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Full Length Research Paper

The potential use of *Prunus africana* for the control, treatment and management of common fungal and bacterial infections

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Traditionally *Prunus africana* stem bark decoctions are used for treating and managing chest pain, malaria, and fever. The present study investigated the antibacterial and antifungal activity of hexane and methanol stem bark extract of *Prunus africana*. Disc diffusion assay was used to evaluate antimicrobial activity against *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosus* ATCC 27852, *Streptococcus pneumoniae,* methicillin resistant *Staphylococcus (MRSA), Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 66031, *Microsporum gypseum* and *Trichophyton mentagrophyte*. The methanol extracts were active against *Trichopyton mentagrophyte*, *Staphylococcus aureus* ATCC 25923, Methicillin resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* at concentrations of 0.039, 0.073, 0.156 and 2.5 mg/ml, respectively. The extract of the above plant had no activity against *Cryptococcus neoformans* ATCC 66031 and *Candida albicans* ATCC 90028. The antifungal and antibacterial activity of *P. africana* demonstrated supports the claimed antimicrobial uses of the plant in the traditional medicine and provides scientific prove for their medicinal uses.

Key words: *Prunus africana*, extracts, antifungal, antibacterial, medicinal plants.

INTRODUCTION

Prunus africana is commonly known as Pygeum or African cherry and belongs to Rosaceae family. It is a medium to large, evergreen tree with a spreading crown of 10 to 20 m when mature. It can become quite huge in forests region, but is usually medium-sized in gardens. It is widely distributed in several provinces of Kenya and especially in Mount Kenya forest. This plant is used in Kenya traditional medicine to treat chest pain, malaria and fever (Kokwaro, 1993). The stem barks are boiled in water and 2 glasses taken in the morning and in the evening. Other traditional uses are diarrhoea, allergies, stomachache, prostrate gland disease and kidney disease (Pujol, 1990; Iwu, 1993). In modern medicine, stem bark water extract is used to manufacture products used for the treatment of prostate gland hypertrophy (enlarged prostate glands) and more serious conditions of benign prostate hyperplasia (BPH). This extracts have been patented and are being sold in a number of pharmacies (Sunderland and Obama, 1999; Schipmarn, 2001). The Western pharmaceutical companies first recognized its medicinal value over 20 years ago and now trade in this medicinal plant is worth millions (Nzilani, 1999, Simons et al., 1998; Schipmarn, 2001). It is estimated that *P. africana* alone has a current market value of around $150 million per annum (Cunningham et al., 1993; 1997; 1999).

Available literature indicates that that phytochemical study has lead to isolation of triterpenic acids including derivatives of ursolic and oleanolic acids (Fourneau et al., 1996). Some previous studies have been carried out on the biological properties of this plant dichloromethane and ethyl acetate extract of the bark and it showed an MIC value of 6.25 and 1.56, respectively, against

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Mycobacterium. However, there are no biological properties of other organic extracts and also on other different strains of bacteria and fungal. The acceptance of traditional medicine as an alternative form of health care by the general public and the development of microbial resistance to the available antibiotics has led many scientist to investigate the antimicrobial activity of several medicinal plants (Ali et al., 2001; Kumarasamy et al., 2002; Hamill et al., 2003; Bii et al., 2008).

The currently, treatment of opportunistic fungal infection is limited and the emerging antimicrobial drug resistant strains among Gram-positive bacteria such like staphylococci and enterococci, calls for an urgent need for search into new therapeutic alternatives and medicinal plants present potential candidates. Therefore we report on the evaluation of *in–vitro* antimicrobial and antifungal properties of hexane and methanol extracts of *P. africana* from our local environment.

MATERIALS AND METHODS

Plant materials

P. africana stem barkwere obtained from Rift Valley Province of Kenya. Taxonomical identification was done at East African Herbarium, National Museums of Kenya where voucher specimen has been deposited.

Extraction procedure

Stem bark of *P. africana* was grounded into a fine powder using a laboratory-grinding mill. The powdered material was soaked in methanol and hexane for 24 h each. The mixture was filtered and the organic solvent was evaporated to near dryness by vacuum evaporation using rotary evaporator.

Phytochemical screening

The components of the different extracts were separated by TLC (Kieselgel 60 F254 0.2 mm, Merck). The separated components were visualized under ultraviolet light (254 and 360 nm) and sprayed with 1% ferric trichloride, natural product reagent (1% methanolic 2-aminoethyl diphenylborate) (Wagner et al., 1984) or aluminium chloride for phenolic compounds, methanolic potassium hydroxide for coumarins (Harbone, 1973), Dragendorff's reagent for alkaloids and anisaldehyde/sulfuric acid for steroids and terpenes (Krebs et al., 1969).

Microbial test cultures

The following microorganisms were used as test cultures, *Staphylococcus aureus* ATCC 25923, *Pseudomonus aeruginosa* ATCC 27852, *Streptococcus pneumoniae,* methicillin resistant *Staphylococcus aureus (MRSA),* fungal strains used were; *Candida albicans* ATCC 90028, *Cryptococcus neoformans* ATCC 66031 (yeasts) and clinical isolates of dermatophytes namely *Microsporum gypseum*, and *Trichophyton mentagrophytes*. The selection of the test strains were based on their significance as opportunistic pathogens and their resistance to conventional drugs. These bacterial and fungal strains were either reference strains, quality control (QC) strains or clinical culture collections at the Mycology Laboratory, Opportunistic Infection laboratory, Kenya Medical Research Institute.

Antimicrobial assay

The antimicrobial activity of the extracts was evaluated by the disc diffusion method (Muanza et al., 1994). Test plates were prepared with Mueller-Hinton agar (Difco) and inoculated on the surface with a cell suspension of vegetative stage of test strain in sterile normal saline. In all cases, the concentration of the inoculum was adjusted to 1.5×10^8 CFU/mL. Test extract (approximately 14 mg) were dissolved in 1 mL of DMSO. Sterile paper discs (6.0 mm) were aseptically impregnated with 10 µL of the resulting solutions and then aseptically deposited on the surface of inoculated plates. After 24 and 72 h of incubation at 37, 35 and 30°C for bacteria, yeast and moulds respectively, the activity was determined by the presence of clear zones of inhibition around the test extracts. Discs of chloramphenicol (30 µg) and fluconazole (25 µg) were used as standard antibacterial and antifungal standards. Similarly, the extraction solvents were set as negative controls. Extracts with activity were serially diluted and re-tested to determine the minimum inhibitory concentrations (MIC). All the assays were carried out in triplicate and subjected to quality control procedures recommended by Clinical Laboratory Standard Institute (CLSI). Fluconazole disc was prepared as described by Klevay et al. (2005).

RESULTS

P. africana extracts demonstrated some antibacterial and antifungal activity against the test bacteria and fungal pathogens. Table 1 shows the zone of Inhibition and minimum inhibitory concentration of hexane and methanol extracts.

The methanol extract was particularly active against gram-positive bacteria *S. aureus* and gram-negative bacteria *P. aeruginosa*. However, its activity against the different test strains from the highest were; *S. pneumoniae, S. aureus* ATCC 25923, *P. aeruginosa* ATCC 27852 and methicillin resistant *Staphylococcus aureus (MRSA).* However, because the method relies of solubility and the rate of extract diffusion, small zones of inhibition does not always indicate low activity. Therefore, the Minimum Inhibitory concentrations were determined using agar and broth dilution method. All the test bacteria and fungal were sensitive to the extracts with MIC values of 0.073 to 2.50 mg/ml. The methanol extracts had the highest activity with a mean zone diameter of 21mm against *T. mentagrophytes* and 20 mm against *M gypseum* with MIC values of 0.0395 and 0.078 mg/ml, respectively, but both extract had no activity against the test yeast. It also had a broad-spectrum activity against the test bacterial isolates namely; *S. aureus* with an inhibition zone diameter of 20 mm and MIC of 0.073 mg/ml, and for Methicillin Resistant *S. aureus,* the zone diameter was 17 mm with MIC of 0.156 mg/ml. The inhibition zone diameter against *P. aeruginosa* was 15 mm and the MIC was 0.3125 mg/ml. *S. pneumoniae* was inhibited by 2.50 mg/ml of extracts with zone diameter of

Table 1. *In-vitro* activity of *P. africana* and minimum inhibitory concentrations against selected bacterial and fungal pathogens

Test strains	*Prunus africana* extracts		Standards
	ZD (mm) and (MIC) mg/ml		
	Hexane$^{\Phi}$	Methanol$^{\Phi}$	(Chloramphenical / fluconazole)
Methicillin resistant *Staphylococcus aureus* (MRSA)	-	17 (0.156)	9 (0.008)
Streptococcus pneumoniae (Clinical isolate)	-	12 (2.50)	22 (0.002)
Staphylococcus aureus ATCC 25923	-	20 (0.073)	21 (0.002)
Pseudomonas aeruginosa ATCC 27853	-	15 (0.3125)	18 (0.008)
Trichophyton mentagrophytes (Clinical)	-	21 (0.0395)	0.008*
Microsporum gypseum (Clinical)	-	20 (0.078)	0.016*
Candida albicans ATCC 90028	-	-	0.001*
Cryptococcus neoformans ATCC 66031	-	-	0.002*

Key: (ZD) zone diameter, (MIC) Minimum inhibitory concentration, – No activity, * MIC to fluconazole as determined by broth microdilution, (Φ) controls Hexane and methanol had no activity on the test strains

12 mm. The hexane extract didn't exhibit any activity against both bacteria and fungi.

DISCUSSION

P. africana extracts have been used for the treatment of chest infections and diarrhea (Kokwaro, 1993). Respiratory infections can be caused by both gram positive and negative bacterial including fungi. *Streptococcus pneumoniae* is the most common etiological agent of pneumonia. In the present study, In-vitro assay indicates that methanol extracts of *P. africana* extracts has activity on *S. pneumoniae* providing proof of the claimed therapeutic value in respiratory conditions. Its activity on gram negative bacteria particularly drug resistant strain demonstrates its potential for control of drug resistance strains. Other studies have demonstrated that extracts of *P. africana* inhibit Mycobacterium spp. (Fourneau et al., 1996). Mycobacterium spp. is important opportunistic respiratory pathogens threatening to develop resistance to all available antibiotics. Phytochemical analysis of *P. africana* extracts by Thin Layer Chromatography showed that the major secondary metabolites present were flavonoids which developed orange and red colors when sprayed with a natural product reagent and terpenes with violet-pink spots. There were no alkaloids in both the hexane and methanol extract however; the hexane extract had mostly the terpenes presence.

Flavonoids are known to be synthesized by plants in response to microbial infection (Dixon et al., 1983). They have been found to be effective antimicrobial substances against a wide array of microorganisms *in-vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins in bacterial cell walls. Lipophilic flavonoids have also been known to disrupt microbial membranes (Tsuchiya et al., 1996). Numerous studies have documented the antimicrobial activity of flavonoids (Pengsuparp et al., 1995).

Previous toxicity studies have demonstrated that *P. africana* extracts is non toxic. *In-vivo* toxicity test in rats indicates that *P. africana* extracts does not cause clinical or pathology abnormalities in rats at daily oral doses of up to 1000 mg/kg for 8 weeks. However, chloroform extracts caused marked clinical signs, organ damage and a 50% mortality rate at a dose of 3.3 g/kg for 6 days. The toxicity is characterized by marked centrilobular hepatocellular degeneration and necrosis, diffuse nephrosis, myocardial degeneration, lymphocytic necrosis and neurol degeneration (Gathumbi et al., 2002). However, in terms of general safety and toxicity, it is good to note that *P. africana* is currently used for treatment of prostrate hyperplasia (Sunderland and Obama, 1999; Schipmarn, 2001). Although *P. africana* is not reported for use in fungal skin conditions, our assay indicates its activity on dermatophytes which presents its potential for control of dermatphytoses.

Conclusion

The methanol extract of *P. africana* showed promising activity against gram negative, gram-positive bacteria and the dermatophytes supporting its traditional uses and providing scientific proof of its claimed efficacy against dermatophytoses and skin condition. Its activity against Methicillin Resistant *Staphylococcus aureus* means it is a potential good source of non toxic antimicrobial agents for both drug sensitive and drug resistant strains. Further investigation is therefore worthwhile.

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