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

A PROJECT REPORT PRESENTED BY

PATHIRAGE ANUSHA GAYANI

to the Board of Study in Chemical Sciences of the

POSTGRADUATE INSTITUTE OF SCIENCE

in partial fulfillment of the requirement
for the award of the degree of

MASTER OF SCIENCE IN ANALYTICAL CHEMISTRY

of the

UNIVERSITY OF PERADENIYA

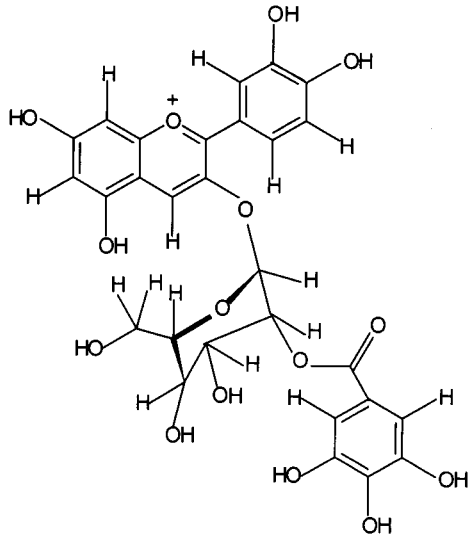
SRI LANKA

2009

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

P. A. Gayani
No. 476, Samudra Mawatha
Hokandara North, Hokandara
Sri Lanka

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 ^{1}H 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 (IC_{50} = 5.44 ± 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 (IC_{50} =12.37 ± 0.25 ppm). All extracts were inferior to that of L-ascorbic acid (IC_{50} =3.61 ± 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 ± 0.23 ppm). Fraction F14 isolated from total ethanol extract also possessed good activity (IC_{50} =6.94 ± 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 ± 48.62 ppm). All extracts were inferior to those of L-ascorbic acid (IC_{50}=74.17 ± 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.