



**EVALUATION OF ANTIOXIDANT  
CONCENTRATIONS AND ACTIVITIES OF MIXED  
SPICES AND HERBS**

**BY**

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## ABSTRACT

Spices and herbs are used in culinary and are also considered medicinally important due to their antioxidant contents. In this project four spices namely, *Zingiber officinale* (Ginger), *Allium cepa* (Onion), *Syzygium aromaticum* (Clove), *Cymbopogon citrates* (Lemongrass) and four herbs such as *Allium fistulosum* (Bunching onion), *Coriandrum Sativum* (Coriander), *Murraya koenigii* (Curry leaves) and *Ocimum tenuiflorum* (Holy basil) were incorporated into three different formulations. These formulations namely F1 (mixed herbs 25% each), F2 (mixed spices 25% each) and F3 (mixed spices & herbs 12.5% each) were studied for their antioxidant/mineral profile. The three formulations were extracted with cold water, hot water & methanol and the extracts were analyzed for antioxidant contents/activities. The antioxidants like total phenolic contents (TPC) were determined by Folin- Ciocalteu method, total flavonoid contents (TFC) were determined by Aluminium chloride complex forming assay. The antioxidants such as gallic acid, catechin and quercetin were quantified chromatographically. Whereas the antioxidant activities of the extracts were determined by DPPH, HRSA and FRAP assays. Minerals such as Magnesium (Mg), Calcium (Ca), Potassium (K), and trace elements such as Manganese (Mn), Chromium (Cr), Copper (Cu), Iron (Fe) and Zinc (Zn) were determined by using ICP-MS. The collected data of the listed parameters was statistically analyzed by using SPSS. The methanolic extract of formulation F3 (mixed spices & herbs) showed significantly ( $p < 0.05$ ) higher TPC with the mean concentration of  $4.45 \pm 0.2$  mg GAE/ml. The methanolic extract of formulation F3 showed significantly ( $p < 0.05$ ) higher TFC being  $3.30 \pm 0.22$  mg QE/ml. Furthermore, the chromatographic evaluation of formulation F2 showed highest concentration of gallic acid, catechin and quercetin that is 86.0, 339.40 & 394.59  $\mu\text{g/ml}$  respectively. The antioxidant activities in DPPH assay of the methanolic extract of formulation F3 showed significantly ( $p < 0.05$ ) stronger antioxidant activity with the  $\text{IC}_{50}$  value of  $65.03 \pm 2.3$   $\mu\text{g GAE/ml}$ . In HRSA the hot water extract of formulation F3 showed significantly ( $p < 0.05$ ) stronger activity with  $\text{IC}_{50}$  value of  $1493 \pm 187.4$   $\mu\text{g AAE/ml}$ . In FRAP assay the cold water extract of F2 showed significantly ( $p < 0.05$ ) stronger activity being the FRAP value of  $98.9 \pm 4.3$   $\mu\text{g TE/ml}$ . Considering the results of mineral analysis, F1 showed significantly ( $p < 0.05$ ) higher Potassium (K) content of  $33589 \pm 841$   $\mu\text{g/g}$ . The results of this study indicate that as a whole the formulations F2 & F3 possessed higher antioxidant contents/activities than F1. Furthermore, significant ( $p < 0.05$ ) associations between the antioxidant contents and activities were observed. As an outcome of this study it could be said that the use of tested formulations of spices and herbs as health supplements could be highly effective to control the drastic repercussions of the oxidative stress.

## ملخص البحث

إن التوابل والأعشاب التي تستخدم في الطهي بوصفها دواءً، قد اعتبرت مضادات للأوكسدة نظرًا لمحتوياتها. وهذا المشروع بالتحديد تناول أربعة أنواع من التوابل، وهي: نبات الزنجبيل (Ginger)، والأليوم CEPA (البصل)، والسيزبية العطري (القرنفل)، وسترات إذخر (عشبة الليمون)، وكذلك أربعة أنواع من الأعشاب، وهي: fistulosum بيسوم (Bunching البصل)، والكزبرة (الكزبرة)، وورقة الكاري (يترك الكاري)، والريحان المقدس (ريحان القدس)، هذا وقد أدرج ثلاث صياغات مختلفة، والمسماة ب: F1 (الأعشاب المختلطة 25٪ لكل منهما)، وF2 (التوابل المختلطة 25٪ لكل منهما)، وF3 (التوابل المختلطة والأعشاب 12.5٪ لكل منهما). يدرس هذا البحث أسباب مضادات الأوكسدة / المعدنية. وقد انتزعت الصياغات الثلاث بالماء البارد، والماء الساخن، والميثانول. ومقتطفات مع تحليل لمحتويات الأنشطة المضادة للأوكسدة. والمواد المضادة للأوكسدة، مثل: محتويات الفينول الكاملة (TPC) التي كانت مصممة على مناهج فولين Ciocalteu، ومحتويات الفلافونويد بمجموع (TFC) التي كانت مصممة على مجمع كلوريد الألومنيوم تشكيل الفحص. المواد المضادة للأوكسدة، مثل: حمض الغال، كاتشين وكيرسيتين وتم قياس الكمية بشكل بياني واضح. حيث إن مستخلصات الأنشطة المضادة للأوكسدة كانت مصممة بفحص DPPH، وFRAP HRSA. المعادن، مثل: الماغنيسيوم (Mg)، والكالسيوم (Ca)، والبوتاسيوم (K)، والعناصر النزرة: مثل: المنجنيز (Mn)، والكروم (Cr)، والنحاس (Cu)، الحديد (Fe) والزنك (Zn)، وحددت من قبل باستخدام ICP-MS. وتم تحليل البيانات والمعلومات الإحصائية باستخدام برنامج SPSS. باستخراج ميثانول من صياغة F3 (التوابل المختلطة والأعشاب)، وأظهرت أهمية أعلى لـ ( $P < 0.05$ ) أعلى TPC مع متوسط تركيز  $0.2 \pm 4.45$  ملغ / GAE / مل. وباستخراج ميثانول من صياغة F3، وأظهرت معنوياً ( $P < 0.05$ ) أعلى نشاطاً وأقواه لـ TFC مع  $0.22 \pm 3.30$  ملغ / GAE / مل. إضافة إلى ذلك، فإن التقييم الكروماتوغرافي من F2 صياغة وأظهرت أعلى نسبة من حمض الغال، كاتشين وكيرسيتين وهذا هو 86.0، 339.40 و 394.59 ميكروغرام / مل على التوالي. الأنشطة المضادة للأوكسدة في DPPH بتقييم ميثانول من صياغة F3 وأظهرت معنوياً ( $P < 0.05$ ) أقوى الأنشطة المضادة للأوكسدة مع TPC بقيمة  $2.3 \pm 65.03$  ميكروغرام / مل. في HRSA استخراج الماء الساخن من صياغة F3 أظهرت معنوياً ( $P < 0.05$ ) مع قيمة IC50 نشاطاً أقوى من  $187.4 \pm 1493$  مجم / مل. وفي تقييم FRAP من استخراج الماء البارد من F2

أظهرت بشكل ملحوظ (ف > 0.05) مقدارًا أعلى لمؤشر عمليات FRAP من  $4.3 \pm 98.9$  ميكروغرام TE / مل. ونتائج تحليل المعادن أظهرت، F1 ( $P < 0.05$ ) أعلى البوتاسيوم (K) المحتوى من  $841 \pm 33589$  ميكروغرام / غ. ونتائج هذه الدراسة تشير إلى أن F2 و F3 أحرزت أعلى مضادات للأكسدة/محتويات/الأنشطة من F1. وبالإضافة، تمت ملاحظة ( $P < 0.05$ ) العلاقات المهمة بين المحتويات والأنشطة المضادات للأكسدة. وخلاصة هذه الدراسة يمكن القول إن استخدام التركيبات باختبار التوابل والأعشاب بوصفها مواد غذائية وصحية تعطي قيمة عالية، وفعالة للغاية للسيطرة على التداعيات الجذرية للأكسدة.

## APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Health Sciences (Nutrition Sciences).

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## DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Signature.....

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*Dedicated to my loving parents; my father Kalim Ullah Shirazi (late) and my mother Farida Shirazi those who always motivated me to obtain higher education and always prayed for my success. A special message of gratitude to my uncle Mian Tufail Bismil, my younger sister Quratulain and my friends Nasyriq, Aa'zam and Badaruddin those who always strengthened my confidence to remain integrated and composed during the entire course of this journey.*

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## LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
NCDs	Non-Communicable diseases
T2DM	Type 2 Diabetes Mellitus
CVD	Cardiovascular diseases
SOD	Superoxide dismutase
ROS	Reactive oxygen species
BHA	Butylatedhydroxanisol
BHT	Butylatedhydroxytoluene
PG	Propyl gallate
TBHQ	Tertiary butyl hydroquinone
NDGA	Nordihydroguaretic acid
UV	Ultraviolet
CNS	Central nervous system
ET	Electron transfer
TEAC	Trolox equivalent antioxidant capacity
ABTS	Azinobisethyl benzothiazoline sulfonate
TPTZ	Tripyridyltriazine
FRAP	Ferric reducing antioxidant power
DPPH	Diphenyl picrylhydrazyl
HAT	Hydrogen atom transfer
ORAC	Oxygen radical absorbance capacity
TRAP	Total radical trapping antioxidant parameter
TOSC	Total oxidant scavenging capacity
KMBA	Keto methiol butyric acid
TBARS	Thiobarbuturic acid reactive substances
MDA	Malondialdehyde
CAA	Cellular antioxidant activity
DCF	Dichlorofluorescein
ABAP	Azobisamidinopropane
HPLC	High Performance Liquid Chromatography
PDA	Photo diode array
SOP	Standard operating procedures
ICP-MS	Inductively coupled plasma mass spectrometry
SD	Standard deviation
TPC	Total phenolic contents
TFC	Total flavonoid contents
GAE	Gallic acid equivalent
QE	Quercetin equivalent
AAE	Ascorbic acid equivalent
TE	Trolox equivalent
HRSA	Hydroxyl radical scavenging assay
F1	Formulation 1
F2	Formulation 2
F3	Formulation 3

CWE	Cold water extract
HWE	Hot water extract
ME	Methanolic extract
UE	Ultrasound extraction
AE	Agitation extraction
SPSS	Statistical package for social sciences
PPM	Parts per million
AUC	Area under curve
ADA	American Diabetes Association
AOAC	Association of analytical communities

# **CHAPTER ONE**

## **INTRODUCTION**

### **1.1 BACKGROUND OF THE STUDY**

Traditionally spices and herbs are used in all cultures of the world for culinary purposes in order to flavor the food and mask the undesirable odor of meat and other constituents used for cooking. Along with the culinary uses of spices and herbs their medicinal importance is a subject of great interest for the modern researchers in the field of health and nutritional sciences (Lai and Roy, 2004). The medicinal importance of spices and herbs is because of the phytochemicals that they contain in considerable amounts. The phytochemicals of nutritional importance are known as antioxidants. The polyphenols such as phenolic acids and flavonoids are the major antioxidants found in spices and herbs (Hinneburg, Damien Dorman and Hiltunen, 2006). In the body they play an important role in the detoxification of metabolic products namely free radicals (Moskovitz, Yim and Chock, 2002). These free radicals are highly reactive and they react with cellular deoxyribonucleic acid (DNA) that may result into its mutations. These mutations in turn cause certain non- communicable diseases (NCDs) such as diabetes mellitus, atherosclerosis, Alzheimer's disease and various types of cancer (Milic et al., 2014). Though the body is equipped with endogenous (internal) antioxidant systems to neutralize these free radicals but these endogenous antioxidants alone cannot cope with the oxidative stress generated by free radicals (Terevinto, Ramos, Castroman, Cabrera, and Saadoun, 2010). An appropriate intake of dietary antioxidants is highly important in order to minimize the free radical hits and to delay the occurrence of NCDs (Kadam et al., 2008).

The major focus of this study was to quantify the probable antioxidants and various minerals in three different formulations of spices and herbs so that they could be used as nutritional supplements to prevent various NCDs. Four spices such as Ginger, Onion, Clove and Lemongrass and four herbs such as Coriander, Bunching onion, Curry leaves and Holy basil were formulated to estimate their phenolic/flavonoid & mineral contents along with their free radical scavenging potential.

## **1.2 STATEMENT OF RESEARCH PROBLEM**

According to the statistics and reports published by various health governing authorities there is a higher prevalence of certain NCDs all over the world (Narayan, Ali and Koplan, 2010). The rapid industrialization and change in lifestyles are considered the main causes for NCDs however they can be prevented to a certain extent by the adoption of healthy lifestyles and dietary practices (Kelishadi et al., 2008). The major emphasis of this project is on the medicinal importance of spices and herbs attributed by their high antioxidant contents (Vallverdú et al., 2014). This project aims to accumulate the medicinal contents (antioxidants and minerals) present in different spices and herbs in the form of different combinations so as to develop a supplement being able to impart a synergistic effect for the maintenance of good health.

## **1.3 SIGNIFICANCE OF THE STUDY**

The recent literature is full of evidences that free radicals are continuously produced in the body and they impart serious toxicity to cellular organelles. These free radicals are cytotoxic in nature and are needed to be neutralized continuously so as to prevent

the cellular damage. Antioxidants being capable to scavenge free radicals must be present in effective concentrations within the body to control the oxidative stress. A sufficient dietary intake of antioxidants provide a better shielding effect against the ever threatening free radicals.

The project under investigation may provide a useful combination of spices and herbs that could be a potent source of antioxidants in the fight against the health challenging toxicities imparted by free radicals. Thus the findings of this project would contribute to design an efficacious supplement the regular intake of which may provide protection against certain NCDs for which free radicals are considered one of the major causes.

One possible implication of this supplement towards the treatment and prevention of NCDs could be its role in minimizing the cost of treatment. As most of the medicines for NCDs are recommended for lifelong use and sometimes in extreme circumstances very high doses are prescribed. So the concurrent use of a supplement based on common spices and herbs could effectively minimize the cost of treatment by minimizing the dose of regular medications through its antioxidant manifestations. Moreover the spices and herbs being used to design the formulations in this project are quite safe for human beings as they are frequently used in culinary. The concurrent use of these formulations could be highly effective in minimizing the adverse effects of the regular medicines used to treat certain NCDs as they may help to decrease the daily dose of the regular medications.

## **1.4 OBJECTIVES**

### **1.4.1 General Objective**

To evaluate the antioxidant profile of three different formulations of mixed spices and herbs mainly designed to be used as nutritional supplements for general health and wellbeing.

### **1.4.2 Specific Objectives**

1. To determine the total phenolic contents (TPC) in the cold water, hot water and methanolic extracts of formulated spices and herbs.
2. To determine the total flavonoid contents (TFC) of cold water, hot water and methanolic extracts of formulated spices and herbs.
3. To determine the antioxidant activities of the formulated mixed spices and herbs.
4. To detect & quantify the probable phenolic acids (gallic acid) and flavonoids (catechin & quercetin) in the formulated spices and herbs.
5. To detect & quantify the essential minerals of nutritional importance in formulated spices and herbs.

## **1.5 HYPOTHESIS**

The formulated spices and herbs are the rich source of antioxidants and they are capable to scavenge the free radicals to combat the oxidative stress.

## **CHAPTER TWO**

### **LITERATURE REVIEW**

#### **2.1 SPICES AND HERBS**

The study of the history of civilizations shows that in all the cultures spices and herbs are used traditionally not only for cooking purposes but also for the treatment of various ailments (Tapsell, 2008). A spice could be a part of the plant such as the dried seed, leave, fruit or bark that is primarily used to impart the flavor to the cooked food by masking the raw odor of constituents used for cooking as well as to preserve the food (Suhaj, 2006). On the other hand herbs are the leafy green plants (including the roots, fruit, bark and seeds) also used for flavoring and elegant presentation of food. In brief herb is a common abbreviation used for a herbaceous plant (Parekh and Chanda, 2007). Despite of a delicate difference between a spice and herb these terms are generally used together as synonyms of each other representing the parts of plants being used for medicinal or culinary purpose. In modern research spices and herbs have attained an utmost importance not due to their role in making the food delicious but more importantly due to their nutritional and health perspective. The medicinal importance of spices and herbs is mainly due to the phytochemicals termed as antioxidants (Hinneburg et al., 2006). The antioxidants found in spices and herbs are mainly the polyphenolic compounds such as the phenolic acids and the flavonoids (Padmanabhan and Jangle, 2012).

## **2.2 ANTIOXIDANTS**

Any substance that has the ability to delay or slow down the oxidation of an oxidizable substrate is called antioxidant (Huang, Boxin and Prior, 2005). During metabolism various oxidation processes take place in the body which generates unstable entities commonly known as free radicals which are able to interact with cellular organelles causing cellular damage (Poyton, Ball and Castello, 2009). Non-communicable diseases for example diabetes, cancer and cardiovascular diseases (CVD) occur due to the oxidative damage of cellular components such as lipids, proteins and DNA (Milic et al., 2014). Antioxidants have the ability to stabilize these free radicals thus hindering their further multiplication (Houston, 2013). Due to this reason the discovery of food sources which can provide a considerable amount of antioxidants has become a subject of major interest for the nutritional scientists (Mayne, 2003).

## **2.3 CLASSIFICATION OF ANTIOXIDANTS**

Antioxidants could be isolated from animal or plant sources or they could be synthesized artificially in laboratory. The antioxidants are mainly classified into two major groups that is the natural antioxidants and the synthetic antioxidants, the classification that is mainly based on the antioxidant source (Hamid, Aiyelaagbe, Usman, Ameen and Lawal, 2010).

### **2.3.1 Natural antioxidants**

The natural antioxidants are further categorized into two major antioxidant systems that are enzymatic and the non – enzymatic antioxidant systems.

### ***2.3.1.1 Enzymatic antioxidants***

The enzymatic antioxidants occur endogenously (inside the body) and play an important role in cellular metabolic reactions by protecting the living cells from the toxic byproducts of cellular metabolism. The second group is the non-enzymatic antioxidants that occur exogenously (outside the body) mainly in edible plants and their dietary intake is highly important as the enzymatic antioxidants alone are insufficient to combat the oxidative stress generated by the free radicals (Rahman, 2007).

Superoxide dismutase (SOD), glutathione peroxidase and catalase are the most important enzymes occurring as endogenous antioxidants so as to maintain a metabolic equilibrium inside the human body. An imbalance of these naturally occurring endogenous antioxidants may cause severe oxidative damage which may lead to chronic diseases (Patil, Kodliwadmath and Kodliwadmath, 2007).

The SOD is a natural intracellular enzyme that dissociates the superoxide ( $O_2^-$ ) radical into hydrogen peroxide and oxygen and it is a natural defense for all the cells at risk due to superoxide free radicals. The SOD is an enzyme which is effective with certain minerals namely zinc, copper, iron and manganese which act as its cofactor sequentially enhancing its efficiency against the superoxide radicals. Cu-SOD, Cu-Zn-SOD and Fe-SOD are some of the important enzyme – cofactor combinations of endogenous antioxidants naturally being deployed to fight against the most common and hazardous superoxide free radicals. In human beings SOD exists in three forms that is SOD1, SOD2 and SOD3 (Zelko, Mariani and Folz, 2002; Wang, Weaver, Zhang and Lei, 2011). The SOD1 is the most important natural enzymatic antioxidant against certain chronic diseases. The inactivation of SOD1 either due to genetic mutations or due to pathological manifestations may lead to serious motor neuronal