-	-	-
	Water	-	*	*	-	-	-	-

* = 7 - 10 mm; ** = 11 - 14 mm; *** = 15 - 18 mm; **** = 19 - 23 mm; ***** ≤ 24 mm.

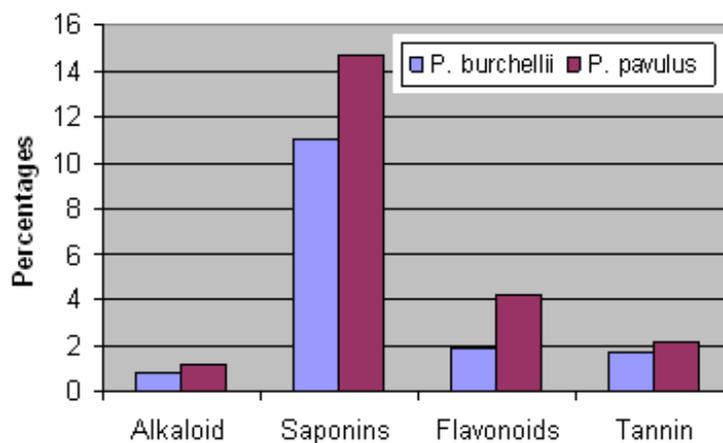


Figure 1. Compositional variation of some secondary metabolites in the two *Phyllanthus* species.

The results of the antimicrobial activity presented in Table 2 and 3 show that all extracts exhibited appreciable antibacterial properties inhibiting the growth of all bacteria (except for *P. burchellii* aqueous and methanol extracts which did not inhibit the growth of *P. aeruginosa* at 2.5 mg/ml). The aqueous extracts of *P. pavulus* *sond* var.

garipensis inhibited *P. mirabilis*, *S. marcescens*, *B. subtilis*, *M. conti* and *K. oxytoca* at very low concentrations (0.16 mg/ml). This is interesting as water is one of the medium through which traditional healers utilize medicinal plants to their clients.

These two South African *Phyllanthus* species seen as

Table 3. Antimicrobial potentials of *P. parvulus* sord var. *garipensis* extracts.

Conc,	Extract	<i>E. coli</i>	<i>S. marcescens</i>	<i>P. mirabilis</i>	<i>P. aerogenosa</i>	<i>K. oxytoca</i>	<i>M. conti</i>	<i>B. subtilis</i>
Control	Acetone	-	-	-	-	-	-	-
	Methanol	-	-	-	-	-	-	-
	Water	-	-	-	-	-	-	-
5 mg/ml	Acetone	***	***	****	****	-	***	**
	Methanol	**	**	**	***	**	**	*
	Water	*	***	*	**	***	*	**
2.5 mg/ml	Acetone	*	*	***	***	-	**	**
	Methanol	**	***	*	**	-	**	*
	water	*	*	*	*	**	*	*
1.25 mg/ml	Acetone	*	*	*	*	-	*	*
	Methanol	-	**	*	*	-	*	*
	Water	-	*	-	*	**	-	*
0.63 mg/ml	Acetone	-	*	-	-	-	*	-
	Methanol	-	*	*	-	-	*	-
	Water	-	*	*	-	*	*	*
0.31 mg/ml	Acetone	-	*	-	-	-	-	-
	Methanol	-	*	*	-	-	-	-
	Water	-	*	-	-	*	-	*
0.16 mg/ml	Acetone	-	*	-	-	-	-	-
	Methanol	-	-	*	-	-	-	-
	water	-	**	*	-	*	*	*

* = 7 – 10 mm; ** = 11 - 14 mm; *** = 15 - 18 mm; **** = 19 - 23 mm; ***** ≤ 24 mm.

weeds are potential medicinal plants from the phytochemical screening and antimicrobial activities. Toxicity investigation of these plants with isolation, identification, characterization and elucidation of bioactive compounds are ongoing and will be communicated later.

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