THE EFFECT OF HEAT PROCESSING ON TRITERPENE GLYCOSIDES AND ANTIOXIDANT ACTIVITY OF HERBAL PEGAGA (*Centella asiatica L. Urban)* DRINK

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ABSTRACT

The health benefit of herbal pegaga drink, which is associated with triterpene glycosides content and antioxidant activity attract a lot of interest from the public and food and herbal industries. The works carried in this research investigated the effect of heat processing at 65°C/15 minutes, 80°C/5minutes and pasteurization at 80°C/5minutes followed by canning and boiling at 100°C/10 minutes on these phytochemicals and compared to untreated herbal pegaga drink or fresh sample. The results revealed that the untreated pegaga drink exhibited much higher ($P<0.05$) antioxidant activity than the heat-treated samples. The Ferric Reducing Ability of Plasm (FRAP) values was 860 μmol/litre for the untreated sample and in the range of 404 - 740 μmol/litre for heat-treated sample. The untreated drink inhibited about 72% of linoleic acid peroxidation and the percentage inhibition of heat-treated samples were in the ranged of 26-56%. The FRAP and Ferric Thiocyanate (FTC) assays were strongly correlated ($r=0.93$) towards the assessment of antioxidant activity in pegaga drink samples. The concentration of ascorbic acid and total polyphenol after heat treatment were 0.7 mg/100ml to 1.76 mg/100ml and 730.27 mg/100ml to 903.23 mg/100ml, respectively. Phenolic compound was found as the major contributor to the antioxidant activity in pegaga drink. Analysis of the triterpene glycosides content was performed using an isocratic High Peformance Liquid Chromatography system (HPLC). Heat processing resulted in a several fold decreased of total triterpene glycosides. The amount in untreated drink was 10.8 to 17.3% higher than those in heat-treated pegaga drinks. The present study indicated that the herbal pegaga drinks samples still retain appreciable amount of madecassoside, madecassic acid, asiaticoside, asiatic acid and polyphenol compounds. These phytochemicals are good sources of antioxidant.

ABSTRAK

Faedah kesihatan bagi minuman herba pegaga yang dikaitkan dengan kehadiran triterpena glikosida dan aktiviti pengantioksidan telah menarik minat yang tinggi daripada orang awam dan pengusaha industri herba serta makanan. Kajian ini dijalankan bagi menyiasat kesan proses pemanasan pada suhu 65°C/15 minit, 80°C/5 minit dan pempasturan pada 80°C/5 minit diikuti dengan pengetinan dan pendidihan pada 100°C/10 minit ke atas perubahan fitokimia tersebut dan dibandingkan dengan minuman tanpa rawatan atau sampel segar. Keputusan menunjukkan minuman pegaga tanpa rawatan menghasilkan aktiviti pengantioksidan yang lebih tinggi ($P<0.05$) berbanding sampel yang dipanaskan. Nilai 'Ferric Reducing Ability of Plasma' (FRAP) adalah 860 μmol/liter bagi sampel tanpa rawatan dan dalam julat 404 - 740 μmol/liter untuk sampel yang dipanaskan. Minuman tanpa rawatan merencat 72% pengoksidaan asid linoleik dan peratus perencatan bagi sampel yang dipanaskan adalah di antara 26-56%. Kaedah FRAP dan 'Ferric Thiocyanate' (FTC) berkorelasi tinggi ($r=0.93$) melalui penilaian aktiviti pengantioksidan di dalam sampel minuman pegaga. Kepekatan asid askorbik dan jumlah polifenol selepas pemanasan adalah 0.7 mg/100ml hingga 1.76 mg/100ml dan 730.27 mg/100ml hingga 903.23 mg/100ml setiap satunya. Sebatian fenolik merupakan penyumbang utama kepada aktiviti pengantioksidan. Analisa bagi kandungan triterpena glikosida dibuat menggunakan sistem isokratik Kromatografi Cecair Berprestasi Tinggi (HPLC). Proses pemanasan turut menyebabkan penurunan beberapa kali ganda amaun triterpena glikosida. Amaun di dalam minuman tanpa rawatan panas adalah 10.8 hingga 17.3% lebih tinggi daripada minuman pegaga yang dipanaskan. Kajian ini menunjukkan bahawa minuman herba pegaga masih mengekalkan amaun madekasosida, asid madekasik , asiatikosida, asid asiatik dan polifenol pada paras yang wajar diterima. Fitokimia ini adalah sumber pengantioksidan yang baik.

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O_2	-	Superoxide radical
H_2O_2	-	Hydrogen peroxide
OH^-	-	Hydroxyl radical
LDL	-	Low debsity lipoprotein
CHO	-	Carbohydrate
HTST	-	High temperature short time
RP	-	Reverse phase
PPO	-	Polyphenol oxidase
DPPH	-	Radical scavenging activity
SS	-	Superoxide free radical scavenging activity
TBHQ	-	*tert*-butylhydroquinone
FDA	-	Food Drug and Administration
TBARS	-	Thiobarbituric acid reactive species
ORAC	-	Oxygen radical absorbance capacity
BCBT	-	β-carotene bleaching test
ABTS	-	2.2', azino-bis(3-ethyl-benz-thiozoline-6-sulfonic acid)
CMC	-	Carboxy methylcellulose
TSS	-	Total soluble solid
TA	-	Total acidity
HCL	-	Hydrochloric acid
GAE	-	Gallic acid equivalent
TPTZ	-	Trypyridyl-s-triazine
UV	-	Ultraviolet-visible
HCL	-	Hydrochloric acid
$Fe_2SO_4.7H_2O$	-	Ferum sulfate
NaOH	-	Sodium hydroxide
K_2S0_4	-	Pottasium sulfate
EDTA	-	Ethylenediamine tetra-acetic acid
DMRT	-	Duncan's multiple range test
SAS	-	Statististical Analysis System
CIE	-	Commision Internationale de L'Eclairage

LIST OF SYMBOLS

R_t	-	Retention time
L	-	Linearity
r^2	-	Correlation coefficient
L*	-	Colour index for lightness
a*	-	Colour index for redness
b*	-	Colour index for yellowness
ppm	-	part per million
rpm	-	rotation per minute
HPLC	-	High Performance Liquid Chromatography
GAE	-	Gallic acid equivalent (mg/100ml)
TSS	-	Total soluble solid
TA	-	Total acidity
°Brix	-	Unit for total soluble solid
NEB	-	Non-enzymatic browning
RDA	-	Recommended Daily Allowance
TLC	-	Thin Layer Chromatography
FTC	-	Ferric Thiocyanate
FRAP	-	Ferric Reducing Ability of Plasma
TBA	-	Thiobarbituric acid
BHT	-	Butylated hydroxytoulene
BHA	-	Butylated hydroxy anisole
MRPs	-	Maillard Reaction Products
ESR	-	Electron Spin Resonance Spectroscopy
SO_2	-	Sodium dioxide
SD	-	Standard deviation
ROS	-	Reactive oxygen species

EGCg	-	epigallocatechin gallate
RE	-	Total vitamin A activity
B1	-	Vitamin B1 (Thiamine)
B2	-	Vitamin B2 (Riboflavin)
E.P	-	Edible portion
Vitamin C	-	Ascorbic acid
Ca	-	Calcium
Fe	-	Iron
Na	-	Sodium
K	-	Pottasium

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CHAPTER 1

INTRODUCTION

In recent year, the production and consumption of fruit and vegetable juice has been increasing. The increased in demand is mainly because of their health benefit (Wong, *et al*., 2001). Lately, attention has been given to pegaga-based products (Faridah, 1998; Brinkhaus, *et al.*, 2000).

Pegaga (C*entella asiatica Linn.*) is widely consumed as herb in different parts of the world. Pegaga is generally used in health food and cosmetic products. This herb is associated with wound healing agents (Vogel, *et al.,* 1990). In Malaysia, it is commonly consume as vegetable or 'ulam' and juice among the Malays and as a cooling drink by the Chinese (Tiek, 1997; Zakaria and Mohd, 1994; Turton, 1993). The interest on herbal beverages such as pegaga drink is because of its pharmacological activity. The pharmacological activity is attributed to its phytochemical constituents such as asiaticoside and antioxidant property.

Currently, several pegaga based herbal products have been developed and marketed by Small and Medium Industries (SMI). They are marketed as herbal drink, cosmetic products and herbal preparation in the form of capsule, tablet and powdered products. Pegaga have also been developed into herbal confectionary.

The health benefit of pegaga is thought to be due to several saponin constituents including triterpene acids (asiatic acid and madecassic acid) and their respective glycosides (asiaticoside and madecassoside). Total triterpenoids; asiatic acid, madecassic acid, asiaticoside and madecassoside have been shown to significantly influence the synthesis of collagen, improve wound healing and ficronectin in human skin fibroblasts culture (Vogel *et al*, 1990; Brinkhaus, *et al*., 2000). Pegaga extract that contains 30 mg of triterpenic acids shows a good wound healing property (Faridah, 1998). Pegaga extract also has anti-ulcer effects especially with reference to its asiatic acid and asiaticoside content (Cheng and Koo, 2000; Somchit, *et al,* 2002; Chatterjee, *et al.*, 1992). Asiaticoside is reported to possess strong antioxidant properties (Shukla, *et al.*, 1999b), act as antimicrobial (WHO, 1998) and anti-inflammatory (Chen, *et al.*, 1999).

Most of the phytochemical from plant extract have been identified to exhibit antioxidant activity. A number of plant constituents have been recognized to have positive effect against the oxygen reactive compounds in biological system (Hemeda and Klein, 1990). There are several evidents indicated that antioxidants in diet provide benefit for health and well-being. The reactive oxygen species (ROS), such as superoxide radical (O_2), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\bullet}$), cause functional damage to man, carcinogenesis, aging and circulatory disturbances (Tagi, 1987). The consumption of fruits and vegetables containing antioxidants has been reported to provide protection against a wide range of degenerative diseases including ageing, cancer, diabetes and cardiovascular diseases (Ames, 1983; Vimala and Mohd Ilham Adenan, 1999; Caragay, 1992). Plants components contain antioxidative properties to counteract ROS (Lu and Foo, 1995).

Antioxidants are compounds that inhibit or delay the oxidation damage in foods and process products. It is well established that lipid peroxidation reaction is caused by the formation of free radicals in cell and tissues. Oxidation reactions are also a concern in food industry. They initiate and promote product deteriorations, thereby limiting the

shelf life of fresh and processed foods (Jadhav, *et al*., 1996). Antioxidants play an important role as inhibitors of lipid peroxidation in food products snd in living cell against oxidative damage (Vimala and Adenan, 1999; Lindsay, 1985).

Synthetic antioxiants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), and natural antioxidants such as tocopherol and ascorbic acid, are widely used in food industries due to their protecting ability against oxidation-reduction reactions (Roberto, *et al.,* 2000). It is known that BHT and BHA retard lipid oxidation, however, due to increasing consumer awareness of health aspect, their used is slowly replaced by alternative antioxidants, which are without toxic effect. Recently, there is growing interest in the used of natural antioxidant in food products. Natural antioxidants are perceived as safe, less toxic and beneficial for human health, however it is very expensive and not widely commercialized. Sources of natural antioxidants are spices and herbs, and such materials have been used throughout history for flavouring and preservative agent (Kikuzaki and Nakatani, 1993).

High concentrations of phytochemical in plant extracts are associated with strong antioxidant activity. Ascorbic acid and phenolic compounds including vitamins, pigments and flavonoids have been identified to be responsible for antioxidant properties in most plants, for example anthocyanin in Roselle extract (Tsai, *et al*., 2002), hydroycinnamic acid in blood orange juice (Arena, *et al*., 2001) and catechins in tea extract (Kikuzaki and Nakatani, 1993). Polyphenols belong to a heterogeneous class of compounds with great variety of effects. These compounds are reported to quench oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical (Yuting, *et al*., 1990). The antioxidant effect of polyhenols has been reported in many in vitro studies including human low-density lipoprotein (LDL) and liposomes (Teissedre, *et al*., 1996). The relationship between antioxidant activity with ascorbic acid content and phenolic compounds has recently been discussed in many research works (Gil-Izquierdo, *et al*., 2002; Arena, *et al*., 2001; Gil-Izquierdo, *et al*., 2001; Dawes and Keene, 1999). The flovonols quercetin was identified as the antioxidant

property in *Polygonum hydropiper*, a medicinal herb (Haraguchi, *et al*., 1992) and onion (Makris and Rossiter, 2001). The antioxidant activity of orange juice, pineapple juice and many fruit juices are found to be associated with the concentration of ascorbic acid (Gardner, *et al*., 2000). On the other hand, ascorbic acid is widely used as an antioxidant in many food products, including processed fruits, vegetables, meat, fish, soft drinks and beverages (Madhavi, *et al.,* 1996b).

Nutritionally, pegaga contains appreciable level of asiaticoside (1-8%), β-carotene (2649 μg), ascorbic acid (48.5 mg) and total phenolic (23000mg/100g) (Brinkhaus, *et al*., 2000; Tee, *et al*., 1997; Fezah, *et al.*, 2000). These compounds play an important role on promoting human health through their antioxidant activity (Velioglu, *et al*., 1998; Gil-Izquierdo *et al*., 2001; Jeniffer, *et al*., 1998; Gazzani, *et al*., 1998). Abdul Hamid, *et al*. (2002), determined that various extracts from different parts of pegaga exhibit antioxidant activity. Phenolic compounds were found out to be the major contributor of antioxidant properties (Zainol, *et al.,* 2003). Since quercetin and kaempferol also appeared as part of major flavonoids components in pegaga (Radzali, *et. al*., 2001; Koo and Suhaila, 2001), it is possible that these constituents may contributed in the antioxidant capacity of pegaga drink. However, the specific phenolic components that involves in antioxidant activity of pegaga are not clearly identified. In other study, Shukla, *et al*. (1999a) investigated the role of asiaticoside as antioxidant property in wound healing activity. Asiaticoside derived from pegaga has been attributed to increase the antioxidant levels at an initial stage of healing. Beside, carotenoid and ascorbate peroxidase are also present as antioxidative constituents in this herb (Yusuf, *et al.*, 2000). In fact, recent traditional applications indicated that a high intake of pegaga is associated with the reduced risk of a number of chronic diseases (Brinkhaus, *et al*., 2000).

Fruits and vegetable products are often subjected to heat treatments in order to preserve their quality and prevent the microbial growth. The most important commercial method of juice and drink preservation is pasteurization. This method is

based on time and temperature relationship (Moyer and Aitken, 1971). The standard pasteurization process destroys harmful bacteria and deactivates detrimental enzymes without adversely affecting the taste, quality and the nutritional value (Nagy and Shaw, 1970). Although, High Temperature Short Time (HTST) processing treatment or flash pasteurization retained most quality and nutrient in processed foods, but the cost of the equipments is high.

The traditional pasteurization processes or known as batch pasteurization often heat the juice or drink for longer periods of time, at slower heat-up rates, using considerably higher temperatures. Most of the vat or batch pasteurization of acidified beverages applied at below 93°C in order to maintain the sensory quality and to reduce the nutrient loss. For example, the mango puree heated under batch process in steam-jacketed kettle until reaches 85°C (Luh, 1970).

The most important factor determining the minimum thermal process is the pH of the product (Noraini, 1984). According to Pederson (1980), for highly acid drink and juice (the pH is lower than pH 4.2) would normally be processed at 71.1°C to 100°C. On the other hand, Chuah (1984) reported that the process of pasteurization usually consists of a process whereby the food is heated to temperature 60-90°C either to destroy the nonsporing pathogens or to prolong the shelf-life of the food, usually but not conjunction with some added preservatives which prevent the spores of microorganisms from germination. High temperature heat processes are unnecessary for acid juices because the heated spores of spore-forming bacteria are unable to germinate at pH 4.2 or lower (Pederson, 1980). The heat treatment of beverages held at 60°C for 10-20 minutes is also recommended for the acidic products (Chuah, 1984). Scalzo (2004) studied the effect of thermal treatments of blood orange juice at 80°C for 6 minutes on antioxidant changes compared to non-thermally treated juice. After pasteurization at 80°C for 6 minutes, the inhibition DPPH (%) was reduced from 49.1% (unheated juice) to 43.2%. The processing of pineapple and “asam jawa” drink at 85 to 90 °C for 1 to 5 minutes still

maintained the sensorial quality of products (Che Rahani, 1998). The carrot juice heated at 82°C for 5 minutes retained 57% of α-carotene (Bao and Chang, 1994). The heating temperature for canned fruit and vegetables beverage is depended on the microbial level of the raw materials, the acidity of the products, the size of the can and the thermal conductivity of the product. Canned mango puree was heated in open steam jacketed kettle to 80°C for 10minutes. After hot-filling, the sealed cans were immersed in boiling water for another 20 minutes (Godoy and Rodriguez-Amaya, 1987). In other processing practice, the guava juice was heated to 87°C for 5 minutes, hot filled and sealed cans pasteurized in boiling water for 30 minutes. (Padula and Rodriguez-Amaya, 1987). The authors found that carotene content was maintained after heating at these processing condition. In other report, Che Rahani (1998) recommended the heat processing of guava drink at 82°C for 5 minutes, followed by canning and immersed in boiling water (100°C) for another 10 minutes.

One of the issues in plant material processing is on the effect of processing method on the phytochemical profile of the products. According to Nicoli, *et al*. (1999), the health benefit of plant material is dependent on their processing methods. Food processing procedures are generally believed to be responsible for the depletion of natural antioxidant and at the same time it is expected to have a lower health protecting capacity than fresh produce. Gazzani, *et al.,* (1998) reported that processing steps significantly influenced the antioxidant activity of plant materials. This is due to the loss of antioxidant or the formation of compounds with pro oxidant action may lower their antioxidant capacity. The naturally occurring antioxidant such as ascorbic acid and phenolic compounds are generally degraded under thermal treatment (Mahanom, *et al*., 1999; Makris and Rossiter, 2001; Fezah, *et. al*., 2000). Thermal treatment also responsible for the reduction of antioxidant activity in processed products (Hunter and Fletcher; 2002; Takeoka, *et al*., 2001). Pro oxidant compounds that formed in early stage of Millard reactions significantly decreased the antioxidant activity (Nicoli, *et al*., 1999).

Thermal treatments are also frequently used in the extraction of phytochemicals substances from fruits and vegetables (Gazzani, *et al*., 1998). Some antioxidant substances are well extracted during preparation of herbs extract at high temperatures. For example, the maximum antioxidant capacity from in vitro studied is associated with the drinking of green tea prepared at high temperatures (90°C) and with long infusion time. However, Langley-Evans (2000) suggested that the black tea is ideally prepared between 70-90°C with infusion times not exceeding 1-2 min for maximum antioxidant recovery. According to Scalzo, *et al*., (2004), thermal treatment generally induced and increased the extractability of the phenolic substances of orange juice, such as anthocyanins and total cinnamates. The presence of intermediate oxidation state of polyphenol is also reported to exert a higher antioxidant activity (Manzocco, *et al*., 1998). On the other hand, alterations to the structure of existing antioxidants, as well as the formation of novel antioxidant components may enhance the initial antioxidant status (Gazzani, *et al*., 1998; Nicoli, *et al*., 1997b; Nicoli, *et al*., 1999). Heat treatment accelerates the oxidation reactions responsible for the formation of compounds with pro oxidant properties and compounds having antioxidant activity. Example of such reaction is Maillard reaction products. The brown-coloured Maillard reaction products formed in advanced stage of non-enzymatic browning reaction have clearly shown to improve antioxidant activity *in vitro.* Complex relations between these variables are generally obtained in multicomponent and in formulated foods (Manzocco, *et al*., 2000). Thus, the heat processing treatment could caused negative effect as well as enhanced their antioxidant activities on the herbalproducts.

The antioxidant potential of herbs dependent on many factors involved in it preparations. The right choice of processing parameters of herbal products may help to retain their phytochemicals content. In most cases, temperature control, minimizing oxygen content and protection from light can help to ensure maximum retention of antioxiants (Lindley, 1998). On the other hand, the eventual processing damage can be minimized by the addition or enrichment of the product with natural antioxidants and/or reconstituted with secondary antioxidants. According to Lindley (1998), the addition of

free radical chain breakers (α-tocopherol), reducing agents and oxygen scavengers (ascorbic acid), chelating agents (citric acid) and 'secondary' antioxidant (carotenoids) may be able to stabilize and prevented oxidation damage in fruits and vegetables. Pokorny (2000) reported that modification of a recipe during preparation of food and ready meals improved the stability against oxidation especially with the addition of spices. Recent studies also indicated that the addition of sulphur dioxide ($S0_2$) or sodium metabisulphite and vitamin C during processing of commercial food products balanced the depletion of natural antioxidant (Tsai, *et al.*, 2002; Majchrzak, *et al.*, 2004). The presence of metabisulphite has been demonstrated to control the spoilage and promote the retention of the natural antioxidant. Sulphites were successfully used to prevent the non-enzymatic browning in food and vegetables (Sapers, 1993), reduction in decoloration of pigments, changes in texture and loss of nutritional quality (Lindley, 1998). Other food additives such as citric acid generally enhanced the antioxidant activity via synergist effect with natural antioxidant like α-tocopherol. Citric acid was also used as metal chelators to inhibit oxidative reactions (Madhavi, *et al.*, 1996). Citric acid is widely used as acidulant and preservatives in food system. The high levels of total soluble solid usually help to stabilize or reduce the deterioration rate of food products. For example, high sugar concentrations are effectively to protect the degradation of anthocyanin (Wrolstad, *et al*., 1990), the strong antioxidant compound in Roselle (Tsai, *et al*., 2002) and berry fruits (Skrede, *et al*., 2000). The effect of sugar concentration is most likely due to lower in water activity (Skede and Wrolstad, 2002).

The impact of food processing and handling on nutrients such as vitamins and minerals are well established. However, the stability and the fate of phytochemicals in processed food have not been investigated to similar extent. It is always believe that phytochemical from pegaga are depleted by processing, particularly where thermal treatments are employed. The level of antioxidant activity and the presence of significant concentration of triterpene glycoside in pegaga are of interest to the herbal industry. However, the effect of processing parameters on both antioxidant activity and triterpene glycoside contents of products from pegaga is yet to be investigated

thoroughly. Since triterpene glycosides such as madecassoside, asiaticoside, madecassic acid and asiatic acid have been reported to contribute to the pharmacological activities, it is important to study the effect of processing treatment of pegaga on the fate of these components.

1.1 Objective

The main objective of the study was to investigate the effect of heat processing on the antioxidant activity and triterpene glycosides content of herbal pegaga drink

1.2 Scope

In order to achieve the objective, the scopes of the study are identified as follows:

1. The herbal pegaga drink was prepared under three different heat processing conditions; 65°C/15 minutes (A), 80°C/5minutes (B) and canned process (heat at 80°C/5minutes followed by canning and boiling at 100°C/10 minutes (C)). The unheated pegaga drink known as fresh sample (F) and two commercial samples, CM1with no thermal treatment and CM2, which heat processed at 90°C for 1 minutes were used as comparison. All pegaga drink samples (F, A, B, C, CM1 and CM2) were used for further assessment.

2. The physico-chemical characteristics of pegaga drink samples (F, A,B, C, CM1 and CM2) including pH, total acidity, total soluble solid, colour, proximate analysis, total polyphenol and ascorbic acid content was

studied. These assessments provide the basic data or information of characteristics of sample studied.

3. The level of antioxidant activity in pegaga drinks prepared under different heat processing conditions was assessed using two antioxidant assays namely Ferric thiocyanate (FTC) method and Ferric reducing ability of plasm (FRAP) methods.

4. The effect of addition of sodium metabisulphite and citric acid, and total soluble solid of fresh herbal pegaga drink on antioxidant activities were evaluated. The contribution of total polyphenol and ascorbic acid on antioxidant activity was also evaluated.

5. The concentration of four components of triterpene glycosides in pegaga drinks; including asiatic acid, madecassic asid, asiaticoside and madecassoside were examined. The contribution of asiaticoside on antioxidant activity of herbal pegaga drinks was also evaluated.

other food components should also be studied in future. Furthermore, their stability under different parameters such as storage conditions, packaging, light, water activity, degree of oxidation and High Temperature Short Time (HTST) processing technology need also be evaluated in future.

- On the other hand, consumers believe that herbal pegaga products that were assumed rich in antioxidants and triterpene glycosides may afford a degree of protection against free radical damage and higher in pharmacological activity. The data on their adsorption in blood stream, pharmacological benefit and toxicity over the range of studies of still remain unknown and further information should be provided.

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