***APPROVAL SHEET***

# PHYTOCHEMICAL AND BIOLOGICAL STUDY ON *PASSIFLORA CAERULEA* FAMILY PASSIFLORACEAE

**BY**

*Amir Alfonce Barsoom*

***B. Pharm. Sci., Ain Shams University***

*Abdel Nasser Badawi Singab*, **Ph.D.** _____

***Professor of Pharmacognosy.***
***Vice Dean for the society service affairs and environmental development.***
***Faculty of Pharmacy, Ain Shams University.***

*Osama Ahmed Badary*, **Ph.D.** _____

***Professor of Clinical Pharmacy.***
***Head of Clinical Pharmacy Department.***
***Faculty of Pharmacy, Ain Shams University.***

*Hesham Ibrahim El-Askary*, **Ph.D.** _____

***Professor of Pharmacognosy.***
***Faculty of Pharmacy, Cairo University.***

*Nahla Abdel Hamid Ayoub*, **Ph.D.** _____

***Ass. Professor, Head of Pharmacognosy Department.***
***Faculty of Pharmacy, Ain Shams University.***

**(Committee in charge)**

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By


## Amir Alfonce Barsoom Takla

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*Under the Supervision of*


**Abdel Nasser B. Singab, Ph.D.**
Professor of Pharmacognosy,
Vice Dean for the society service affairs and environmental development.

**Nahla Abdel-Hamid Ayoub, Ph.D.**
Associate professor of Pharmacognosy,
Head of the Department of Pharmacognosy.

**Department of Pharmacognosy**

**Faculty of Pharmacy**

**Ain Shams University**

**Cairo, Egypt**.


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# Contents

# List of Abbreviations

| | |
|---|---|
| Ach | Acetylcholine |
| AcOH-6 | 6% Acetic acid |
| ALP | Alkaline phosphatase |
| BAW | *n*-butanol: acetic acid: water (4: 1: 5, top layer) |
| bGP | β- glycerophosphate |
| BZF | Benzoflavone |
| Cg | Carrageenan |
| CC | Column chromatography |
| CoPC | Comparative Paper Chromatography |
| $^{13}$C-NMR | Carbon-13 Nuclear Magnetic Resonance |
| *d* | Doublet |
| DMSO | Dimethylsulfoxide |
| DMSO-*d6* | Deuterated Dimethylsulfoxide |
| D-PBS | Dulbecco's Phosphate Buffered Saline |
| DPPH | 2, 2-diphenyl-1-picrylhydrazyl |
| FBS | Fetal bovine serum |
| HBSS | Hank's buffered saline solution |
| $^{1}$H-NMR | Proton Nuclear Magnetic Resonance |
| HPLC | High performance liquid chromatography |
| $HR_f$ | Hundred retardation factor ($R_f$ x100) |
| Hz | Hertz |
| $IC_{50}$ | Inhibitory concentration by 50 % |
| IL-1b | Interleukin-1 beta |
| IMDM | Iscove's modification of Dulbecco's medium |
| *J* value | Coupling constant |
| *m* | Multiplet |
| MIP-2 | Macrophage inflammatory protein-2 |
| MMN | Monomorphic nuclear cells |
| MPO | Myeloperoxidase |
| MSFAE | Methanol soluble fraction of the aqueous extract |
| nm | Nanometer |
| NR | Neutral red |
| OD | Optical density |
| PC | Paper Chromatography |
| PFP | Purple passion fruit peel |
| PPC | Preparative paper chromatography |
| PPM | Part Per Million |

| | |
|---|---|
| PTZ | Pentylentetrazole |
| $R_f$ | Retardation factor |
| *s* | Singlet |
| SD | Standard deviation |
| *t* | Triplet |
| TBARS | Thiobarbituric acid reactive substances |
| TFF | Total flavonoid fraction |
| TLC | Thin Layer Chromatography |
| TMS | Tetramethylsilane |
| TNFα | Tumor necrosis factor- alpha |
| µg/ml | Microgram per milliliter. |
| UV | Ultraviolet |
| δ | Chemical shift |
| $\Delta^9$-THC | $\Delta^9$-tetrahydrocannabinol |
| λ | Wave length |
| 2D-PC | Two dimensional paper chromatography |

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## Introduction:

Medicinal plants have been used to cure human illness since a time immemorial, long before recorded history. In the last few decades, increasing public dissatisfaction with the cost, efficacy and potential side-effects of prescription medications, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of herbal medicines. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Recent trends show that the discovery rate of active novel chemical entities is declining. Therefore, there is a need to bioprospect new sources and if possible from less explored regions and habitats to maximize the discovery of novel bioactive metabolites.

The family Passifloraceae is a small family of herbaceous or woody vines with alternate, generally stalked, lobed and stipulate leaves, climbing by means of tendrils borne in the leaf-axil. It comprises about 12 genera and 580 species wide-spread in the warmer temperate regions of the world being quite common in the southern United States (Gathercoal, 1949; Youngken, 1951; Bailey, 1958; Hangar, 1984; Hickey and King, 1988, and Maoute and Decaisne, 1876).

The genus *Passiflora* (from the latin Passus, suffering and flos, a flower) includes about 400 species of tender tendril climbers, the majority

of which with beautiful blossoms and some with edible fruits. It is so named from the supposed resemblance of the finely-cut corona in the centre of the blossoms to the crown of thorns and of the other parts of the flowers to the instruments of the passion of the lord. The genus *Passiflora* includes about three-fourths of the species of the family Passifloraceae, of which 7 are native to the southeastern part of the United States and one (*P. lutea*) extends north into Missouri and Pennsylvania. A few species of this genus are native to Asia and Australia and one to Madagascar (Cavendish, 1978; Lawrence, 1958, and Rendle, 1959).

***Passiflora incarnata*** has a long history in folk medicine as a calmative agent for nervous unrest and as a sedative. The herb is usually administered in the form of a tea; an extract is also employed in a number of pharmaceutical speciality products marketed in Europe. It is official in several pharmacopoeias (Belg.P., Chil.P., Fr.P., Span., Swiss P. as well as in the Martindale). It is reputed to have antispasmodic and sedative properties. It has been used as a nerve sedative in various neuralgias as a liquid extract (1 in 1; dose 0.5 to 1ml.), and as a tincture (1 in 5; dose 0.5 to 2 ml) (Wade, 1979, and Tyler, 1981).

***Passiflora caerulea*** (native to Brazil) is the subject of the present study. Synonyms of *Passiflora caerulea* in other languages includes : Abû sabʿ at alwân ابو سبعة ألوان ; Charkh el falâk چرخ الفلاك ; Zahrat es sâʿah زهرة الساعه (Arabic), Passiflore bleue; Fleur de la passion (French), Blaue passionsblume (German), Fiore della passione (Italian) and Çarki felek (Turkish) (Bedevian, 1994).

The Spanish Roman Catholic priests arriving in newly colonized South America found in *Passiflora caerulea* features which they regarded as symbols of the Cruscifixion. “ In an old Spanish tradition it was the passion flower that climbed the cross and fastened upon the scars in the wood where the nails had been driven through the hands and feet of the Sufferer. The early fathers saw in the flower in full bloom, the five wounds, the nails, the hammer, the spear, the pillar of scourging and the crown of thorns; in its leaves the spearhead and in its tendrils the cords that bound the Lord” (Wilder, 1974; Cavendish, 1978, and Hangar, 1984).

Though many phytochemical constituents and valuable medicinal uses were reported from different plants belonging to family Passifloraceae, yet only few reports concerning the phytochemical composition and the biological activities of ***Passiflora caerulea*** were traced in literature. Thus it was felt necessary to explore the biological activity as well as the phytoconstituents of the entitled plant.