PHYTOCHEMICAL AND BIOLOGICAL STUDY ON *Heimia Myrtifolia* FAMILY LYTHRACEAE

A Thesis Submitted

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To the Soul of My Brother, "Tamer"

And my "Grandmother"

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Abstract

Investigation of phenolic leaf extract of *Heimia myrtifolia* (Lythraceae): Pharmacological properties (stimulation of mineralization of SaOS-2 osteosarcoma cells) and identification of polyphenols

Evaluation of the activity of an aqueous alcoholic extract obtained from the leaves of *Heimia myrtifolia* (Lythraceae) by determining its stimulating effect on two human osteoblastic cell lines HOS58 and SaOS-2 proved possible prevention and treatment of osteoporosis. In addition, the extract was found to increase significantly the mineralization of cultivated human bone cell SaOS-2, whereby a strong dose dependent increase was observed. On the other hand, a phytochemical investigation of the extract confirmed that *H. myrtifolia* is capable of synthesizing and accumulating appreciable amounts of several phenolics, thus leading to the isolation and characterization of sixteen of these constituents. Among these isolates the new natural product, 1,6-di-*O*-dehydrotrigalloyl-β-D-$^{4}C_{1}$-glucopyranose and the rare natural product (secondly reported) 5,7,4'-trihydroxy-3-methoxyflavanone; dihydrokaempferol-3-O-methyl ether were fully identified. All structures were elucidated on the basis of conventional methods of analysis and confirmed by ESI/MS and, ^{1}H and ^{13}C-NMR analysis.

Keywords:*Heimia myrtifolia*, phenolics, osteoporosis, 1,6-di-*O*-dehydrotrigalloyl-β-D-$^{4}C_{1}$-glucopyranose

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Enhancement Activity of Calcium Deposition of SaOS-2 Human Osteosarcoma Cells by Phenolic Leaf Extract of *Heimia myrtifolia*

Evaluation of the activity of an aqueous methanolic extract obtained from the leaves of *Heimia myrtifolia* (Lythraceae) by determining its stimulating effect on the two human osteoblastic cell lines HOS58 and SaOS-2 proved possible prevention and treatment of osteoporosis. In addition, the extract was found to increase significantly the mineralization of cultivated human bone cell SaOS-2, whereby a strong dose dependent increase was observed.

On the other hand, a phytochemical investigation of the extract confirmed that *Heimia myrtifolia* is capable of synthesizing and accumulating appreciable amounts of several phenolics, thus leading to the isolation and characterization of sixteen of these constituents. Among these isolates the new natural product, 1,6-di-*O*-dehydrotrigalloyl-β-D-$^{4}C_{1}$-glucopyranose was fully identified. All structures were elucidated on the basis of conventional analytical methods and confirmed by ESI/MS, ^{1}H and ^{13}C-NMR analysis.

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CONTENTS

Page

LIST OF ABBREVIATIONS

AcOH-6%	6% acetic acid
ALP	Alkaline phosphatase
ax.	axial
bGP	B-glycero phosphate
BMD	Bone mineral density
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
CC	Column chromatography
conc.	concentrated
CoPC	Comparative paper chromatography
d	doublet
dd	doublet of doublet
dil.	Diluted
DMSO-d_6	Deutrated Dimethylsulfoxide-d_6
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
EC_{50}	Efficient Concentration by 50%.
ECM	Extracellular matrix
eq.	equatorial
ESI-MS	Electro-Spray Ionization Mass Spectrometry
Fig.	figure
^{1}H-NMR	Proton Nuclear Magnetic Resonance
HHDP	Hexahydroxydiphenoyl
HMBC	Heteronuclear Multiple Bond Correlation
HOS58 cells	Human Osteogenic Sarcoma cell line
Hz	Hertz
IC_{50}	Inhibitory concentration by 50 %.
IMDM	Iscove's Modification of Dulbecco's Medium
in.	inch
IR	Infrared
***J* value**	Coupling constant

MHz	Mega hertz
mM	Millimole
MS	Mass spectrometry
m/z	Mass to charge ratio
nm	Nanometer
NR	Neutral red
OD	Optical Density
PC	Paper Chromatography
PPC	Preparative paper chromatography
ppm	Part Per Million
q	quartet
R_f	Retardation factor
R_t	Retention time
s	singlet
SaOS-2 cells	Sarcoma Osteogenic cell line
t	triplet
TDPC/UV	Two Dimensional Paper Chromatography/ Ultraviolet
TLC	Thin Layer Chromatography
TMS	Tartemethylsilane
µg/ml	Microgram per milliliter
µM	Micromole
UV	Ultraviolet
δ	Chemical shift by delta value
λ	Wave length
2D-PC	Two dimensional paper chromatography

LIST OF FIGURES

Figure **Page**