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Preliminary report on the *in vitro* antibacterial activity of *Bryophyllum pinnatum* leaf juice

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Summary

The juice from the leaves of *Bryophyllum pinnatum* S. Kurtz (Crassulaceae) was tested for antibacterial activity. The extract at 5% v/v was bactericidal to a wide spectrum of Gram-positive and Gram-negative bacteria such as *Bacillus subtilis, Staphyllococcus aureus, Streptococcus pyogenes; Streptococcus faecalis; Escherichia coli; Proteus* spp; *Klebsiella* spp; *Shigella* spp; *Salmonella* spp; *Serratia marcescens*; and *Pseudomonas aeruginosa* including the clinical isolates of these organisms possessing multiple antibiotic resistance.

Résumé

Le jus des feuilles de *Bryophyllum pinnatum* S. Kurtz (Crassulaceae) était expérimenté pour l'activité anti-bactérienne. L'extrait à 5% v/v était bactéricide envers un échantillon à la fois de la bactérie Gram-positive et Gram-negative telle que *Bacillus subtilis; Staph aureus; Strep pyogenes; Strep faecalis; Escherichia coli; Proteus* spp; *klebsiella* spp; *Shigella* spp; *Salmonella* spp; *Serratia marcescens* et *Pseudomonas aeruginosa* y compris celle (c'est-à-dire la bactérie) qui est isolée dans la clinique et qui possède de la multiple résistance antibiotique.

Introduction

Bryophyllum pinnatum S. Kurtz (Crassulaceae) leaves are commonly used by Nigerians of Edo tribe as an antitussive and antibacterial agent in the healing of septic wounds. In such therapy, the leaves are warmed over a flame such as a candle flame for a few minutes, the juice of the succulent leaves expressed by hand and applied as a remedy. The whole plant of *Bryophyllum pinnatum* has been documented to contain saponins, flavonoids and tannins, and also to have diuretic, antipyretic, carminative, antiseptic and analgesic properties (Adesina, 1983). As far as we know, the antimicrobial property of *Bryophyllum pinnatum* has not been studied. This study is an evaluation of the potential antimicrobial activity of the crude juice of the *Bryophyllum pinnatum* leaves as claimed in traditional medicine in Nigeria.

Materials and methods

Juice extract

Authenticated samples of the succulent leaves of *Bryophyllum pinnatum* were collected during the rainy season (April–July), macerated using a pestle and mortar and centrifuged at 4500 rpm for 10 min. The supernatant was concentrated by distilling off the water content at about 90°C under an atmosphere of dry nitrogen to a quarter of the original volume and stored in sterile bottles at −5–0°C.

Organisms

The organisms used in this study and their properties are listed in Table 1.

Antibacterial agents

Ampicillin (Glaxo laboratories Ltd.); erythromycin stearate B.P. (Abbot labora-

Table 1. Organisms used to evaluate the antimicrobial activity of the honey distillate

Organism	Relevant properties	Source and reference
Escherichia coli K12 J53	Wild type antibiotic susceptible	Crumplin & Smith (1981)
Escherichia coli K12 (pR222)	R-plasmid-mediated resistance to Sm, Cm, Tc, Su	Crumplin & Smith (1981)
Escherichia coli K12 J53 (pBN100)	R-plasmid-mediated resistance to NT, Tp, Sm	Laboratory stock
Escherchia coli W667 (pH(pJR225)	R-plasmid-mediated resistance to Gm, Ap, Tc, Cp, Ka, Nm	J. Davies, Biogen, SA, Switzerland
Proteus mirabilis	Resistant to Ap, Tc, Su, Sm, cfx, Cm, Ey	Clinical isolate
Proteus rettgeri	Resistant to Ap, NT, Sm, cfx, Tc, Ey	Clinical isolate
Klebsiella aerogenes	Resistant to Tc, Ap, Su, Na, Sm, Ey	Clinical isolate
Pseudomonas aeruginosa	Resistant to Gm, Cb, Ap, Sm, Tp, Na, Ey	Clinical isolate
Pseudomonas aeruginosa NCTC 6749	Ap, Sm, Na, Ey	Laboratory stock
Coagulase-positive *Staph. aureus*	Cb, Tc, NT, Ap, Cm, Na, Ey	Clinical isolate
Oxford *Staph aureus* NCTC 6571	Wild-type strian susceptibility, Na	Laboratory stock
Serratia marcescens	Resistant to Ap, Tc, NT	Clinical isolate
Strept. faecalis	Resistant to Tc, Su, Na, Ey	Clinical isolate
Strept. pyogenes	Resistant to Ap, Tp, Sm, Na, Ey	Clinical isolate
Salmonella spp	Resistant to Ap, Ey	Clinical isolate
Shigella spp	Resistant to Tc, Su, Ey	Clinical isolate
Bacillus subtilis	Resistant to Su, Ap	Clinical isolate
Candida albicans		Laboratory stock
Penicillium spp		Laboratory stock
Aspergillus niger		Laboratory stock

Sm, streptomycin; Gm, gentamicin; Ka, kanamycin; Nm, neomycin; Ap, ampicillin; Tc, tetracycline; Su, sulphonamide; cfx, cefotaxime; Cm, chloramphenicol; Cb, carbenicillin; NT, nitrofurantoin; Na, nalidixic acid; Tp, trimethoprim, Ey, erythromycin.

tories, England); gentamicin sulphate (Nicholas laboratories Ltd., England).

Media

Diagnostic Sensitivity Test agar (Oxoid) pH 7.4; nutrient broth No. 2 (Oxoid) pH 7.4; sabouraud liquid medium (Oxoid) pH 5.4; sabouraud dextrose agar (Oxoid) pH 5.4.

Determination of minimum inhibitory concentration (M.I.C.)

10^5–10^6 bacteria colony forming cells of overnight broth cultures or 10^2 dilution in sterile saline of the 2-day fungal cultures were spotted respectively onto a series of overdried D.S.T. or sabouraud agar plates containing progressively increasing concentrations of the antibacterial agents. The plates inoculated with bacterial organisms were incubated at 37°C while those with fungal organisms were incubated at 25°C for a period of about 48 h.

Antimicrobial assay

Appropriate agar plates were seeded with the appropriate organisms. A standard cork borer was used to make uniform and equidistant wells on the surface of the agar, and known dilutions of the antimicrobial agents were added. The plates were suitably incubated at 35°C for 24 h and the zones of inhibition measured.

Phytochemical tests

Standard basic methods were employed to test for the presence of sugar, protein, volatile oil, glycoside, alkaloid, saponin, tannin, etc (Sofowora, 1982) in the extract.

Results

The juice was purple in colour, bacteriologically sterile on aseptic extraction and of pH 4.2. The yield of the extract residue after distillation was about 20% w/w. The antibacterial activity of the juice after removing the water content was twice that of the juice on extraction. The juice exhibited a broad spectrum antibacterial activity (Table 1). The bactericidal M.I.C. of the distilled residue fraction was 5% v/v irrespective of the presence of chromosomal or R-plasmid-mediated antibacterial resistance genes on the bacterial strains. The juice did not possess antifungal activity against *Aspergilus niger*, *Penicillum* spp and *Candida albicans*.

The antibacterial activity of this extract was not pH dependent. The effect of the extract's acidity was critically investigated by determining the antibacterial activity at varying pH values using citric acid/phosphate buffered D.S.T. agar pH 4.0–8.0.

A comparison of the antibacterial activity of this extract and some standard antibacterial agents such as ampicillin, erythromycin and gentamicin was determined (Table 2). The result indicated that the antibacterial activity of this extract at 5% w/v compared favourably with 2 µg/ml ampicillin; 1.5 µg/ml erythromycin and 0.8 µg/ml gentamicin. Bacterial resistant mutants could not be selected at 5% v/v and at bacteriostatic concentrations of 2–3% v/v. The extract was found to contain saponin and alkaloid.

Discussion

The results basically justify the scientifically undetermined reason for the use of this juice as

Table 2. Zone of inhibition (mm) of the organisms by the test antimicrobial agents

Organisms	Extract (5% v/v)	Ampicillin (2 µg/ml)	Gentamicin (0.8 µg/ml)	Erythromycin (1.5 µg/ml)
E.coli K12 J53	18.5	18	19	18
E.coli K12 J53(pR222)	19	19	20	18
E. coli K12 J53(pBN100)	18	20	19	19
E.coli W667 (pJR225)	19	resistant	resistant	20
Proteus mirabilis	20	resistant	21	resistant
Proteus rettgeri	21	resistant	21	resistant
Klebsiella aerogenes	19	resistant	21	resistant
Pseudomonas aeruginosa	19	resistant	resistant	resistant
Pseudomonas aeruginosa NCTC 6749	20	resistant	resistant	resistant
Staph. aureau	18	resistant	20	resistant
Oxford *Staph. aureus* NCTC 6571	18.5	20	21	19
Serratia marcescene	17	resistant	20	18
Strept. faecalis	19	20	20	resistant
Strept. pyogenes	18.5	19	21	resistant
Salmonella spp	20	resistant	21	resistant
Shigella spp	20	20	21	resistant
Bacillus subtitis	18	resistant	21	18
Candida albicans	resistant	NT	NT	NT
Penicilium spp	resistant	NT	NT	NT
Aspergilus niger	resistant	NT	NT	NT

NT, Not tested.

an antimicrobial agent. The antimicrobial activity does not seem to be due to intracellular lytic enzyme(s) of the leaf, released because of the rupture of the leaf morphology during maceration or expression. The antibacterial activity of the juice was retained after subjecting it to the distillation process indicating thermal stability of the antibacteriologically active principles. The antimicrobial activity does not seem to be due to the acidity of the juice because on exposure to buffered agar and broth antibacterial activity was still retained. The antibacterial activity of this extract seemed seasonal because the juice of the leaves obtained at dry season had no antibacterial activity. The broad spectrum antibacterial activity, bactericidal activity against *P. aeruginosa* and the inability to select resistant mutants qualify the extract to be further examined as a potential antibacterial agent. Since the juice has been used since time immemorial by Nigerians as an antitussive and in the healing of wounds with no reported or adverse toxic effects, this juice could be improved for varying chemotherapeutic purposes. Presently, phytochemistry and mode of antibacterial action of this juice is in progress.

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