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Antioxidative and anti-α-amylase activities of four wild plants consumed by pastoral nomads in Egypt

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SUMMARY

In this study, four plants grown naturally in Egypt that are usually consumed by pastoral nomads were screened for their total phenolic contents, antioxidative, and anti-α-amylase activities. Dried powder of plant's part was extracted in absolute or 70% ethanol. A polar extract of *Panicum turgidum* (PTPE) had the highest total polyphenol content {92.5 mg gallic acid equivalents (GAE)/g}, followed by an alkaloid extract of *Withania somnifera* (WSAlk; 77.5 mg GAE/g), and an ethanol extract of *Leptadenia pyrotechnica* (LPEE; 59.1 mg GAE/g). By employing different assays such as DPPH radical scavenging, reducing power, Fe^{+} chelating, H_2O_2 scavenging and total antioxidant capacity, it was shown that PTPE, WSEE (ethanol extract of *W. somnifera*), WSAlk and LPEE had promising antioxidant activity, though, their potency varied according to the different tests. WSAlk had the highest level of α-amylase inhibition (40.2%) *in vitro*, followed by WSEE (30.5%). Therefore, it can be concluded that these plants, especially extracts of PTPE, and WSAlk are beneficial to physiological health, and could be used in food and pharmaceutical industries to prepare dietary supplements, functional foods or food preservatives.

Key words: Antioxidant; Anti-amylase; Wild plants; Fruits; Polyphenol

INTRODUCTION

Fruits, flowers, and vegetative parts of plants are the major sources of health-promoting agents in the body. Among these agents, antioxidants are the most important because they inhibit lipid peroxidation, scavenge free radicals, and chelate divalent cations (Shon *et al.*, 2004). Reportedly, the pathogenesis of many diseases such as cardiovascular disorders, cancer, aging, inflammation, and brain dysfunction is accompanied by the production of free radicals leading to oxidative stress. Various epidemiological studies have suggested that consumption of fruits and vegetables has been associated with reduced risk of cardiovascular diseases and cancers (Kris-Etherton *et al.*, 2002) and neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases (Di Matteo and Esposito, 2003) as well as inflammation and aging (Ames *et al.*, 1993). Moreover, according to the recent estimates, the human population worldwide appears to be in the midst of an epidemic of diabetes mellitus, a metabolic disorder characterized by chronic hyperglycaemia. Retardation of the digestion of starch by key gastrointestinal enzymes

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can be of benefit to the diabetic patients (Puls and Keup, 1973). Since glucose release from food sources is the main factor affecting post-prandial hyperglycaemia, the use of á-amylase inhibitors is essential to control it. However, free radicals have been implicated in the pathophysiology of diabetes, and oxidative stress may be a common pathway linking diverse mechanisms for diabetes complications such as vascular dysfunctions, nephropathy, neuropathy and retinopathy (Baynes, 1991). Furthermore, diabetes mellitus results in a reduction of endogenous antioxidants and an increase in oxidative stress in the human body. Reportedly, antioxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and improve some of the associated complications. Phenolic compounds in fruits, vegetables and foods, such as catechin and its derivatives are important to health because they have a wide range of health-promoting effects. Natural antioxidants extracted from fruits and vegetables could be used in food and pharmaceutical industries instead of synthetic antioxidants. Their use in food industries will certainly improve the quality and nutritive value of foods. Therefore, it is necessary to search out natural antioxidants that could be used not only to preserve food but also in the treatment and management of some diseases.

Pastoral nomads in Egypt are using some plants as a source of health-promoting agents such as vitamins, minerals, dietary fibers and so forth. People in desert areas usually consume these plants fresh, and sometimes by means of various preparations. *Launaea capitata* is used as an alternative good source in salads. The slimy fruits, young twigs, seeds and the tuberous root of *Leptadenia pyrotechnica* are often eaten by nomads (Belal *et al*., 1998) and reportedly, in countries from Senegal to Nigeria it is used in folk medicine. Kassas (1967) described the collection and human consumption of *Panicum turgidum* grains by desert-dwellers, especially in dry years when cultivated crops may fail. Smoke of *P. turgidum* is believed to relieve women's pain during delivery. *Withania somnifera* (Ashwagandha) is also known by the names Winter Cherry, Indian Ginseng, and Withania. This plant has been used for medicinal and narcotic purposes in Mesopotamia and is well known in ancient Egypt. It is useful in the treatment of cognitive dysfunction, dyspepsia, epilepsy, gout, inflammation, insomnia, oxidative-stress, rheumatism, and tumor (Mishra *et al*., 2000; Russo *et al*., 2001). However, a plant is largely affected by environment, especially in its production of secondary metabolites. Therefore, *W. somnifera* of Egypt may have dissimilarities in respect of secondary metabolites with the *W. somnifera* of other countries. In Egypt, despite the common consumption of these plants there is no scientific data confirming their biological activities. The present research is aimed at investigating and recording the polyphenol content, antioxidative, and antiamylase activities of four wild plants in Egypt. Another aim is to investigate plants which could be used in food or pharmaceutical industries to prepare dietary supplements, functional foods or food preservatives.

MATERIALS AND METHODS

Chemicals

Folin-Ciocalteu's phenol reagent and ferrozine were purchased from Sigma-Aldrich Co (St. Louis, MO). DPPH, and α-amylase from bacteria were purchased from Wako Pure Chemical Industry, Ltd., Osaka, Japan. Ascorbic acid was purchased from Katayama Chemicals, Osaka, Japan. Gallic acid was purchased from Nacalai Tesque, Kyoto, Japan. All of these chemicals and reagents were of analytical grade.

Plant collection and extraction

Three desert plants growing in Wadi Allaqi, 180 km south-east of Aswan city used by local people (Bedouins), *Launaea capitata* L., *Panicum turgidum* Forssk. as whole plants and green fruits of *Leptadenia pyrotechnica* (Forssk.) Decne. were collected and air dried. Ripe fruits of *Withania somnifera* (L.) Dunal.

were collected from plants growing in the cultivated fields along the Nile bank at Aswan city and air dried. All plants were identified according to Bolous (1995), and voucher samples were deposited in the herbarium of Aswan faculty of Science. The dried plant materials were then powdered and defatted with n-hexane to remove non-polar compounds, and then the defatted materials were extracted with ethanol followed by extraction of very polar constituents with 70% ethanol.

A portion of defatted fruit material of *W. somnifera* was extracted with acidic ethanol (pH 3.5) and filtered resulting in an alkaloidal fraction. The pH of the filtrate was then raised up to 9 with ammonia to precipitate alkaloids. Alkaloids were removed by centrifugation and dried. Ethanol extract of *L. capitata*, *L. pyrotechnica*, *P. turgidum*, and *W. somnifera* are denoted as LCEE, LPEE, PTEE and WSEE respectively. The 70% ethanol extract of *L. pyrotechnica*, *P. turgidum*, and *W. somnifera* are referred to as LPPE, PTPE and WSPE respectively. The alkaloid fraction of *W. somnifera* is referred to as WSAlk.

Determination of total phenolics (TPH)

The total concentration of phenolics (TPH) in the extracts was determined according to the Folin-Ciocalteu method (Ough and Amerine, 1988) with gallic acid (GA) as the standard and expressed (mg) as gallic acid equivalents (GAE)/g of extract (Aoshima and Ayabe, 2007). One milliliter of diluted extract was mixed with 1ml of Folin-Ciocalteu's reagent and vortexed for 5 s. Then, 1ml of a 10% (w/w) sodium carbonate aqueous solution was added to the mixture. The mixture was incubated at room temperature for 1 h, after which colorimetric measurements were made at 700 nm. Each experiment was conducted three times.

DPPH radical scavenging activity

The reaction mixture (total volume, 3 ml), consisting of 0.5 ml of a 0.5 M acetic acid buffer solution at pH 5.5, 1 ml of 0.2 mM DPPH in ethanol, and 1.5 ml of a 50% (v/v) ethanol aqueous solution, was shaken vigorously with the extracts (Blois, 1958). After incubation at room temperature for 30 min, the amount of DPPH remaining was determined by measuring absorbance at 517 nm (Aoshima *et al.*, 2004). Mean values were obtained from triplicate experiments.

Reducing power activity

The reducing power of the extracts was determined according to the method of (Oyaizu, 1986). Briefly, different concentrations of the extracts were mixed with 2.5 ml of 0.2 M phosphate buffer, pH 6.6 and 2.5 ml of 1% potassioum ferricyanide solution. After incubation at 50°C for 20 min, the mixtures were mixed with 2.5 ml of 10% trichloroacetic acid followed by centrifugation at 650 g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride. The absorbance of this solution was measured at 700 nm. Ascorbic acid served as positive control.

Measurement of chelating activity

The activity of extracts to chelate Fe^{2+} was measured according to the method of (Carter, 1971). Briefly, the extract was incubated with 0.05 ml of 2.0 mM $FeCl_2.4H_2O$. Then, 0.2 ml of 5.0 mM ferrozine was added, and the volume was adjusted to 0.8 ml with methanol. After 10 min, the absorbance of the mixture was measured at 562 nm. EDTA served as the positive control, and a sample without extracts and EDTA served as the negative control.

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging ability of the extracts was determined according to the method of Ruch *et al.* (1989). Different concentrations of extract in 0.1 M phosphate buffer (pH 7.4) mixed with H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. For each concentration, a separate blank sample was used for background subtraction. The percentage of H_2O_2 scavenging of the extracts and standard compounds was calculated.

Determination of total antioxidant capacity

The assay was done according to Prieto *et al.* (1999). The tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 90°C for 90 min. The antioxidant capacity was expressed as ascorbic acid equivalent (AAE) and gallic acid equivalent (GAE).

α-Amylase assays *in vitro*

α-Amylase activity was carried out using the starch-iodine method. The reaction mixture contained 10 μl of α-amylase solution (10 mg/ml), phosphate buffer (0.02 M, pH 7.0) with 0.006 M NaCl (0.4 ml) and 1% starch solution (0.1 ml). After incubation at 37°C for 10min, the starch solution was added, and the mixture was re-incubated for 1 h. Hereafter, 0.1 ml of 1% iodine solution was added, and after the adding of 5 ml distilled water the absorbance was taken at 565 nm. Sample, substrate and α-amylase blank determinations were undertaken under the same conditions. The above experiment was conducted using different starch solutions.

RESULTS

TPH

The TPH content of the different extracts are shown in Table 1. The amount was largest in PTPE (92.5 mg GAE/g) followed by WSAlk (77.5 mg GAE/g) and LPEE (59.1 mg GAE/g).

DPPH radical scavenging activity

The DPPH radical scavenging activities of the different extracts are also shown in Table 1. The level of free radical (DPPH) scavenging activity ranged from 79.5 to 12.3% at 100 μg/ml of extract. At this concentration, WSEE showed the highest DPPH radical scavenging activity (79.5%) followed by PTPE (72.6%) and WSAlk (69.5%). Fig. 1(A) shows the dose-dependent DPPH radical scavenging activities of LPEE, PTPE, and WSEE with IC_{50} values of 89.3, 47.2, and 44.7 μg/ml respectively. WSEE had the lowest IC_{50}, which means that among the tested extracts, it had the strongest radical scavenging activity.

Reducing power

Reportedly, the activity of antioxidants is concomitant with the development of reducing power (Duh *et al.*, 1999). Table 1 shows the reducing power of the extracts determined using the potassium ferricyanide reduction method. Since PTPE and WSEE had the high reducing activity among the extracts, their dose-dependent increase of reducing power was examined, Fig. 1(B).

Chelating activity

Table 1 shows the chelating effect of the extracts on

Table 1. Total phenolic content, DPPH radical scavenging, reducing power, chelating activity, and H_2O_2 scavenging activity of the different plant extracts[a]

Name of extracts	Total phenolic mg GAG/g	DPPH activity (%) at 0.1 mg/ml	Reducing power (O.D.) at 0.2 mg/ml	Chelating activity (%) at 2.0 mg/ml	H_2O_2 scavenging activity (%) at 40 μg/ml
LCEE	38.2 ± 0.3	36.8 ± 0.5	0.12 ± 0.00	Nill	27.5 ± 0.9
LPEE	59.1 ± 0.4	57.2 ± 1.6	0.16 ± 0.01	3.7 ± 5.2*	55.8 ± 7.8
LPPE	37.2 ± 0.9	51.8 ± 0.5	0.15 ± 0.00	12.9 ± 1.4	15.6 ± 2.1
PTEE	42.1 ± 0.7	47.8 ± 3.3	0.17 ± 0.01	Nill	31.3 ± 4.3
PTPE	92.5 ± 1.3	72.6 ± 1.1	0.41 ± 0.01	16.2 ± 0.5	38.5 ± 9.4
WSEE	37.1 ± 0.2	79.5 ± 0.6	0.27 ± 0.05	13.4 ± 2.4	19.7 ± 3.2
WSPE	7.3 ± 0.3	12.3 ± 1.2	0.05 ± 0.00	75.5 ± 2.0	9.5 ± 2.1*
WSAlk	77.5 ± 1.0	69.5 ± 0.4	0.12 ± 0.01	76.7 ± 3.6	62.7 ± 11.6

$P < 0.01$ by Student's t test for values between the sample and the control in DPPH, chelating, and H_2O_2 experiments. *Not significant. [a]Values are the mean of three replicates ± S.D.

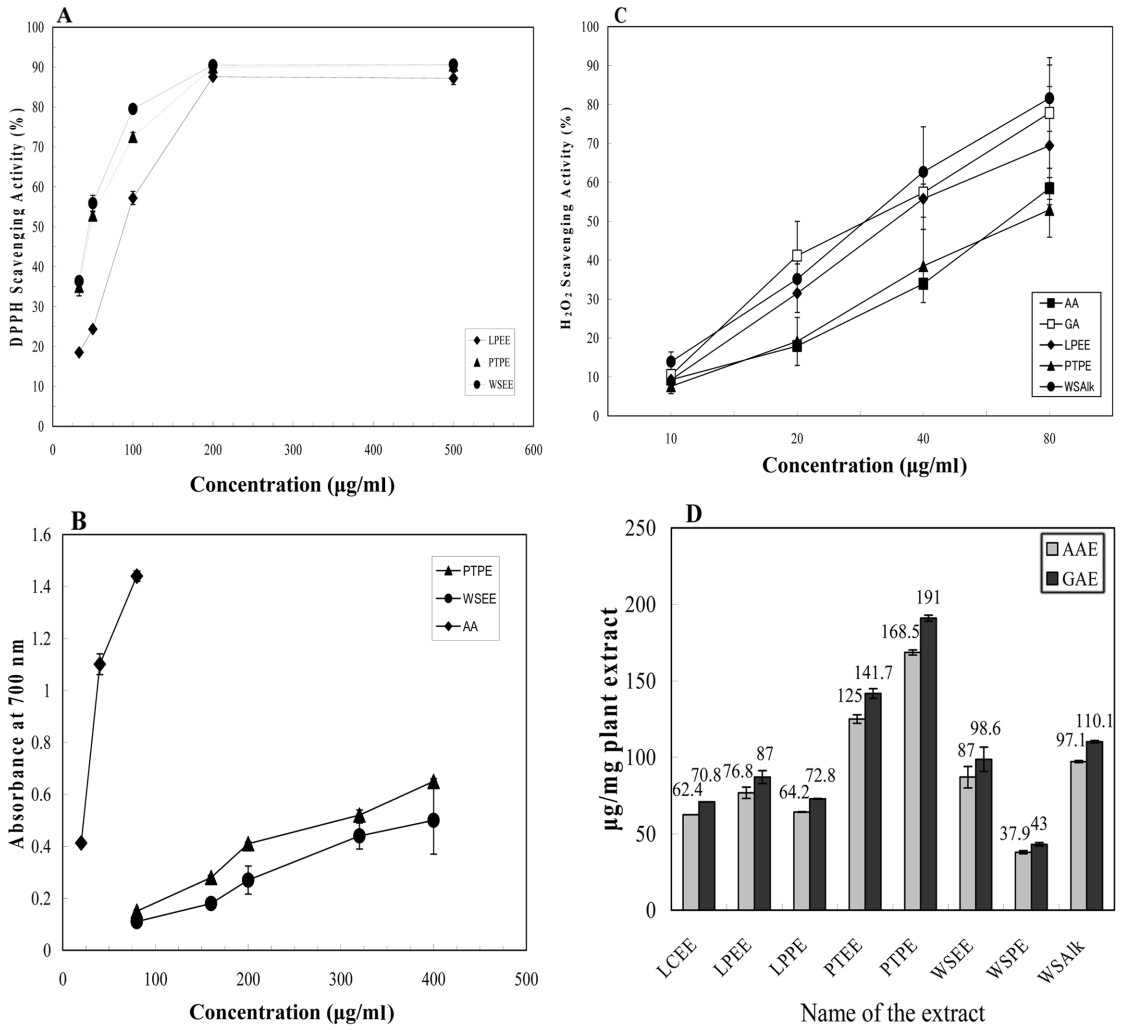

Fig. 1. Antioxidant activity of the extracts. (A): dose-dependency of the DPPH free radical scavenging activity of LPEE, PTPE, and WSEE; (B): dose-dependent increase of reducing power of PTPE, and WSEE (AA: ascorbic acid, positive control); (C): H_2O_2 scavenging ability of LPEE, PTPE, and WSAlk at different concentrations (AA: ascorbic acid, GA: gallic acid, positive control); (D): comparison of total antioxidant capacity of the extracts (AAE: ascorbic acid equivalent, GAE: gallic acid equivalent). The data are presented as mean ± S.D., n = 3 - 5.

ferrous ions. Among the extracts, WSPE and WSAlk were the most promising chelators, and their dose-dependent activities were examined. The IC_{50} values of WSPE and WSAlk were 355 and 395 μg/ml respectively. WSPE and WSAlk exhibited chelating effects on ferrous ions, suggesting that they minimize the concentration of metal in the Fenton reaction.

Hydrogen peroxide scavenging activity

The H_2O_2 scavenging ability of the extracts is shown in Table 1. At a concentration of 40 μg/ml in phosphate buffer, WSAlk showed the highest (62%) activity followed by LPEE (55%) and PTPE (38%). The dose-dependent scavenging effects of LPEE, PTPE, and WSAlk along with standards (AA, ascorbic acid; GA, gallic acid) are shown in Fig. 1(C).

Total antioxidant capacity

Total antioxidant capacities, which are expressed

as the ascorbic acid (AAE), and gallic acid equivalents (GAE) of the different extract are shown in Fig. 1(D). The capacity was highest in PTPE followed by PTEE, and WSAlk; whereas the lowest in WSPE.

Inhibition of α-amylase activity *in vitro*

In the present study, all extracts were found to possess significant ($P < 0.01$) inhibitory effects on starch break-down *in vitro* as shown in Fig. 2. At a concentration of 1 mg/ml in phosphate buffer, the highest inhibitory activity of α-amylase was in WSAlk, followed by WSEE and LPEE. They also showed dose-dependent increase in α-amylase inhibitory activity. At the concentrations of 500, 1000 and 2000 μg/ml, WSAlk showed inhibitory activity of 25.3, 40.2 and 36.7% respectively, and that of in case of LPEE were 15.6, 27.4 and 35.8% respectively. Increasing the concentration of starch 1% to 5% had no effects on the inhibitory activities, indicating non-competitive inhibition.

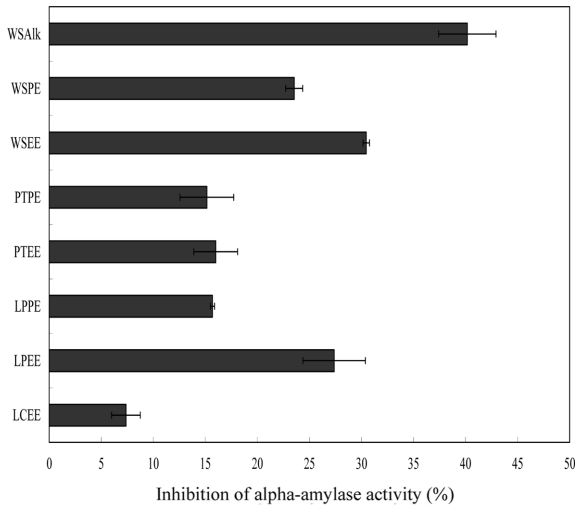

Fig. 2. Effect of the extracts on the inhibition of α-amylase activity (%). α-Amylase without any extract was taken to be 100% activity (control). Inhibition (%) of the activity was studied in presence of 1 mg/ml extract. The data are presented as mean ± S.D., $n = 3 - 5$, $P < 0.01$.

DISCUSSION

Fruits and vegetables are natural sources of dietary fibers, trace elements, antioxidants and bioactive compounds beneficial to health. Polyphenols are the most abundant antioxidants in the diet. Among the plant-originated dietary intake of antioxidants they are at the top. In this study, extract with the highest proportion of polyphenols, displayed the highest antioxidant activity, particularly in case of PTPE. The polar extract of *P. turgidium* consisted of higher polyphenols than ethanol extract whereas *L. pyrotechnica*, and *W. somnifera* had the opposite result (Table 1). Ethanol is more efficient to degrade cell components resulting release of polyphenols from cells. Alkaloid fraction of *W. somnifera* produced considerably larger amounts of polyphenols than its ethanol, and polar extracts because secondary metabolites of plants include various alkaloids as well as polyphenols. However, the common notion that antioxidant activity strictly correlates with total phenolic concentration does not always hold true (Chinnici *et al.*, 2004). In this study, correlation (r) between total polyphenol and DPPH radical scavenging activity, reducing power or total antioxidant capacity is 0.73, 0.65 or 0.79 respectively. It is probable that unknown components in these wild plants, other than polyphenols, might contribute in part to their antioxidant activity. Polar (WSPE) and alkaloid (WSAlk) fractions of *W. somnifera*, displayed a remarkable capacity to bind iron, suggesting that they may protect against peroxidation. An affinity for ferrous ions minimizes the concentration of the catalyzing transition metal needed in a lipid peroxidation reaction. Owing to the complexity of the oxidation-anti-oxidation process, no single testing method is capable of providing a comprehensive view of the anti-oxidative profile of a sample (Parejo *et al.*, 2002). Therefore, multi-method approach is necessary to assess anti-oxidative activity. Moreover, various oxidase enzymes, such as superoxide dismutase produce H_2O_2 *in vivo*. It is toxic to cells because of the production of hydroxyl

radicals, which oxidized a number of molecules including DNA. Thus, removing H_2O_2 is very important for protecting cell components from oxidation. Since phenolic compounds present in the extract are good electron donors, they show the reducing power and may accelerate the conversion of H_2O_2 to H_2O (Ruch *et al*., 1984).

Fruits and vegetables that reduce post-prandial hyperglycaemia by suppressing the hydrolysis of carbohydrates may be helpful in the control of diabetes mellitus. In the present study, α-amylase activity was significantly ($P < 0.01$) inhibited by all extracts (Fig. 2). In dose-dependent effects, at high concentrations saturation of component(s) may have occurred thereby causing no further increase in inhibition. These extracts probably noncompetitively bind to the active site of the enzyme. Promising anti-oxidative, anti-α-amylase, and iron-chelating activities of *W. somnifera* (WSAlk) indicate that it might be used to prevent onset of various diseases including diabetes, cancer, inflammation etc. Reportedly, various natural products inhibit α-amylase and α-glucosidase activities (Hossain *et al*., 2008) such as flavone, flavonoids (Havsteen, 1983; Kim *et al*., 2000) etc. Moreover, polyphenols also have antihyperglycemic effects (Hossain *et al*., 2002; Hanamura *et al*., 2006; Hossain *et al*., 2007), and inhibit the development of diabetes (Zunimo *et al*., 2007).

The results support that these plants, which are consumed by pastoral nomads in Egypt, contribute to their health. Attention should be paid especially to *P. turgidum* and *W. somnifera* because of their high antioxidative activity in addition to considerable inhibition of α-amylase activity. It is necessary to elucidate potential cytotoxic effects when they are used for the preparation of dietary supplements, since some polyphenols perturb the membrane structure (Hossain *et al*., 2002; Aoshima *et al*., 2005). Moreover, fractionation of potential extracts is essential to know the phenolic or nonphenolic compound(s) responsible for the antioxidative and antiamylase activities.

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