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Conducted with the permission and endorsement of Guringai elders

Alternative antibacterial treatments through native Australian plants

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Abstract

This study investigated the antibacterial properties and phytochemical contents of seven native Australian plants used in traditional Indigenous medicine. Methanolic plant extracts were tested for antibacterial activity against the bacteria *S. epidermis* and *E. coli* and measured for their phenolic and flavonoid contents. All extracts were found inhibitory of the bacteria, with greater inhibition correlating with higher phenolic contents and unexpectedly lower flavonoid contents. The greatest inhibition and phenolics were observed in *Angophora costata* and *Persoonia linearis*, which were both understudied. Further research is required to isolate the bioactive compounds and evaluate their large scale therapeutic efficacy.

Literature review

Introduction: The global health crisis of antimicrobial resistance (AMR) is estimated to cause 10 million deaths annually by 2050 (UNEP, 2023). As traditional antibiotics become increasingly ineffective, the need for alternative treatment strategies becomes more demanding (Plackett, 2020). Medicinal plants serve as a promising avenue for antibacterial drug discovery, and despite their widespread usage to treat infection, most have not yet been researched (Wangchuk et al., 2020). Their antibacterial effects are due to bioactive compounds called phytochemicals (Khameneh et al., 2021). Out of the main classes of phytochemicals, polyphenols, which include flavonoids and non-flavonoids, have the most potent antibacterial properties (Hochma et al., 2021).

Indigenous medicine:
For millennia, the Indigenous peoples of Australia have used native flora in the treatment of wounds and infections (Simpson et al., 2013). Yet only a limited number of these species have been scientifically studied (Packer et al., 2012). A review of traditional uses, known phytochemicals, and antibacterial activity of the medicinal plants selected for this study was conducted to collate with the experimental findings. For example, Smilax glyciphylla leaves (*Figure 1)* were used as a tea to treat septic infections and skin wounds. Polyphenols had previously been isolated, and some antibacterial activity had been observed (Packer et al., 2015; (Akter et al., 2016).

Figure 1: Smilax glyciphylla, one of the plants examined

Methodology

Samples of A. costata (Sydney red gum), C. saligna (Willow leaved crowea), S. glomulifera (Turpentine), L. petersonii (lemon-scented tea tree), P. linearis (Geebung), C. citriodora (lemon-scented gum), and S. glyciphylla (Sarsaparilla) were collected and extracted with anhydrous methanol. Antibacterial activity was quantified through a disc diffusion assay against *S. epidermis* and *E. coli*, measuring zone of inhibition (ZOI) compared to an antibiotic positive control and negative control. Total phenolic content (TPC) was determined through the Folin-Ciocalteu method (*Figure 2)* and expressed as a gallic acid equivalent (GAE) concentration. Total flavonoid content (TFC) was determined through the aluminium chloride method and expressed as a quercetin equivalent (QE) concentration.

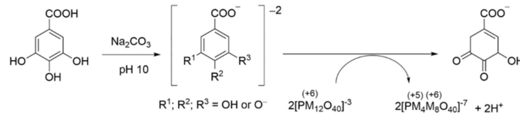

Figure 2: oxidation of gallic acid through the Folin-Ciocalteu method, forming large metal complexes that can be detected by measuring absorbance at a specific wavelength. The aluminium chloride method has a similar principle.

Scientific research question:
Do medicinal plants traditionally used by Indigenous Australians exhibit *in vitro* antibacterial activity, and is this correlated with their phytochemical profiles?

Hypothesis: All plant extracts will exhibit antibacterial activity compared to control groups. Greater antibacterial activity will be correlate with higher polyphenol content.

Null hypothesis: Plant extracts will not exhibit antibacterial activity compared to control groups and this will not be related to their polyphenolic contents.

Results & analysis

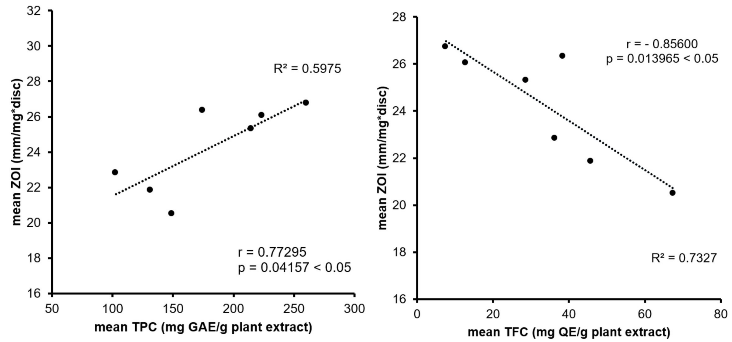

Figure 3: Correlation of total phenolic content (TPC) (left) and total flavonoid content (TFC) (right) with mean zone of inhibition

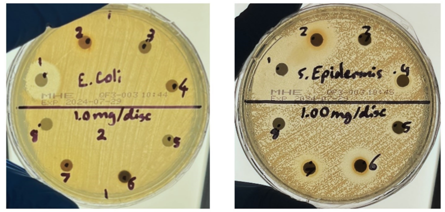

Figure 4: Agar plates of 1.00 mg/disc samples against E. coli (left) and S. epidermis (right). 1: Cephalexin, 2: A. Costata, 3: C. Saligna, 4: S. Glomulifera, 5: L. Petersonii, 6: P. Linearis, 7: C. Citriodora, 8: S. Glyciphilla.

Figure 4 shows that moderate antibacterial activity was observed across all extracts, with S. epidermis displaying greater susceptibility then E. coli. Single-factor analysis of variance (ANOVA) revealed statistically significant differences in zones of inhibition between the extracts and control groups ($p < 0.001$), suggesting they exhibited prominent antibacterial activity. As shown in *Figure 3,* higher antibacterial activity correlated with higher TPC ($r = 0.773$; $p<0.05$), consistent with prior studies, but it also correlated with lower TFC ($r = -0.856$; $p<0.05$); an unexpected result suggesting lower antibacterial potency from the flavonoids present.

Conclusion

Medicinal plants used by Indigenous Australians are a potentialsource of antibacterial phytochemicals in the fight against drug resistant bacteria. This primary study aimed to compare the antibacterial activities and phytochemical profiles of selected medicinal plants, finding that plant extracts exhibited statistically significant inhibitory effects likely due to the presence of phenols. Future research directions include spectrometric analysis to isolate phytochemicals of interest and *in vivo* testing to assess their antibacterial effects in live organisms.

Read the full paper with references here: