CHEMISTRY AND IMMUNOMODULATORY PROPERTIES OF AEGLE MARMELOS, L. CORREA.

BY

PATHMASIRI ANANDA KUMARA SENEVIRATNE

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I DEDICATE THIS THESIS TO MY PARENTS.

ABBREVIATIONS

AP	Alternative pathway
CL_{lum}	Luminol dependent chemiluminescence
CP	Classical pathway
DTH	Delayed type hypersensitivity
Dx.S	Dextran sulphate
EGTA-VB	Ethylene bis(oxyethylenenitrilo)tetraacitic acid-supplemented vernol saline buffer containing 2.5mM of Mg^{2+}.
FAB-MS	Fast atom bombardment - Mass spectroscopy
GC-MS	Gas chromatography - Mass spectroscopy
GLC	Gas liquid chromatography
GPC	Gel permeation chromatography
HBSS	Hank's balanced salt solution
HPLC	High performance liquid chromatography
HPS	Human pooled serum
HX	Hypoxanthine
IC_{50}	50% Inhibition concentration
IC	Intra cutaneous
IP	Intra peritoneal
I.STD.	Internal standard
MAC	Membrane attack complex
MAF	Molar adjustment factor
$\overline{Mn}$	Number average molecular weight

$\overline{Mp}$	Peak average molecular weight
MPO	Myeloperoxidase
NMR	Nuclear magnetic resonance
PBS	Posphate buffered saline
PMNL	Polymorphonuclear leucocytes
Ra.E.	Rabbit erythrocytes
RF	Response factor
ROS	Reactive oxygen species
SDS	Sodium dodecyl sulphate
Sh.E	Sheep erythrocytes
SOD	Superoxide dismutase
STZ	Serum treated zymosan
TFA	Tri fluoro acetic acid
THF	Tetra hydro furan
TLC	Thin layer chromatography
UV	Ultra violet
VSB	Vernol saline buffer containing 0.5 mM Mg^{2+} and 0.15 mM Ca^{2+}
XO	Xanthine oxidase

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ABSTRACT

In the initial screening of immunomodulating compounds from Sri Lankan medicinal plants, it was pronounced that Aegle marmelos was one of the most effective plants on our test models.At the initial stage of this research programme a three month field survey was carried out and the various medicinal uses, applications and prepared drugs of A. marmelos were collected.These ethnopharmacological results collected from both field work and literature survey are described in Chapter 1.

The broad range of application of the unripe fruit in Ayurveda and in other indigenous systems of medicine lead us to carry out investigations on the unripe fruit.In the experimental immunopharmacognostic phase, immunomodulatory compounds were isolated, purified and characterized from the aqueous extract, through action guided fractionation procedures.

In this thesis, the chemistry and the immunomodulatory properties of isolated compounds are discussed.The results described in this dissertation refer to activities found on human complement activation and on Polymorphonuclear leucocytes (PMNL) activation.

Two polymeric compounds namely, a polysaccharide and a proanthocyanidin were isolated from the aqueous extract and the polysaccharide showed potent alternative pathway (AP) anticomplementary activity while the latter showed classical pathway (CP) anticomplementary activity.Further, mechanistic complement assays revealed that the anticomplementary activities are not due to the chelation of bivalent ions but due to the enzymetic activity related complement consumption.It is much interesting to report that the polysaccharide showed potent immunological adjuvant activity on intraperitoneal administration to mice.Both polymers were characterized using various chromatographic and spectroscopic methods of analysis.These results suggested that the structure of the proanthocyanidin is more likely to be a new compound.The proanthocyanidin was a polymer of afzelechin (as the monomer unit)with -C- glucosyl moieties.

The PMNL are particularly involved in acute inflammatory responses and are able to perform phagocytosis.The ethyl acetate fraction which was obtained by partioning the aqueous extract, showed strong inhibition of luminol dependent chemiluminescence (CL_{lum}) produced by zymosan activated human polymorphonuclear leucocytes (PMNL).CL_{lum} is measured by means of a luminometer.The ethyl acetate fraction was subjected to further fractionation and purification based on the CL_{lum} activity.Various chromatographic methods were used and consequently , five low molecular weight constituents were isolated.Four out of five constituents were coumarins and the other was a flavonoid compound.Two coumarins, namely xanthotoxol and scopoletin, showed strong CL_{lum} activity.The flavonoid namely rutin (Quercetin-3-rutinoside), was not earlier reported from A. marmelos fruit.^{1}H-, ^{13}C-NMR, GCMS and MS spectroscopic data of the pure compounds were used for their characterization and structure elucidation.

The mechanistic assays for cytotoxicity and for scavenging of reactive oxygen species were performed as control experiments and results showed that the activity is not due to either the cytotoxicity or scavenging of ROS.

On a weight basis, the proanthocyanidin (0.84 %) and the polysaccharides (0.125 %) isolated from the unripe fruit were found to be the most active immunomodulatory constituents. These two polymeric constituents are the compounds most responsible for the total activity of the aqueous extract of the unripe fruit. Xanthotoxol, scopoletin and rutin are the most active compounds in the CL_{lum} assay.