Evaluation of antimicrobial activity of Parankipaddai Kudineer (decoction) used in skin diseases

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Parankipaddai kudineer (PPK) is a decoction used in Siddha medicine. It is recorded in the 12 volume classic "Pararajasekaram siraroganithanam", written in poetic form. It is mentioned as a treatment for skin diseases in this classic work. It is prepared from parts of 12 medicinal plants, namely, *Smilax chinensis, Indigofera aspalathoides, Enicostemma littorale, Cassia angustifolia, Azima tetracantha, Embelia ribes, Zingiber officinale, Piper nigrum, Piper longum, Myristica fragrans* (seed and leaves), *Syzygium aromaticum* and *Hyoscyamus niger.* Although PPK is used in the treatment of skin diseases, its antimicrobial activity has not been previously reported. Therefore, the effectiveness of this decoction was evaluated to determine its antimicrobial activity including resistant bacteria against skin pathogens and identify the antimicrobial active compound(s). The study of the decoction as well as its individual plant components is a timely study due to the rapidly increasing demand for detection of novel antimicrobial agents from plant sources, particularly for the treatment of infections caused by multiresistant antibiotic resistant pathogens.

The PPK decoction, methanol extracts of PPK decoction and extracts of individual plants were prepared. Preliminary phytochemical screening of PPK and individual plant extracts was carried out. To identify the active components, sequential extraction of PPK plants was carried out using hexane, dichloromethane (DCM), ethyl acetate, methanol and water, based on their polarity from less polar to high polar. Using TLC, chemical components of each extract was identified. For further identification, active extracts were fractionated using vacuum liquid chromatography. Nine fractions from the hexane extract and 8 fractions from the DCM extract were obtained. Extractions using non polar/less polar solvents suggested that the activity is associated with non/less polar compounds. As plant oils also contains similar compounds, oils were distilled from PPK and its component plants, using the Clevenger apparatus. GC-MS analysis of PPK oil confirmed the presence of compounds. Of the individual plants tested, *S. aromaticum* was identified as a highly active plant,

and its oil was subjected to column chromatography. Eugenol was isolated from the fraction by preparative TLC. Screening of extracts for antibacterial activity was performed using the cut well agar method for decoctions and methanol extracts, and disc diffusion method for plant fractions and oils. The minimum inhibitory concentration (MIC) of decoction and methanolic extracts was determined by the agar dilution method, for the decoctions and methanolic extracts, a broth dilution method was used for the fractions and oils. All experiments were conducted using standard aseptic techniques. Antibacterial activity of decoctions and methanolic extracts were evaluated against eight bacterial isolates (*Staphylococcus aureus* NCTC 6571, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 10662 and five wild strains of Methicillin Resistant *Staphylococcus aureus* (MRSA). The oils of PPK and its component plants were evaluated against a spectrum of organisms including sensitive, standard organisms, Gram positive cocci, multi resistant Gram negative bacilli and 5 species of *Candida*. The test organisms were obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya.

Both, PPK decoction and methanol extracts of PPK plants inhibited the growth of methicillin sensitive as well as resistant *S. aureus*. Methnol extracts of the 12 plants showed inhibitory activity (ZOI 14-32 mm; MIC 0.2–15 mg/ml) against *S. aureus*. However, only 9 of the 13 plant decoctions showed activity against *S. aureus* at dilutions ranging from 1/5 to 1/160 (Table 1). Both, the decoction and methanolic extract of *S. aromaticum* inhibited *E. coli*. Methanolic extracts of *E. ribes* and *C. angustifolia* also inhibited *E. coli*. Both, the decoction and methanol extracts of *S. aromaticum* and *E. ribes* inhibited *P. aeruginosa*. Decoctions of *S. chinensis, I. aspalathoides* and *P. longum* and methanolic extract of *C. angustifolia* also inhibited *P. aeruginosa*. Preliminary phytochemical analysis of PPK (Fig. 2) and its individual plants showed that terpenoids are consistently present in 11 of the 12 plant species that showed inhibitory activity against *S. aureus* (Table 1).

The MICs of hexane, dichloromethane (DCM) and ethyl acetate extracts for *S. aureus* were less than

Table 1. Phytochemicals in PPK decoction, methanol extracts of PPK and extracts of individual plants with corresponding minimum inhibitory concentration (MIC)

Plants	Water soluble chemicals (decoction)			*Methanol soluble chemicals*					*Sensitive S. aureus*		MRSA	
	Terpenoids	tannins	saponin	Terpenoids	tannins	saponin	*flavonois*	*alkaloids*	decoction	Methanolic extract MIC mg/mL	decoction	Methanolic extract MIC mg/mL
S aromaticum	+	+	-	+	+	-	-	-	1/160	0.2	1/160	0.2
E.ribes	+	+	+	+	+	+	+	+	1/80	0.2	1/80	0.2
C.angustifolia	+	+	+	+	+	+	-	+	1/80	0.7	1/80	0.7
P.longum	+	-	+	+	-	+	-	+	1/40	5	1/40	5
I.aspalathoides	+	+	-	+	+	-	+	+	1/20	0.7	1/20	0.7
M.fragrans leaf	+	+	-	+	+	-	+	+	1/10	1	1/10	1
PPK	+	+	+	+	+	+	+	+	**1/10**	**1.7**	**1/10**	**1.7**
S.chinensis	+	-	+	+	-	+	+	+	1/5	3.2	1/5	3.2
Z.officinale	+	-	+	+	-	+	+	+	1/5	3.3	1/5	3.3
P.nigrum	+	-	-	-	-	-	-	+	1/5	4.5	-	4.5
M.fragra.seed	+	-	-	+	-	-	+	+	1/5	5	1/5	5
E.litorale	+	+	+	+	+	+	+	+	1/5	10	1/5	10
A.tetracantha	-	-	+	-	-	+	-	+	-	10	-	15
H.niger	+	-	-	+	-	-	--	+	-	0.8	-	1.5

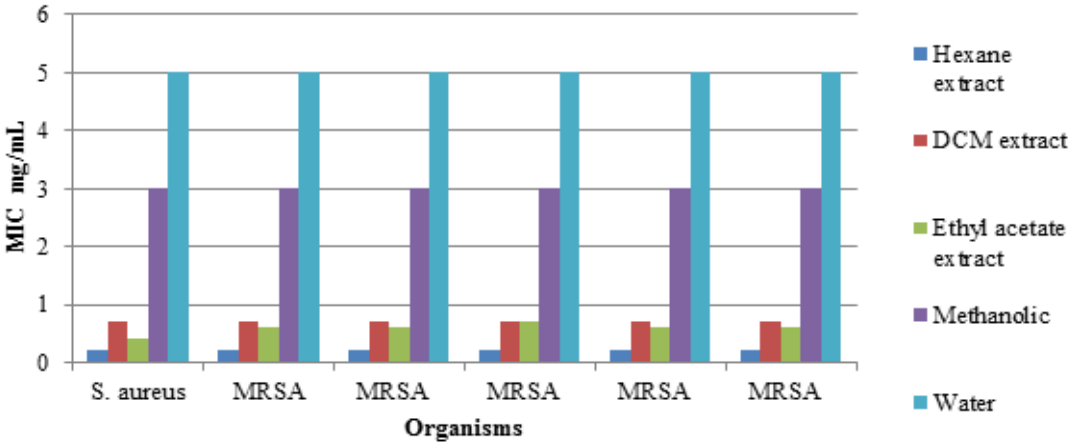

Figure 1. Minimum inhibitory concentration (MIC) (mg/mL) of the sequential extracts of PPK (Mean ± SD)

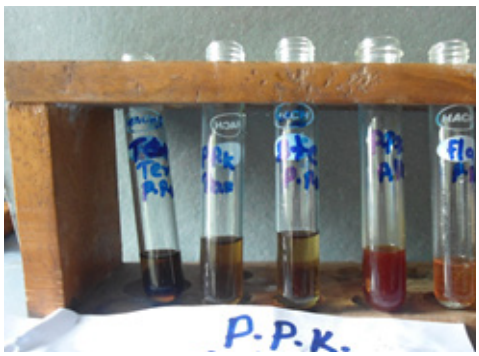

Figure 2. Phytochemical screening of PPK

the MICs of the methanol and water extracts. Mean ± SD of MICs of hexane, DCM and ethyl acetate extracts for all tested strains of *S. aureus* ranged from 0.2±0.0 to 0.7±0.3 mg mL^{-1} suggesting that the active ingredients were less polar or medium polar compounds(Fig 1). Fractions Hexane 1, DCM 1, Hexane 4, Hexane 5 and DCM 5 possess inhibitory activity against *S. aureus* and *Candida* sp. H1 and D1 contain β-caryophyllene, β-pinene and limonene. Fraction H4, 5 and D5 contain several compounds including eugenol.

Oil obtained from PPK and 6 of the 12 individual plants were tested for antimicrobial activity against a wide spectrum of multidrug resistant (MDR) bacteria and *Candida* sp. *S.aromaticum* was the most active with MICs of 0.005 – 0.037 µg/ml for *S. aureus*, 0.018–0.15 µg/mL for MDR Gram negative Bacilli and 0.004 µg/ml

Table 2. Minimum inhibitory concentration (MIC) of PPK oil, S. aromaticum oil and isolated Eugenol

Plant oils	**Panel-1-Control strains**			**Panel-2-Gram positive cocci**		**Panel-3-MDR Gram negative bacilli**				**Pael-4-Candida sp**
	S.aureus NCTC 6571	*E.coli* NCTC 10418	*P.aeruginosa* NCTC 10662	5 MRSA strains	VRE	*K.pneumonea*	*E.cloacae*	*Acinetobacter*	*Proteus*	*Candida sp*
PPK oil	0.05	0.32	0.64	0.160 –0.320	0.640	0.310-1.250	0.310	0.150	0.310	0.075 – 0.310
S.aromaticum oil	0.005	0.018	0.31	0.018 –0.037	0.075	0.037–0.075	0.150	0.018	0.037	0.004
Isolated eugenol	0.009	0.018	0.31	0.037 –0.310	0.009	0.018	0.009	0.0045	0.009	0.002

for *Candida* sp (Table 2). Eugenol was identified as the main component in PPK and *S. aromaticum* oil, using GC–MS analysis and column chromatography.

The study infers that PPK decoction (Fig. 3) contains many compounds at very low Minimum inhibitory concentrations (MICs), important to human pathogens.

Figure 3. Parankipaddai Decoction

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Thesis Reference

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