

PROTEIN PROFILING OF *Orthosiphon stamineus*

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## ABSTRACT

*Orthosiphon stamineus* (cat whiskers) is a medicinal herb belonging to the family of Lamiaceae, where the leaves are commonly used as herbal tea known as “Java tea”. It is used as a diuretic agent and for the treatment of heat rheumatism. Currently there is no protein profile data available for this important plant species. This study focus on the optimization of total protein extraction by employing four different methods of extraction protocols; QB buffer, Phenol/SDS with three prewashed steps, Phenol/SDS without prewashed steps and Sigma protein extraction kit with the aim of determining the best protein extraction method, protein pattern of the two varieties of *O.stamineus* (white and purple flower) and bioinformatics analysis to identify the proteins. Overall, Phenol/SDS with three prewashed steps result in a better protein quality and pattern of separation for both plant varieties. A total of 104 functional proteins were identified with each containing at least one unique peptide. Among the identified proteins, Rubisco activase and Triosephosphate isomerase corresponds to the chloroplastic protein of photosynthesis/carbohydrate metabolism while Phosphoglycerate kinase and Glyceraldehyde are cytosolic enzymes of glycolysis pathway which were found to be major housekeeping proteins in a leaf tissue. The result of this study will provide an insight for advanced pharmaceutical research and a baseline information for further proteomics work on *O. stamineus* and similar plant species.

## ABSTRAK

*Orthosiphon stamineus* (misai kucing) ialah sejenis herba ubatan yang berasal dari keluarga Lamiaceae. Daun herba ini selalu dijadikan teh herba yang dikenali sebagai "Teh Java". Ia bertindak sebagai agen penggalakan urin dan untuk rawatan "heat rheumatism". Pada masa kini, masih belum lagi wujud data profil protein yang untuk spesis ini. Kajian ini memfokuskan kepada pengoptimuman pengekstrakan protein dengan menggunakan empat jenis kaedah yang berbeza iaitu penimbal QB, Fenol/SDS menggunakan tiga pra-basuhan, Fenol/SDS tanpa menggunakan tiga prabasuhan dan protein ekstrak menggunakan kit Sigma dengan tujuan untuk menentukan kaedah pengelestrakan protein yang terbaik, corak susunan protein daripada dua jenis pokok (bunga warna putih dan ungu) dan analisis bioinformatik untuk mengenalpasti protein. Secara keseluruhan, Fenol/SDS bersama dengan tiga pra-basuhan menghasilkan kualiti protein dan corak pemisahan yang lebih baik untuk kedua-dua jenis tumbuhan. Sejumlah 104 protein yang berfungsi telah dikenal pasti dan setiap daripadanya mengandungi sekurang-kurangnya satu peptida yang unik. Di kalangan protein yang telah dikenal pasti, Rubisco activase dan Triosephosphate isomerise adalah sepadan dengan protein kloroplas bagi metabolisme fotosintesis/karbohidrat manakala Phosphoglycerate kinase dan Glyceraldehyde adalah enzim sitosol yang terlibat dalam proses glikolisis yang boleh didapati sebagai protein penanda perumah utama dalam tisu daun. Hasil daripada kajian ini akan memberi manfaat kepada bidang penyelidikan farmaseutikal yang mendalam dan juga maklumat asas untuk kajian proteomik yang lebih lanjut mengenai *O. stamineus* dan spesies tumbuhan yang sama.

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**LIST OF SYMBOLS AND ABBREVIATIONS**

ACN	-	Acetonitrile
ADP	-	Adenosine Diphosphate
<i>Arai</i>	-	<i>Arabidopsis lyrata</i>
<i>Arath</i>	-	<i>Arabidopsis thaliana</i>
Arg	-	Arginine
Asp	-	Asparagine
ATP	-	Adenosine Triphosphate
B	-	Biological
BSA	-	Bovine Serum Albumin
C	-	Cellular
°C	-	Degree Celsius
CID	-	Collision Induced Dissociation
Cys	-	Cysteine
Da	-	Dalton
DDT	-	Dichlorodiphenyltrichloroethane
DIGE	-	Difference Gel Electrophoresis
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylenediaminetetraacetic acid

ESI-MS/MS	-	Electrospray Ionization-Tandem Mass Spectrometry
EST	-	Expressed Sequence Tag
g	-	Gram
Glu	-	Glutamine
GO	-	Gene Ontology
GTP	-	Guanosine Triphosphate
HPLC ESI	-	High Performance Liquid Chromatography- Electrospray Ion
IEF	-	Isoelectric Focusing
IR	-	Infrared Radiation
IPG	-	Immobilized pH Gradient
KPO <sub>4</sub>	-	Potassium phosphate
L	-	Liter
LC-MS/MS	-	Liquid Chromatography–Tandem Mass Spectrometry
Lys	-	Lysine
M	-	Molecular
mM	-	Millimolar
MALDI-MS	-	Matrix-Assisted Laser Desorption-Ionization Mass Spectrometry
MALDI-TOF	-	Matrix-Assisted Laser Desorption-Ionization Time-of- Flight
MeOH	-	Methanol
mg	-	Milligram
min	-	Minutes
mL	-	Milliliter
MS	-	Mass Spectrometry
MW	-	Molecular Weight
NCBI	-	National Center for Biotechnology Information
NH <sub>4</sub> HCO <sub>3</sub>	-	Ammonium bicarbonate
nm	-	Nanometer
pI	-	Isoelectric Point
PMF	-	Peptide mass fingerprinting
PMSF	-	Phenylmethanesulfonyl fluoride
PVP (PVPP)	-	Polyvinylpolypyrrolidone

QB	-	Quick Buffer
RNA	-	Ribonucleic acid
rpm	-	Revolution per Minute
SEM	-	Standard Error Mean
SDS-PAGE	-	Sodium dodecyl Sulphate Polyacrylamide Gel Electrophoresis
SK	-	Sigma Kit
TAIR	-	The Arabidopsis Information Resource
TCA	-	Trichloroacetic acid
UNIPROT	-	Universal Protein Resource
UV	-	Ultraviolet Radiation
V	-	Voltage
v/v	-	Volume by Volume
w/v	-	Weight by Volume
<i>x g</i>	-	Gravity
1-D	-	One dimensional
2-DE	-	Two dimensional Electrophoresis
2-ME	-	Two-Mercaptoethanol
$\beta$	-	Beta
$\mu\text{g}$	-	Microgram
$\mu\text{L}$	-	Microliter
%	-	Percentage

## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of the Study

*Orthosiphon stamineus* (cat whiskers) or misai kucing as locally called in Malaysia is a medicinal herb belonging to the family of Lamiaceae and is mostly grown in Southeast Asia. This herbaceous shrub grows to a height of 1.5m and the leaves are arranged in opposite pairs and are simple, green with a lancet leaf blade and a serrate margin (Basheer and Majid, 2010). The leaves of this plant were commonly used in Southeast Asia and other European countries as an herbal tea known as “Java tea” Traditionally, the leaves are used as a diuretic and for the treatment of heat rheumatism, abdominal pain, edema, kidney and bladder inflammation (Awale *et al.*, 2004, Akowuah *et al.*, 2004).

Studies have demonstrated that *O. stamineus* leaves exhibit a number of pharmacological properties such as anti-inflammatory, antioxidant, antibacterial and antiangiogenic properties (Yam *et al.*, 2009, Ueda *et al.*, 2002). On top of all, the plant has shown synergistic bio-enhancing ability of tamoxifen against human breast cancer and was proven to be safe with no *in vitro* and *in vivo* toxicity (Basheer and Majid, 2010). Despite all its medicinal values, *O. stamineus* protein profile which may pave way for further proteomics work is yet to be scientifically established.

Proteomics, which deals with the analysis of complete genomes, provide a qualitative and quantitative nature of the proteins that directly correlates with cellular, biochemical function and accurate system changes during growth, development and environmental factor response (Pandey and Mann, 2000). It is one of the fastest growing areas of biological researches and its objectives has over the recent years goes beyond only cataloguing to the study of functional and regulatory aspects of proteins which includes protein-protein interaction, sub cellular localization, comparative expression, activities as well as structures (Patterson and Aebersold, 2003).

While proteomics research has gone an advanced stage in model microorganisms such as yeast, *Escherichia coli* and mammals, plant proteomics may be at its early stage. With the completion of model plant *Arabidopsis*, rice and Poplar genome projects along with the available expressed sequence tags (ESTs) and gene indices of some plant species, plant proteomics is said to be gaining momentum (Chen and Harmon, 2006). Despite the above and the much exploited medicinal value of *O. stamineus*, to date, there is no protein profile data available for this important plant species which hinders any advancement on its further proteomics work.

## **1.2 Statement of Research Problem**

The leaves of *Orthosiphon stamineus* plant have been taken largely by a lot of people in Southeast Asia, and part of Australia as a local drink in the form of infusion or concoction for the cure of some diseases, including heat rheumatism, abdominal pain, kidney and bladder inflammation, edema, gout and hypertension (Akowuah *et al.*, 2004, Awale *et al.*, 2004)

Many works on *O. stamineus* have shown that it has antimicrobial activity, antioxidant, diuretic, cyto-toxic, hepatoprotective and anti-inflammatory properties.

However, to date little to none is known about its protein content (Basheer and Majid, 2010, Yam *et al.*, 2009, Ueda *et al.*, 2002). Therefore, the valuation of protein profile from *O. stamineus* needs to be carried out as well as possible via total protein extraction from the plant to pave way for further proteomics work.

### **1.3 Significance of the Study**

Establishment of the first protein profile of *the Orthosiphon stamineus plant* will also provide information for advancement in *O. stamineus* and similar species proteomics work. Further exploration on other potential benefits and the generation of baseline data about the plant could be carried out in which other studies can be built upon that can be useful for research and pharmaceutical industries.

### **1.4 Objectives of the Study**

- i. To determine the best protein extraction method.
- ii. To determine the protein pattern of two varieties of *Orthosiphon stamineus* (white and purple flowered) by protein gel (SDS-PAGE) analysis.
- iii. To identify the proteins in *Orthosiphon stamineus*

## 1.5 Scope of the Work

This study focused on the optimization of total protein extraction from purple *Orthosiphon stamineus* plant variety by employing four different methods of extraction; Quick Buffer, Phenol/SDS with prewashed steps, Phenol/SDS without prewashed steps and Sigma kit using leaves as the starting material. Subsequent protein quantitative analysis by Bradford assay and quality check through SDS-PAGE were performed for both purple and white varieties. Finally, the extracted proteins were digested by trypsin and analysed using LC-MS/MS.

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