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*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 it use in ethnomedicine. Leaves extracts of *Phyllanthus parvulus sond* 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 sond* 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 genuses 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 cedrifoluis* 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.

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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* antimalaria properties, which validates other traditional uses of the genus (Veeramuthu et al., 2006). Extracts of *Phyllanthus* had been used as antiviral source to treat hepatatis 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).

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

| Secondary metabolite | *P. burchellii* | *P. pavulus sond* var. *garipensis* |
|---|---|---|
| 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 berghei* 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$^{o}$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, flavoniods 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 | *E. coli* | *S. marcesens* | *P. mirabilis* | *P. aerogenosa* | *K. oxytoca* | *M. conti* | *B. subtilis* |
|---|---|---|---|---|---|---|---|---|
| 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.

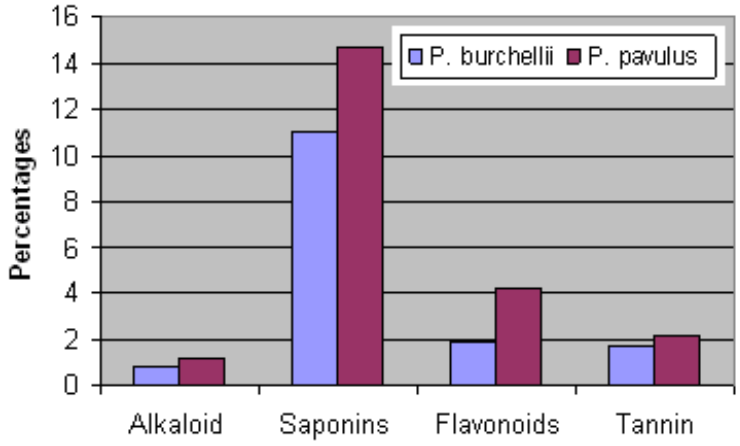


**Figure 1.** Compositional variation of some secondary metabolites in the two *Phyllanthus* species.

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. aeroginosa* at 2.5 mg/ml). The aqueous extracts of *P. pavulus sond* var. *garipensis* inhibited *P. mirabilis, S. marcesens, 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 sond* var. *garipensis* extracts.

| Conc, | Extract | *E. coli* | *S. marcesens* | *P. mirabilis* | *P. aerogenosa* | *K. oxytoca* | *M. conti* | *B. subtilis* |
|---|---|---|---|---|---|---|---|---|
| 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 phyto-chemical 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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### REFERENCES

Archer RH (2000). Euphorbiaceae In: *Seed Plants of Southern Africa* (ed. O.A. Leistner) *Strelitzia* National Botanical Institute, Pretoria. 10: 249-262.

Blumberg BS, Millman I, Venkateswaran PS, Thyagarajan SP (1989). Hepatitis B virus and heptaocellular carcinoma-treatment of HBV carrier with *Phyllanthus amarus*. Cancer Detect. Prev. 14: 195-201

Boham BA, Kocipal-Abyazan R (1974). Flavonoids and condensed tanins from the leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. Pac. Sci., 48: 458-463.

Calixto JB, Santos AR, Cechinel FV, Yunes RA (1998). A review of the plants of the genus *Phyllanthus*: their chemistry, pharmacology, and therapeutic potential. Med. Res. Rev. 18: 225-258

Chan ECS, Pelczar MJ, Krieg RN (1993). Laboratory exercises in microbiology, 6th edition, McGraw Hill.

Dhiman RK, Chawla YK (2005). Herbal Medicine for Liver Diseases. Dig. Dis. Sci. 50: 1807-1812.

Edeoga HO, Okwu DE, Mbaebie BO (2005). Phytochemical constituents of some Nigerian medicinal plants. Afr. J. Biotechnol. 4: 685-688

Harbone JB (1973). Phytochemical Methods, Chapman and Hall, Ltd., London, pp. 49-188.

Kumaran A, Karunakaran RJ (2007). *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT Food Sci. Technol. 40: 344-352

Lam WY, Leung KT, Lee SM, Chan HL, Fung KP, Ooj VE, Waye MM (2006). Antiviral Effects of *Phyllanthus nanus* extracts Against Hepatitis B Virus. Cell Biochem., pp. 795-812.

Obadoni BO, Ochuko PO (2001). Phytochemical studies and comparative efficacy of the crude extracts of some Homostatic plants in Edo and Delta State of Nigeria. Global J. Pure Appl. Sci. 8b: 203-208.

Pettit GR, Schaufelberger DE, Nieman RA, Dufresne C, Saenz-Renauld JA (1990). Antineoplastic agents, 177. Isolation and structure of Phyllanthostatin 6, J. Nat. Prod. 53: 1406-1413.

Powis G, Moore DJ (1985). High-performance liquid chromatographic assay for the antitumor glycoside phllanthoside and its stability in plasma of several species, J. Chromatograph. 342: 129-134.

Steentoft PM (1988). Flowering Plants in West Africa. University Press, Cambridge, pp. 130-131.

Thyagarajan SP, Subramarnian S, Thirunalasundari T, Venkateswaran PS, Blumberg BS (1988). Preliminary Study: The effect of *Phyllanthus amarus* on chronic carriers of hepatitis B virus. The Lancet II: 764-950.

Unander DW, Webster GL, Blumberg BS (1990). Record of usage or assays in *Phyllanthus* (Euphorbiaceae) I. Subgenera Isocladus, Kirangella, Cicca amd Emblica. J. Ethnopharmacol. 30: 233-264.

Unander DW, Webster GL, Blumberg BS (1991). Usage and bioassays in *Phyllanthus* (Euphorbiaceae): a compilation II. The subgenus Phyllanthus. J. Ethnopharmacol. 34: 97-133.

Unander DW, Webster GL, Blumberg BS (1995). Usage and bioassays in *Phyllanthus* (Euphorbiaceae): IV. Clustering of antiviral uses and other effect. J. Ethnopharmacol. 45: 1-18.
Unander DW (1996). *Phyllanthus* species: *In vitro* culture and production of secondary metabolites: In Biotechnology in Agriculture and Forestry (Y.P.S. Bajaj, ed.), Springer-Verlag, Berlin, 37: 304-318.
Van Coller A, Rogers K, Hertiage G (1997). Linking Riparian Vegetation Types and Fluvial geomorphology along the Sabie River within the Kruger National Park, South Africa. Afr. J. Ecol. 35: 194-212.
Van-Burden TP, Roberson WC (1981). Formation of complexes between protein and tannin acid. J. Agric. Food Chem. 1: 77.
Veeramuthu D, Muniappan A, Savarimuthu I (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from TamiNadu, India. India BMC Complement. Altern. Med., 6: 35.
Venkateswaran PS, Millman I, Blumberg BS (1987). Effect of an extract from *Phyllanthus niruri* on hepatitis B and woodchuck hepatitis viruses: *In vivo* and in viro studies. Proc. Natl. Acad. Sci. (USA) 84: 274-278