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**ANTI-OXIDANT AND ANTI-TYROSINASE
NATURAL PRODUCTS OF *NYMPHAEA STELLATA***

A PROJECT REPORT PRESENTED BY

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MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

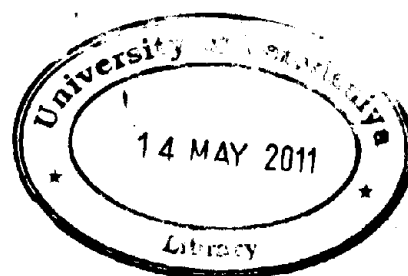
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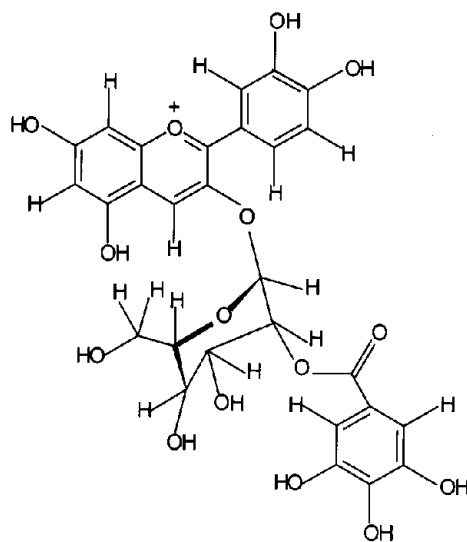
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In search of anti-oxidants and anti-tyrosinase natural sources, the anti-oxidant and anti-tyrosinase activities of *Nymphaea stellata* stamens (Manel, Nilmanel, *Nymphaeaceae*) were identified. Air-dried stamens of *N. stellata* were sequentially extracted into hexane, ethyl acetate and ethanol. Bio-assay guided fractionation of the sequential ethanol extract by chromatography followed by recrystallization afforded six compounds. Structure elucidation of compound **6** was achieved by ^1H NMR, ^{13}C NMR and DEPT spectroscopic techniques. The structure of compound **6** was tentatively established as cyanidin derivative, which is cyanidin 3-*O*-(2''-*O*-galloyl- β -galactopyranoside) and exhibited very good anti-oxidant activity ($\text{IC}_{50} = 5.44 \pm 1.02$ ppm).



Compound **6**, cyanidin 3-*O*-(2''-*O*-galloyl- β -galactopyranoside)

Anti-oxidant activity of extracts and fractions were evaluated using in vitro 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The sequential ethanol extract of *N. stellata* demonstrated the highest anti-oxidant activity ($\text{IC}_{50} = 12.37 \pm 0.25$ ppm). All extracts were inferior to that of L-ascorbic acid ($\text{IC}_{50} = 3.61 \pm 0.05$ ppm). Among the chromatography fractions, F1 to F13 obtained from the sequential ethanol extract, the

fraction F11 possessed the highest anti-oxidant activity ($IC_{50} = 5.97 \pm 0.23$ ppm). Fraction F14 isolated from total ethanol extract also possessed good activity ($IC_{50} = 6.94 \pm 0.37$ ppm).

Anti-tyrosinase activity of extracts of *N. stellata* was evaluated using 3,4-dihydroxy-L-phenylalanine (L-DOPA) as a substrate and the sequential ethanol extract demonstrated the highest anti-tyrosinase activity ($IC_{50} = 378.58 \pm 48.62$ ppm). All extracts were inferior to those of L-ascorbic acid ($IC_{50} = 74.17 \pm 5.46$ ppm).

Polyphenol content of stamen extracts of total ethanol and sequential extracts of hexane, ethyl acetate and ethanol was determined by Folin-Ciocalteu method using gallic acid as a standard. Sequential ethanol extract showed the highest polyphenol content (254.55 ± 3.99 GAE mg/g).

The Highest anti-oxidant and anti-tyrosinase activity of the sequential ethanol extract may be correlated to the phenolic content of this extract. The activity data further suggest that sequential ethanol extract could be developed as a natural ingredient for cosmetic products such as natural skin-whitening agent. However, the toxicity of these extracts needs to be evaluated before use in such products.