Bioactivity of extracts and components of *Pteleopsis myrtifolia*

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"It can seem a formidable task, faced with a litre of fermentation broth - a dark, viscous sludge - knowing that in there is one group of molecules that has to be separated from all the rest. Those molecules possibly represent only about 0.0001%, or 1 ppm of the total biomass and are dispersed throughout the organism, possibly intimately bound up with other molecules. Like the proverbial needle in a haystack, you have to remove lot of hay to be left with just the needle, without knowing what the needle looks like or where in the haystack it is."

Richard J.P. Cannell

SUMMARY

Combretaceae contain several species with bioactive properties - especially the genera *Combretum* and *Terminalia*. *Pteleopsis myrtifolia* and *Quisqualis littorea* belong to this family and have not previously been thoroughly investigated for their bioactivity. Leaf and fruit extracts of *P. myrtifolia* and leaf extracts of *Q. littorea* were separated by three different thin layer chromatography eluent systems. For all leaf and fruit material, the largest amount of acetone soluble material was extracted with extractants of intermediate polarity. Antibacterial activity of 30 extracts was investigated using a microplate serial dilution method and bioautography. The four most important nosocomial pathogens that are used worldwide namely two Gram-positive: *Staphylococcus aureus* and *Enterococcus faecalis*, and two Gram-negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli* were used as test organisms. Areas of growth inhibition were best defined after an eluent system that separates compounds of intermediate polarity had been used. The Gram-positive bacteria were most sensitive to some extracts of *P. myrtifolia* leaves. Fruit extracts exhibited minimum inhibitory concentrations (MIC) values as low as 0.04 mg/ml, less than that of the allopathic antibiotics, ampicillin and chloramphenicol. *Q. littorea* leaf extracts had an average MIC value of 0.32 mg/ml for Gram-negative bacteria. The average antibacterial activity expressed as total activity for each bacterium was higher in the leaves than in the fruit of *P. myrtifolia*. After considering the amount of antibacterial compounds extracted, toxicity of extractants to test organisms and miscibility of several extractants, acetone was eventually chosen as the best extractant for future extractions.

Results obtained in this investigation showed clearly that *P. myrtifolia* leaves, fruit, and *Q. littorea* leaves contain several antibacterial compounds.

Five different extracts of *P. myrtifolia* leaves were tested for growth inhibitory effects on different

human cell lines (MCF-12, MCF-7, H157, $WHCO_3$, HeLa). The non-cancerous MCF-12A cell line's growth was not inhibited extensively, and the cancer cell lines - MCF-7, H157, $WHCO_3$ and HeLa, differed in their sensitivity to the plant extracts. This indicated that the plant extracts' effects were selective and not due to general toxicity. The effect of some extracts on certain cell lines, especially $WHCO_3$, was growth inhibitory but not lethal. This is the desired effect – to inhibit growth of cancer cells, but not to be toxic to cells in general. The presence of tannin in extracts either promoted or inhibited growth inhibition of different cell lines.

The same extractants that were used for cytotoxic tests were investigated for their antioxidant activity. All extracts gave positive scavenging capacity with the 1,2-diphenyl-2-picrylhydrazyl assay. The cold water, methanol and hot water extracts had vitamin C equivalents of 0.34, 0.20 and 0.147 mg/g respectively, all more than that of black tea.

The solvent-solvent separation of *P. myrtifolia* leaves was started with acetone as an initial extractant. Separation was undertaken with immiscible solvents of different polarities. All fractions had antibacterial activity against the Gram-positive bacteria. The chloroform fraction was antibacterial to all bacteria tested, and had the largest amount of antibacterial compounds.

Pure compounds were isolated from the chloroform fraction by column chromatography. One pure compound's structure was elucidated as a pentacyclic triterpenoid, taraxerol ($C_{30}H_{50}O$).

Taraxerol had MIC values of 0.04, 0.016, 0.63 and 0.31 mg/ml for the bacteria *S. aureus*, *E. faecalis, P. aeruginosa* and *E. coli* respectively. It significantly inhibited growth of the human lung cancer cell line H157 and did not display free radical scavenger activity.

This is the first report of the antibacterial activity of several extracts from *P. myrtifolia* and *Q. littorea,* growth inhibition effects of several *P. myrtifolia* leaf extracts on the human cell lines

MCF-12, MCF-7, H157 and $WHCO_{3,}$ the isolation of taraxerol from *P. myrtifolia* leaves, taraxerol's antibacterial activity for above-mentioned test organisms, and growth inhibition effects on human cancer cell lines MCF-7, H157, $WHCO_3$ and HeLa.

OPSOMMING

Combretaceae word gekenmerk deur die teenwoordigheid van baie spesies met bioaktiewe eienskappe, veral van die genusse *Combretum* en *Terminalia*. *Pteleopsis myrtifolia* en *Quisqualis littorea,* wat tot hierdie familie hoort, is nog nie voorheen deeglik vir bioaktiwiteit ondersoek nie. Blaar- en vrugekstrakte van *P. myrtifolia,* en blaarekstrakte van *Q. littorea* is met drie verskillende elueermiddels en dunlaagchromatografie (DLC) ontwikkel. Van al die blaar- en vrugmateriaal is die grootste hoeveelheid asetoon-oplosbare materiaal deur ekstraheermiddels met intermediêre polariteit geëkstraheer. Antibakteriële aktiwiteit van 30 ekstrakte was ondersoek met serie verdunnings in multi-putjie plate en bioautografie. Die vier belangrikste nosokomiale patogene wat wêreldwyd gebruik word, naamlik twee Gram-positiewe: *Staphylococcus aureus* en *Enterococcus faecalis*, en twee Gram-negatiewe bakterië: *Pseudomonas aeruginosa* en *Escherichia coli* was as toetsorganismes gebruik. Areas van groei-inhibisie was die duidelikste ná ontwikkeling met ‘n elueermiddel van intermediëre polariteit. Die Gram-positiewe bakterië was die sensitiefste vir sommige ekstrakte van *P. myrtifolia* blare en vrugte met sommige minimum inhibitoriese konsentrasie (MIK) waardes so laag as 0.04 mg/ml - minder as die van die allopatiese antibiotika, ampisillien en chlooramfenikol. *Q. littorea* blaarekstrakte het ‘n gemiddelde MIK waarde van 0.32 mg/ml vir Gram-negatiewe bakterië gehad. Die gemiddelde aktiwiteit voorgestel as totale aktiwiteit, was vir elke bakterium hoër in die blare as in die vrugte van *P. myrtifolia*. Nadat hoeveelheid antibakteriële verbindings geëkstraheer, toksisiteit vir toetsorganismes en mengbaarheid van verskeie ekstrakte in ag geneem is, is daar eventueel besluit dat asetoon die beste ekstraheermiddel vir toekomstige ekstraksies is.

Resultate uit hierdie ondersoek verkry, toon duidelik dat *P. myrtifolia* blare, vrugte en *Q. littorea* blare verskeie antibakteriële verbindings bevat.

Vyf verskillende *P. myrtifolia* blaar ekstrakte is vir groei-inhiberende effekte teen menslike sellyne (MCF-12, MCF-7, H157, $WHCO_3$ en HeLa) getoets. Die nie-kankeragtige MCF-12A sellyn se groei is nie ekstensief geïnhibeer nie, en die kanker sellyne - MCF-7, H157, $WHCO_3$ en HeLa, het verskil in hul sensitiwiteit vir verskillende plant ekstrakte. Dit toon dat die plantekstrakte se effek selektief was en nie as gevolg van algemene toksisiteit nie. Die effek van sommige ekstrakte op spesifieke sellyne, veral $WHCO_3$, was groei inhiberend maar nie dodelik (toksies) nie. Hierdie is die gewensde effek – dat kankerselle se groei geïnhibeer sal word, maar nie sterf as gevolg van toksisiteit in die algemeen nie. Die teenwoordigheid van tanniene in ekstrakte het die sellyne se groei minder of soms meer geïnhibeer.

Dieselfde ekstrakte as wat vir sitotoksiese toetse gebruik was, is ondersoek vir hul antioksidant aktiwiteit. Alle ekstrakte het ‘n positiewe suiwerings aktiwiteit met 1,2-difeniel-2-pikrielhidrasiel getoon. Die koue water, metanol and warm waterekstrakte het vitamien C ekwivalente van of 0.34, 0.20 en 0.147 mg/g respetiewelik gehad, almal meer as dié van swart tee.

Die groepskeiding van *P. myrtifolia* blare is met asetoon as inisiële ekstraheermiddel begin. Skeiding is bewerkstellig met onmengbare vloeistowwe van verskillende polariteite. Alle fraksies was aktief teen Gram-positiewe bakterië. Die chloroformfraksie was antibakteriëel vir all die bakterië, en het die meeste antibakteriële verbindings gehad.

Suiwer verbindings is deur kolomkromatografie uit die chloroformfraksie geïsoleer. Een suiwer verbinding se struktuur is geïdentifiseer as ‘n pentasikliese triterpenoïed, taraxerol ($C_{30}H_{50}O$).

Taraxerol het MIK waardes van 0.04, 0.016, 0.63 en 0.31 mg/ml vir die bakterië *S. aureus*, *E. faecalis, P. aeruginosa* en *E. coli* respektiewelik gehad. Dit het die groei van die menslike longkanker sellyn H157, betekenisvol geïnhibeer en nie antioksidant aktiwiteit getoon nie.

Hierdie is die eerste verslag van antibakteriële aktiwiteit van verskeie ekstrakte van *P. myrtifolia* en *Q. littorea,* inhibisie van groei van die menslike sellyne MCF-12, MCF-7, H157 en $WHCO_3$ deur verskeie *P. myrtifolia* blaarekstrakte, die isolasie van taraxerol uit *P. myrtifolia* blare, taraxerol se antibakteriële aktiwiteit vir bogenoemde toetsorganismes, antioksidant aktiwiteit en groei-inhibeerende effekte op die bogenoemde menslike kanker sellyne.

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List of abbreviations

Abbreviation	Explanation
ATCC	American Tissue Culture Collection
BEA	benzene: ethanol: ammonia (36:5.4:4)
^{13}C-NMR	carbon-nuclear magnetic resonance
CEF	chloroform: ethyl acetate: formic acid (5:4:1)
COSY	correlated spectroscopy
DMBA	7,12-dimethylbenz[a]anthracene
DMSO	dimethylsulfoxide
DPPH	1,2-diphenyl-2-picrylhydrazyl
E. coli	*Escherichia coli* (ATTC 27853)
EMW	ethyl acetate: methanol: water (40:5:4.4)
GI_{50}	growth inhibition by 50%
^{1}H	proton
HMQC	heteronuclear multiple quantum correlation
^{1}H-NMR	proton-nuclear magnetic resonance
INT	p-iodonitrotetrazolium violet
LC	lethal concentration
MIC	minimum inhibitory concentration
MS	mass spectroscopy
MWP	multiwell plate
MWP-96	96-multiwell plate
E. faecalis	*Enterococcus faecalis* (ATTC 29212)
NCCLS	National Committee for Clinical Laboratory Standards
NMR	nuclear magnetic resonance (spectroscopy)

P. aeruginosa	*Pseudomonas aeruginosa* (ATTC 25922)
rpm	revolutions per minute
S. aureus	*Staphylococcus aureus* (ATTC 29213)
TLC	thin layer chromatography
TMS	tetramethylsilane
UV	ultra violet