IDENTIFICATION AND CHARACTERIZATION OF *Stevia rebaudiana* EXTRACTION FOR TOTAL PHENOLIC COMPOUND USING SOXHLET EXTRACTION AND MICROWAVE-ASSISTED EXTRACTION TECHNIQUES

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ABSTRACT

This study focused on identifying extraction condition (e.g. sample concentration, extraction time) of total phenolic compound (TPC) from leaves, stems and flowers of *Stevia rebaudiana*. Two method processes were used: 1) using Soxhlet Extraction (SE) and 2) Microwave-Assisted Extraction (MAE). Possible mechanisms of productivity enhancement by both methods were investigated. The extract were analyzed with an appropriate Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR). The reproducibility of extraction and of chromatographic analysis was proved.

The identification of extraction in both extraction technique has been intensively studied. In comparison of these two techniques to extraction of TPC in leaves, stems and flowers of *Stevia rebaudiana*, MAE had a higher extraction efficiency with reduces extraction time. The yield of TPC in leaves of *Stevia rebaudiana* was 100.385 mg GAE/g dw and in stems was 18.702 mg GAE/g dw at concentration 0.1 g/ml. Meanwhile, the yield TPC in flowers was 64.461 mg GAE/g dw at the concentration 0.06 g/ml after 12 min running using MAE.

The qualitative GC-MS analysis of the extract from leaves, stems and flowers of *Stevia rebaudiana* showed that total ion chromatogram (TIC) of Soxhlet and MAE shows some valuable constituents of extracts such as Octadecanoic acid, *n*-Hexadecanoic acid, 1-Octadecene, Taraxasterol, Tridecanoic acid, D-allose, Morpinan, Benzene rings, Stigmasterol, *β*-Sitosterol, *γ*-Sitosterol, α-Amyrin, Longifolenaldehyde, Allo-inisitol, Neo-inositol, Azulene, and Phenol. The results suggest that *Stevia rebaudiana* can also be consider as a potential source of essential oil for the use of cosmeceutical, medicinal and pharmacological application.

FTIR analysis of raw and extracted from leaves, stems, and flowers of *Stevia rebaudiana* showed a great abundance of alcohols, phenols, aromatic rings, carboxylic acids, ketones, aldehydes, alkanes, ethers, ester, acetylenic as well as olefinic.

Heavy metals accumulation in the *Stevia rebaudiana* from leaves, stems, and flowers is reported. The analysis of sample was carried out by using an Inductively Coupled Plasma Mass-Spectrophotometer (ICP-MS) to analyze the Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Magnesium (Mg), plumbum (Pb), Selenium (Se), Zinc (Zn), aluminium (Al), silver (Ag), Cobalt (Co), calcium (Ca), manganese (Mn) and nickel (Ni). The accumulation of heavy metals in leaves, stems, and flowers of *Stevia rebaudiana* contain fifteen heavy metals below tolerance limit in plant and can be used as food products.

Keywords: *Soxhlet Extraction, Microwave-assisted extraction, Stevia rebaudiana, GC-MS, FTIR, ICP-MS, Heavy metals.*

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LIST OF SYMBOLS

Ag	= Silver
Al	= Aluminium
As	= Arsenic
Ca	= Calcium
Cd	= Cadmium
Co	= Cobalt
Cr	= Chromium
Cu	= Copper
Fe	= Iron
FTIR	= Fourier Transforms Infra-Red
GAE	= Gallic acid equivalent
GC-MS	= Gas Chromatography – Mass Spectrometry
ICP-MS	= Inductively Couple Plasma – Mass Spectrophotometer
MAE	= Microwave-assisted extraction
Mg	= Magnesium
Mn	= Manganese
Ni	= Nickel
Pb	= Plumbum
Se	= Selenium
SE	= Soxhlet extraction
SEM	= Scanning Electron Microscopy
TIC	= Total ion chromatogram
TPC	= Total phenolic compound
UV	= Ultra violet
Zn	= Zinc

CHAPTER 1

INTRODUCTION

1.1 Background

Stevia is a genus of about 240 species of herbs and shrubs in the sunflower family (Asteraceae), native to subtropical and tropical regions of Western North America to South America. The species *Stevia rebaudiana*, commonly known as sweet leaf, sugar leaf, or simply stevia, is widely grown for its sweet leaves. As a sweetener and sugar substitute, stevia's taste has a slower onset and longer duration than that of sugar, although some of its extracts may have a bitter aftertaste at high concentrations.

With its steviol glycoside extracts having up to 300 times the sweetness of the sugar. Stevia has garnered attention with the rise in demand for low-carbohydrate, low-sugar food alternatives. Stevia has a good potential for the human health especially for negligible effect on blood glucose, it is attractive as a natural sweetener for people on carbohydrate-controlled diets.

Stevia rebaudiana is an outstanding herb bearing leaves of very refreshing sweet taste and remarkable health qualities. The sweetness of Stevia leaves is largely due to its complex molecules called stevioside and the second compound called rebaudioside. Stevia leaves have a taste that is unique and very sweet with a slight bitter aftertaste. The sweetness of Stevia leaves is much different from the sweetness of other natural sweeteners, sugar, or artificial sweeteners, but it is delicious. Stevia has many favorable and exciting health benefits and it is completely non-toxic. The herb is nutrient rich, containing substantial amounts of protein, calcium, phosphorous, sodium, magnesium, zinc, rutin, vitamin A, vitamin C, and other nutrients, yet has no caloric value.

The availability of *Stevia rebaudiana* varies from country to country. In a few countries, it has been available as a sweetener for decades or centuries; for example, *Stevia rebaudiana* is widely used as a sweetener in Japan where it has been available for decades. In some countries, Stevia is restricted or banned. In other countries, health concerns and political controversies have limited its availability; for example, the United States banned stevia in the early 1990s unless labeled as a dietary supplement, but in 2008 approved rebaudioside extract as a food additive. Over the years, the number of countries in which Stevia is available as a sweetener has been increasing (McCaleb, 1997). Approximately 80% of the world populations depending exclusively on plants for their health and healing. Whereas in the developed world, reliance on surgery and pharmaceutical medicine is more usual however in the recent years, more and more people are complementing their treatment with natural supplements (Dursum *et al*., 2004).

Recently there has been an upsurge of interest in the therapeutic potentials of plants, as antioxidants in reducing free radical induced tissue injury. Although several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are commercially available, but are quite unsafe and their toxicity is a problem of concern. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Natural plant based antioxidants especially phenolics and flavonoids have been exploited commercially either as antioxidant additives or as nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu *et al*., 2000). However there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant ability.

Many herbs and some common medicinal plants are good sources of antioxidant compounds. Many of the biologically active substances found in plants, including phenolic compounds are known to possess potential antioxidant properties. The antioxidant activity of medicinal plants depends on the concentration of individual antioxidant entering into the composition (Larson, 1988).

The protective effects of plant products are due to the presence of several components which have distinct mechanisms of action, some are enzymes and proteins, other are low molecular weight compounds such as vitamins, carotenoids and phenolic compounds (Tadhani *et al*., 2007; Zhang and Wang, 2002). The beneficial health-related effects of some phenols or their potential antioxidant properties, especially when these compounds are present in large quantities in foods, are important to be consumers. Therefore, it is of great interest to evaluate the total phenolic compounds of *Stevia rebaudiana*. For this purpose, leaves, stems and flowers of *Stevia rebaudiana* were selected in the present study for the analysis of the total phenolic compounds.

More than 100 compounds have been identified in *Stevia rebaudiana*, the best known of which are the steviol glycosides, particularly stevioside and rebaudioside A, being the most abundant (Kennelly, 2002). Many review articles on the composition of Stevia *rebaudiana* have been published. In general, it has reported that *Stevia rebaudiana* is nutrient rich, containing substantial of protein, vitamins, antioxidants and other important nutrients has made this very interesting field for chemical investigations of *Stevia rebaudiana* extract or essential oil. *Stevia rebaudiana* are being intensively investigated because the search for new compounds is still ongoing.

According to the literature, many of these compounds are known for their antioxidant proprieties (pinene, carvacrol, caryophyllene) also the essential oil constituents of the plants varied to several factors: geographical, culture conditions, collect time, altitude, climate, different type and nutritional status; all these factors can influence their composition (Kapoor *et al*., 2009, Ozkan *et al*., 2010). *Stevia rebaudiana* has become rather widespread over a wide range of climatic location around the world and can apparently be successfully grown under different cultivation condition. Since the chemical composition of the *Stevia rebaudiana* extract is dependent on the applied condition of plant cultivation, thus, they have become the subject of many research projects.

It has been observed that agricultural soils have been contaminated due to use chemical fertilizers, pesticides, irrigation water and disposal of chemicals nearby.

Thus, the environmental conditions of the region and the agricultural practices decide the level of heavy metals that would accumulate in the plant of Stevia as herbal raw materials are also often used as food, functional food, nutritional or dietary supplements. It is clear that quality control of herbal materials and medicinal plants is very important, not only for the safety of the herbal medicines themselves, but also for food safety. It should be borne in mind that although controlling the contaminants in plant of the herb has certain similarities, there may also be many differences. Therefore, more research is needed in order to establish the scientific evidence criteria for herbal medicines.

Stevia has become rather widespread over a wide range of climatic location around the world and can apparently be successfully grown under different cultivation condition, although often by seedling establishment in a greenhouse before planting in the field. Stevia plant is easily contaminated during growth, development and processing, and for this, an extensive research is needed to explore the characteristics of the heavy metal produced by a plant of Stevia. The heavy metals produced from the herb and its tolerance of heavy metals in Stevia plant is not well documented, and scientific evidence is limited to establishing Stevia plant as a medicinal plant. Although there is a great concern about heavy metal contamination of herbal raw materials, information from the World Health Organization (WHO) regarding permissible limit is available only for Pb and Cd. Since heavy metal accumulation of the *Stevia rebaudiana* extract is dependent on an obtained heavy metals from the soil and water. Heavy metals from plant sources may be also vary from place to place because soil heavy metals content varies geographically, thus, they have become the subject of many research projects.

Classical techniques for the solvent extraction of nutraceuticals from plant matrices are based on the choice of solvent coupled with the use of agitation and/or heat. Traditional extraction methods include maceration, Soxhlet extraction, and percolation; however, they are often time-consuming, require relatively large quantities of solvents, and the active compounds sometimes degrade (De Castro and Garcia-Ayuso, 1998).

In order to increase the productivity and extraction performance, several intensification techniques like ultrasonic waves, supercritical fluids or microwaves were associated with extraction of plant compounds will be implemented to improve the yield and quality of extracted products (Wang and Weller, 2006). From these, ultrasound and microwave assisted-extractions emerged as two promising techniques from an economical point of view, being inexpensive, simple and efficient. These procedures increase at least one of the major parameters governing extraction: the kinetic, through the partial mass transfer rates, the interfacial area or the driving force (Cravotto *et al.*, 2008). Microwave-assisted extraction widely used to selectively extract target compounds from various raw materials. The principle of microwave-assisted extraction is based on two processes, which is ionic conduction and dipole rotation. The heating mechanism of microwave-assisted extraction can significantly reduce the extraction time as compared to soxhlet extraction.

Despite the great variety of extraction applications already in use, it is difficult to interpret and transfer theory to application at the industrial scale. Even in recent application of extraction, optimal extraction or separation condition is obtained empirically. One primary goal of food process engineering is finding out how an extraction process works best achieving the optimum in respect of yield, quality, or cost and consequently finding adjustable processing factor value that produces the optimum.

Optimization of extraction processes is needed either for extraction processes that are already in use or for future process designs for certain applications in food engineering. Consequently, it is important for the food industry to optimizations (yield, quality, operation time, and cost) using appropriate method techniques.

1.2 Problem Statement

1. Long time extraction and procedure by using conventional methods.
2. There are few reports on the chemical composition of extract *Stevia rebaudiana* leaves, but there is no report on examining the chemical composition of the extracts from stems and flowers of *Stevia rebaudiana*.

3. The heavy metals produced from the herb and its tolerance of heavy metals in *Stevia rebaudiana* plant is not well documented and scientific evidence is limited to establishing *Stevia rebaudiana* plant as a medicinal plant.

1.3 Objectives of the Research

1. To investigate the various effects of different concentration and time to optimize the yield of total phenol compound from the leaves, stems and flowers of *Stevia rebaudiana* using soxhlet extraction and microwave-assisted extraction techniques.
2. To characterize the chemical composition of extracts from leaves, stems and flowers of local *Stevia rebaudiana*.
3. To investigate the heavy metal accumulation from leaves, stems and flowers of *Stevia rebaudiana* extracts.

1.4 Research Scope

1. Investigate the various effects of different concentration and time on phenol yields from leaves, stems and flowers of *Stevia rebaudiana* using UV-Spectrophotometer from soxhlet extraction and microwave-assisted extraction techniques.
2. Optimization of total phenolic compounds from leaves, stems, and flowers of *Stevia rebaudiana*.
3. Characterization the chemical composition of extracts from leaves, stems, and flowers of *Stevia rebaudiana* by FTIR, GC-MS and SEM.
4. Investigate the heavy metal accumulation of leaves, stems and flowers of *Stevia rebaudiana* extract using ICP-MS.

1.5 Rationale and Significant of Research

1. Governments Malaysia focuses on growth *Stevia rebaudiana* plants and the investigation about *Stevia rebaudiana* plants as raw materials for phenol has been done continuously.

2. In an attempt to explore such untapped sources of good nutrition, the present investigation has been undertaken to identify the chemical composition of the extracts in the Malaysia species *Stevia rebaudiana* Bertoni.
3. *Stevia rebaudiana* stems and flowers discarded as unused materials, whereas the stems and flowers could be processed as an alternative raw material for natural supplements.
4. Separations of total phenolic compounds from leaves, stems and flowers of *Stevia rebaudiana* using soxhlet extraction and microwave assistance-extraction has never been done, whereas these methods could be applied for phenol and give economical value.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter would contents the related literature of the previous studies on the subject of interest which are optimization and characterization of *Stevia rebaudiana* extracts for total phenolic compound using sohxlet extraction and microwave-assisted extraction techniques.

2.2 Medicinal Plants

Medicinal plants as herb medicine, are plants that have at least one of their part are leaves, stems, barks, roots , tubers and flowers used for therapeutic purpose and active principles in medicinal plants plays a strategic role in the phytochemical investigation of crude plant extract and very important to their potential pharmacological effects (Pascual *et al.,* 2002). Medicinal plants are of great importance to the health of individuals and communities, and this plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, flavonoids, tannins, and phenolic compounds (Edeoga *et al.,* 2005).

Medicinal plants have been used in almost all cultures as a source of medicine. The use of traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed (Hoareau and DaSilva, 1999). Moreover, in the nowadays motivation of people towards herbs is increasing due to the concern about the side effects of synthetic chemical drugs. People want to concern their own health rather than submitting themselves to the impersonal health care system.

Tadhani and Subhash (2006a) subjected leaves of *Stevia rebaudiana* to qualitative phytochemical screening for the identification of various classes of active chemical constituents. The powdered leaf subjected to preliminary phytochemical screening using chemical methods showed the most abundant compounds in the leaf extract to be tannins and alkaloids, followed by cardiac glycosides, saponins, sterols and triterpenes, reducing compounds and anthraquinones. Tests for cyanogenetic glycosides, however, showed negative results.

The World Health Organization (WHO), estimated that eighty percent (80%) of the population of developing countries rely on traditional medicine, most plant drug, for their primary health care need medicinal plants being natural, non-narcotic, having no side effect of arrange of safe, cost effective, preventive and curative therapies could be useful in achieving the goal of health for all, in the coat effective manner (Tewari, 2000). The World Health Organization (WHO) has now recognized that stevioside is not genotoxic and assigned a temporary acceptable daily intake for steviol glycosides of 0–2 mg/kg body weight (Beneford *et al*., 2006). Given the impact that steviol glycosides have had on society, and the plant's remarkable metabolic capability the study of their biosynthesis is more than warranted.

Stevia rebaudiana, native of Paraguay, has been used as an herbal sweetener for over 1500 years. Extracts of *Stevia rebaudiana* are part in weight-loss programs because of its ability to reduce the cravings for sweet and fatty foods, to treat the diseases' diabetes, hypoglycaemia, candidasis, high blood pressure, skin abrasions and inhibiting growth and reproduction of bacteria-like plaque. Stevia's greatest appears to be a natural alternative to artificial sweeteners (such as aspartame or sodium saccharin). The sweetness in *Stevia rebaudiana* is mainly attributed to two glycoside compounds: stevioside (3-10% of dry leaf weight) and rebaudioside A (1-3%) which can be up to 250 times sweeter than sucrose (Duke, 2006).

The Paraguayan herb *Stevia rebaudiana* (Bertoni), also known by natives as Kaa-He-é o "hierba dulce," has captured the growing interest as a potential source of natural non caloric sweeteners for use as a possible substitute for synthetic sweeteners. Its use has been approved in Brazil, Argentina, and Paraguay, as well as

in China, Korea, and Japan (Kolb *et al*., 2000). Nowadays, *Stevia rebaudiana* plant and stevioside are being used as sweeteners in South America, Asia, Japan, China, and in some countries in Europe. In Canada and the USA, Stevia products have been used as dietary supplements since 1990 and 1995, respectively (Geuns, 2003).

As the Malaysian herbal medicine market is experiencing an extraordinary growth, the research approaches taken have recently included activities to develop herbal medicine into quality efficacious and safe products for human consumption. More people are turning to herbal products as alternatives to the conventional therapy medicine or as nutritional and dietary supplements. To date, in past five decades of medicinal plant research in Malaysia, efforts to discover new bioactive agents from flora for the use as chemicals have taken the lead in the development. In Malaysia, the medicinal plants use as chemical templates to produce new candidates for drugs is developing herbal medicine into quality, efficacious and safe products for human consumption. Furthermore, the tropical rain forest plants are biologically and chemically diverse resources as they syntheses various chemicals, as defense agents against diseases and predator (Ibrahim, 2004).

2.3 *Stevia rebaudiana*

In 1899 Swiss botanist Moisés Santiago Bertoni, while conducting research in eastern Paraguay, first described the plant and the sweet taste in detail (Bertoni, 1899). Initially called *Eupatorium rebaudianum*, its name changed to *Stevia rebaudiana* (Bertoni) Bertoni in 1905. The sweet principle was first isolated in 1909 and only in 1931 was the extract purified to produce stevioside, the chemical structure of which was established in 1952 as a diterpene glycoside. Stevioside is described as a glycoside comprising three glucose molecules attached to an aglycone, the steviol moiety. During the 1970s, other compounds were isolated, including rebaudioside A, with a sweetening potency even higher than stevioside (Barriocanal *et al*., 2008).

Stevia rebaudiana (Bertoni) as shown in Figure 2.1 family Asteraceae is an herbaceous perennial plant indigenous to Paraguay and Brazil where its leaves are used by the local Guarani Indians as a natural sweetener for hundreds of years. About 150 stevia species are known, among them *Stevia rebaudiana* is the only one with

significant sweet-tasting properties (Soejarto *et al*., 1983). This plant is of worldwide importance today because its leaves are used as non-nutritive high-potency sweetener primarily in Japan, Korea, China and South America. The consumption of Stevia extracts in Japan and Korea is about 200 and 115 tons/year, respectively (Kinghorn *et al*., 2000).

Figure 2.1: *Stevia rebaudiana* Bertoni

Classification of *Stevia rebaudiana* plant is:

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivisio	: Spermatophyta
Divisio	: Magnoliophyta
Class	: Magnoliopsida
Sub-class	: Asteridae
Ordo	: Asterales
Familia	: Asteraceae
Genus	: Stevia Cav

All sweeteners within the leaves of *Stevia rebaudiana* contain steviol (ent-13-hydroxykaur-16-en-19-oic acid) as the sole aglycone contributor, which has two hydroxyl groups, one attached to the C-19 of the C-4 carboxyl and the other attached

to the C-13, both of which in theory can be glycosylated. Elaboration of the aglycone steviol in *Stevia rebaudiana* by various glycosyltransferases creates up to at least eight different steviol glycosides and may vary from 4% to 20% of the leaf dry weight, depending on variety and growth conditions, although most field crops contain around 10% by leaf dry weight. Besides sweet glycosides, sterebins A to H, flavonoids, triterpenes, volatile oil constituents, pigments, gums, and inorganic matter have been isolated from *Stevia rebaudiana* (Brandle *et al*., 1998).

2.4 Antioxidant

Antioxidants are compounds that have gained importance in recent years due to their ability to neutralize free radicals (Devasagayam *et al*., 2004). Antioxidants have been reported to prevent oxidative damage caused by free radicals. They can interfere with the oxidation process by reacting with the free radicals, chelating catalytic metals and also acting as oxygen scavengers (Buyukokuroglu *et al*., 2001). The antioxidant compounds present in edible plants have recently been promoted as food additives since they display little or no toxic side effects (Seong *et al*., 2004). Many of the biologically active substances found in plants, including phenolic compounds (flavonoids, phenolics) are known to possess potent antioxidant properties. The antioxidant activity of medicinal plants depends on the concentration of the individual antioxidant entering into the composition (Shukla *et al*., 2011).

Stevia leaf extract exhibits a high degree of antioxidant activity and has been reported to inhibit hydroperoxide formation in sardine oil with potency greater than that of either DL-α-tocopherol or green tea extract. The antioxidant activity of Stevia leaf extract has been attributed to the scavenging of free radical electrons and superoxides (Thomas and Glade, 2010). A recent study assessing the in vitro potential of ethanolic leaf extract of *Stevia rebaudiana* indicates that it has a significant potential for use as a natural antioxidant (Shukla *et al*., 2009). However there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been directed towards the identification of plants with antioxidant potential (Shukla *et al*., 2011).

Cioni *et al* (2006) examined the composition of the essential oil on the area parts of five different *Stevia rebaudiana* genotypes from Brazil and Paraguay cultivated in the coastal area of Tuscany (Italy). Forty different components were identified and the main constituents in all the samples were spathulenol, caryophyllene oxide, *β*-caryophyllene and *β*-pinene.

Yoda *et al* (2003) used supercritical fluid extraction with carbon dioxide to investigate the active compounds in Stevia. They identified six classes of compounds, *i.e*., sesquiterpenes, alcohols, labdanic diterpenes, aliphatic hydrocarbons, sterols and triterpenes. In the investigated extract, the main compounds were diterpenes, of which the most abundant was austroinulin. The other labdanic diterpene represented at a higher level was jhanol. Significant portions of the contents of non-polar components belonged to the hydrocarbons *n*-tetracosane and *n*-pentacosane.

2.5 Phenolic Compound

Phenolic compounds are secondary plant metabolites, which are important determinants in the sensory and nutritional quality of fruits, vegetables and other plants. (Tomas-Barberan *et al*., 2000). In vascular plants, more than 4000 phenolic and polyphenolic compounds were the most antioxidants which have been identified, namely, phenolic acids, tannins, coumarins, anthraquinones, flavonoids, phenolic diterpens and anthocyanin (Middleton and Kandaswami, 1993; Trease and Evans, 1989), which have the ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations (Pietta *et al*., 1998). A high correlation was reported between the antioxidant capacity and total phenol and flavonoides contents of plants (Silva *et al*., 2007; Tawaha *et al*., 2007). Besides antioxidant capacity, phenolic compounds exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, immune-stimulating agents, antiallergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, anti-stress, anti-hyperglycemia, cardioprotective and vasodilatory effects (Balasundram *et al*., 2006). Therefore, supplementing a food product with antioxidant plant phenols may provide a health benefit as well.