General Summary

Premna odorata Blanco (Lamiaceae or Labiatae) popularly known as “alagaw” is a tree native to temperate and tropical Asia including the Philippines. In the Philippines, the decoction of the leaves considered diuretic, carminative and febrifuge and used for treatment of endemic tuberculosis disease, vaginal irrigation; coughs; beri-beri; abdominal pains and dysentery. *Premna odorata* is one of seven plants present in a commercialized Philippine herbal preparation called “Pito-Pito” used for headaches, fever, cough, colds, migraine, asthma, abdominal pains and diarrhea.

The present study includes two main parts as follows:

Part I: Phytochemical Study

Chapter 1: Investigation of *Premna odorata* volatile oil isolated from leaves, young stems and flowers

The aim of this chapter was to compare the volatile oils compositions of the leaves, young stems and flowers of *Premna odorata* cultivated in Egypt using GC/MS analysis. GC/MS analysis results revealed the presence of 20, 25 and 20 compounds represented as monoterpenes, sesquiterpenes, diterpenes and higher alkanes in the leaves, young stems and flowers oils, respectively. Monoterpenes and sesquiterpenes represented the major oils fractions. The main compounds in the oils of the leaves and young stems were showed to be trans-caryophyllene (29.403% & 14.638%) and *β*-phellandrene (22.390% & 11.701%); respectively. *α*-pinene (38.160%) and trans-caryophyllene (24.488%) were represented the main compounds in the flowers oil.

Chapter 2: Preliminary phytochemical screening and TLC investigation of *Premna odorata* leaves

Air-dried *Premna odorata* leaves powder (5kg) was successively extracted three times by cold maceration for three days using 70% ethanol till exhaustion, filter, and dry by evaporating under vacuum at 45º C. The plant give 750 g crude extractive, 500 gm was suspended in 150 ml distillated water, successively extracted with *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol. The residues left after distillation the solvents were weighted to give 38, 28, 37 and 250 g residue, respectively. They were subjected to TLC investigations. The percentage yield and organoleptic characters of the different extractives were recorded.

From results of preliminary phytochemical screening and TLC investigation showed the following

1- Presence of carbohydrates and/or glycosides, flavonoids and sterols and/or triterpenes.
2- Presence of alkaloids and/or nitrogenous bases in traces.
3- Absence of crystalline sublimate, steam volatile substances, tannins, saponins, coumarins, anthraquinones and cardiac glycosides.
4- TLC investigation showed that the presence of sterols and/or triterpenoids in the *n*-hexane and dichloromethane extracts. On the other hand, carbohydrates and/or glycosides and flavonoids were predominated in ethyl acetate and *n*-butanol extractives.

Chapter 3: Investigation of *n*-hexane extractive

GC/MS analysis of *n*-hexane extractive

GC/MS analysis for *n*-hexane extractive showed 25 identified compounds represented 72.15% of total identified compounds. Linolenic acid ethyl ester and linolenic acid were the major identified compounds in *n*-hexane fraction represented 19.00% and 18.58%, respectively.

Isolation of the major constituents of *n*-hexane fraction of *Premna odorata*

The *n*-hexane extractive was fractionated using CC and ended with the isolation of two major compounds *β*-sitosterol and tricosan-1-ol primary alcohol which were identified using different spectroscopic data.

Chapter 4: Investigation of ethyl acetate extractive

The ethyl acetate extractive was fractionated using polyamide CC with the isolation of six compounds which were identified using different spectroscopic techniques (^{1}H-NMR, DEPT-Q, HSQC, HMBC and HREMS). Two isolated compounds (1-*O*-trans-p-hydroxycinnamoyl-2-*O*-trans-caffeoyl-*α*-L-rhamnopyranose and 1-*O*-trans-caffeoyl-3-*O*-trans-*p*-hydroxycinnamoyl-*α*-L-rhamnopyranose) are new in nature. The isolated compounds verbascocide and diosmetin were previously isolated from these species, while luteolin and apigenin compounds were firstly reported for *Premna odorata* Blanco.

Chapter 5: Investigation of *n*-butanol extractive

The *n*-butanol extractive was fractionated using polyamide CC with the isolation of nine compounds which were identified using different spectroscopic techniques (^{1}H-NMR, DEPT-Q, HSQC, HMBC and HREMS). Three isolated compounds (6-*O*-*α*-L-(3",4"-di-*O*-trans-*p*-hydroxycinnamoyl) rhamnopyranosylcatalpol, 6-*O*-*α*-L-(3",4"-di-*O*-trans-*p*-methoxycinnamoyl) rhamnopyranosylcatalpol and 6-*O*-*α*-L-(3"-*O*-trans-*p*-hydroxycinnamoyl, 4"-*O*-trans-*p*-caffeoyl) rhamnopyranosylcatalpol) are new in nature. The isolated compounds 6-*O*-*α*-L-4"-*O*-trans-*p*-hydroxycinnamoyl rhamnopyranosylcatalpol and premnoside B and C were previously isolated from these species, while 6-*O*-*α*-L-(2",3"-di-*O*-trans-*p*-hydroxycinnamoyl) rhamnopyranosylcatalpol, 6-*O*-*α*-L-(2",3"-di-*O*-trans-*p*-methoxycinnamoyl) rhamnopyranosylcatalpol and vitexin compounds were firstly reported for *Premna odorata* Blanco.

All compounds isolated from *Premna odorata* Blanco leaves were summarized in Table 34.

Part II: Biological Study

Chapter 1: *In vitro* and *in vivo* study of the antituberculosis activity of *Premna odorata* Blanco volatile oils isolated from leaves, young stems and flowers

MeDipro *M. tuberculosis* Antigen ELISA technique showed that at concentration of 100 µL mL^{-1} *in vitro* and 300 µL mL^{-1} *in vivo*, the leaves, young stems and flowers oils exerted antituberculosis activities with measured values > 1.5 ng mL^{-1} *Mycobacterium* antigen; while the combination (1:1:1) of the three oils showed better antituberculosis activity with measured value < 1.5 ng mL^{-1} *Mycobacterium* antigen and negative result for PCR analysis. Because of *Premna odorata* volatile oil is rich in cyclic and acyclic monoterpenes and sesquiterpenes explained the oil activity against antituberculosis.

Chapter 2: *In vivo* anti-inflammatory and antioxidant study of different extractives of *Premna odorata* leaves

This study was aimed to evaluate the anti-inflammatory and antioxidant activities of plant extractives (total 70% ethanol, defatted total 70% ethanol and *n*-hexane) against alcoholic inflamed liver Wister albino rats using liver and kidney

function tests, oxidative stress markers and antioxidant tests, pro-inflammatory mediators and adhesion molecules tests. According to the biological results; using dose of 500mg/kg; total 70% ethanol extractive improved liver and kidney functions tests. Defatted total 70% ethanol extractive improved GSH and TAC activities; while *n*-hexane and defatted total 70% ethanol extractives opposite the effect of pro-inflammatory mediators and adhesion molecules tests. Consequently; *Premna odorata* showed potent anti-inflammatory and antioxidant properties which improved liver organ function in alcoholic inflamed liver disease.

Chapter 3: *In vitro* study of cyclo-oxygenase inhibition activity (COX-I and COX-II) for selected isolated compounds

The *in vitro* screening results of cyclo-oxygenase inhibition (COX-I and COX-II) for the isolated compounds revealed that all tested compounds from ethyl acetate and *n*-butanol extractives showed COX-I and COX-II inhibition activities with different IC_{50} values. Compound **P11** and **P12** exhibited the most potent suppression effect with IC_{50} –COX-I/COX-II- (4.5/0.87) for compound **P11** and IC_{50} (4.2/0.83) for compound **P12**.

Table 34. Compounds isolated from *Premna odorata* Blanco leaves cultivated in Egypt

Compound	Compound name	Class	Note
P1	Tricosan-1-ol	Primary alcohol	New in genus
P2	*β*- Sitosterol	Sterol	Isolated before
P3	1-*O*-trans-*p*-hydroxycinnamoyl-2-*O*-trans-caffeoyl-*α*-L-rhamnopyranose	Acylated rhamopyranoside	New in nature
P4	1-*O*-trans-*p*-hydroxycinnamoyl-3-*O*-trans-caffeoyl-*α*-L-rhamnopyranose	Acylated rhamopyranoside	New in nature
P5	Verbascoside	Phenyl ethanoid	Isolated before
P6	Diosmetin	Flavone	Isolated before
P7	Luteolin	Flavone	New in species
P8	Apigenin	Flavone	New in species
P9	6-*O*-*α*-L-(2", 3"-di-*O*-trans-*p*-hydroxycinnamoyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	New in genus
P10	6-*O*-*α*-L-(3", 4"-di-*O*-trans-*p*-hydroxycinnamoyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	New in nature
P11	6-*O*-*α*-L-(3", 4"-di-*O*-trans-*p*-methoxycinnamoyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	New in nature
P12	6-*O*-*α*-L-(2", 3"-di-*O*-trans-*p*-methoxycinnamoyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	New in genus
P13	6-*O*-*α*-L-(4"-*O*-trans-*p*-methoxycinnamoyl)rhamnopyranosylcatalpol	Acylated iridoid glycoside	New in genus
P14	6-*O*-*α*-L-(2"-*O*-trans-*p*-caffeoyl, 3"-*O*-trans-*p*-hydroxycinnamoyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	Isolated before
P15	6-*O*-*α*-L-(3"-*O*-trans-*p*-hydroxycinnamoyl, 4"-*O*-trans-*p*-caffeoyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	New in nature
P16	6-*O*-*α*-L-(2"-*O*-trans-*p*-caffeoyl, 3"-*O*-trans-feruloyl) rhamnopyranosylcatalpol	Acylated iridoid glycoside	Isolated before
P17	Vitexin	Flavone C-glycoside	New in genus