COMPOSITION FOR MULTIPLE HEALTH BENEFITS AND METHODS THEREOF

FIELD OF THE INVENTION

The present invention relates to a composition having antioxidant, anti-diabetic, anti-pigmentation, antistress and liver protective effects produced by the method. The composition produced by the method also having immunostimulatory effects on proliferation of immune cells and cytotoxic effects on human cancerous cells.

BACKGROUND ART

Mung bean (*Vigna radiate*), has excellent source of protein (approximately 27%), minerals, vitamins and its essential amino acids composition with respect to other legumes, mainly cultivated in East Asia and South Asia. This legume is believed to have multiple medical properties such as detoxifying, hypolipemic and diuretic properties (Leong, 1995). A non-specific lipid transfer protein extracted from mung bean seeds was known to have antifungal and antibacterial activity (Wang et al., 2004). By means of fermenting ground mung bean powder using Rhizopus oligosporus, Randhir and Shetty (2007) disclosed a solid state bioconversion process to increase phenolic content with improved antioxidant activity that contribute to α-amylase inhibition relevant to potential diabetic management and H. pylori inhibition linked to peptic ulcer management. However, there is no specific anti-diabetic assay conducted to prove its effectiveness in diabetic management. Boiling water extracts from mung bean itself and *Lentinus edodes* has been claimed to contain effective amount of anti-malignancy agent (US Patent No. 5437866) and phytochemicals like isoflavone glycosides and their derivatives (US Patent No. 7182972 B2). From surveys, the nutritive value of fermented foods was seen improved much after gone through fermentation process. For example, mung bean extracts from lactic acid bacteria fermentation was claimed to promote the collagen synthesis and alleviating the skin inflammation (US Patent Application No. 2009/0068150 A1).

US Patent Application No. 2009/0068150 A1 disclosed is a lactic acid bacteria culture of mung bean obtained by culturing lactic acid bacteria in a culture medium containing mung bean extract. The culture contains the mung bean extract and GABA, so that it exhibits effects of promoting collagen synthesis and alleviating inflammation. Accordingly, the cosmetic composition containing the culture can be usefully used as a cosmetic composition for promoting collagen biosynthesis, preventing or improving skin senescence, anti-inflammatory and preventing or improving skin injury.

Another application, US Patent Application No. 2011/0038969 disclosed the invention provides a method for forming a fermented composition of mung bean hulls, including: mixing mung bean hulls and water to form a mixture; adding a fungus into the mixture, wherein the fungus includes *Rhizopus s*p. or *Aspergillus* sp.; and fermenting the mixture to form a fermented composition. The invention also includes a fermented composition of

mung bean hulls by the method and an anti-oxidation and anti-inflammation composition containing the fermented composition of mung bean hulls.

Interest to improve the nutritional and health functional foods has increased globally. Fermented food with high protein content and other nutritional properties has been safely consumed by millions of people in tropical communities as cheap staple foodstuff. During the fermentation, some features like lipid, carbohydrate, protein profile and several vitamins are improved. Fermentation brings changes in texture, aroma, flavour of the product and improves the nutritional quality such as formation of γ-linolenic acid, vitamin B_{12} and the presence of abundant free amino acids including GABA and etc. which has been proven to have pharmaceutical effects on human body (Liem et al., 1977; Nout and Rombouts, 1990; Bisping et al., 1993; Aoki et al., 2003). Other health benefits of fermented foods arise from the antioxidant activity of phenolic compounds were also improved during fermentation (Watanabe et al., 2007 Babu et al., 2009). The present study showed that fermented soybean with 0.1% γ-aminobutyric acid (GABA) has antihypertensive effect activity in spontaneously hypertensive rat (Aoki et al., 2003). Most of the above studies were directed to soybean. Although, there are several studies on the nutritional properties of mung bean as describe above. There is still a quest in identifying the potential nutritional properties of mung bean.

Having described the above, there is still a need in the art for a composition rich in polyphenolic, flavanoid and GABA carries multiple bioactivities including antioxidant, anti-diabetic, anti-pigmentation, antistress, and liver protective effects, The present art will also cover the immunostimulatory effects on immune cells proliferation, cytotoxic effects on human cancerous cells and a method of preparing the same.

SUMMARY OF INVENTION

According to a 1st aspect of the present invention, we provide a method for preparing a germinated mung bean extract comprising the steps of: (a) soaking mung bean in water; (b) incubating the mung bean anaerobically for a period of time to obtain germinated mung bean; (c) drying and grinding the germinated mung bean to obtain fine powder of germinated mung bean; and (d) extracting the germinated mung bean extract rich in bioactive from the fine powder of step (c).

Step (d) further may comprise the steps of: (a) dissolving the fine powder of germinated mung bean in a solvent; (b) separating a layer containing solubles; and (c) freeze drying the layer containing soluble to obtain the germinated mung bean extract rich in bioactive.

The germinated mung bean may comprise moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 2.5%, protein content ranges from 27 to 33%, crude fibre content ranges from 1.5 to 6.0%; and carbohydrate content ranges from 50 to 60%.

There is provided, according to a 2nd aspect of the present invention, a mung bean extract produced by a method as set out above.

The mung bean extract may comprise antioxidant activity, anti-pigmentation activity, anti-diabetic activity, immune cell proliferation activity, immunostimulatory activity, cytotoxic activity, antistress activity or liver protective activity.

We provide, according to a 3rd aspect of the present invention, a mung bean extract as set out above for use as a medicament or in the treatment, co-treatment or prevention of diabetes, immunodeficiency, stress, liver damage or skin pigmentation.

As a 4th aspect of the present invention, there is provided a nutraceutical comprising a composition as set out above and a nutraceutically acceptable carrier.

The nutraceutical may comprise a food product, foodstuff, dietary supplement, nutritional supplement or a supplement composition for a food product or a foodstuff.

We provide, according to a 5th aspect of the present invention, a cosmetic composition comprising a composition as set out above and a cosmetically acceptable carrier.

The cosmetic composition may comprise a skin care preparation.

We also describe a composition for promoting health comprising extract of fermented mung bean; wherein the extract is a product of fermentation of mung bean with *Rhizopus* sp. The provision of fermentation of mung bean with *Rhizopus* sp. is advantageous as it produces fermented mung bean extract high in GABA, free amino acids, polyphenols, wherein the composition exhibits antioxidant, anti-diabetic, anti-pigmentation, immunostimulatory effects on immune cells proliferation and cytotoxic effects on human cancerous cells, liver protective effects, antistress activities which are beneficial in promoting health.

The *Rhizopus* sp. may comprise *Rhizopus microsporus*.

The fermented mung bean may comprise moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 4.0%, protein content ranges from 27 to 37 %, crude fibre content ranges from 1.5 to 3.0%; and carbohydrate content ranges from 45 to 60%.

We further describe a composition for promoting health comprising extract of germinated mung bean. Advantageously, the germinated mung bean extract high in GABA, free amino acids, polyphenols, wherein the composition exhibits antioxidant, anti-pigmentation, anti-diabetic, liver protective effects, and antistress activities. Meanwhile, the composition also possess immunostimulatory effects on immune cells proliferation and cytotoxic effects on human cancerous cells, which are beneficial in promoting health.

The germinated mung bean may further comprise moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 2.5%, protein content ranges from 27 to 33%, crude fibre content ranges from 1.5 to 6%; and carbohydrate content ranges from 50 to 60%.

The composition as set out above may be used as an agent for the treatment, co-treatment or prevention of diabetic, immunodeficiency, stress, liver damage and skin pigmentation .

We describe a nutraceutical comprising a composition as defined above and a nutraceutical acceptable carrier.

The nutraceutical may comprise a food product, foodstuff, dietary supplement, nutritional supplement or a supplement composition for a food product or a foodstuff.

The composition as described above may be used as a medicament.

We further describe a cosmetic composition comprising a composition as described above and a cosmetically acceptable carrier.

The cosmetic composition may comprise a skin care preparation.

The composition as described above, may, when consumed by an animal or human, have an effect on the animal or human selected from the group consisting of; as antioxidant, anti-pigmentation, immunomodulation therapy and cytotoxic effects, antistress, anti-diabetic and liver protective effects.

We further describe a method for preparing a fermented mung bean extract comprises the steps of: (a) preparing the mung bean; (b) inoculating the mung bean with *Rhizopus* sp.; (c) incubating the mung bean aerobically; (d) fermenting the mung bean anaerobically for a period of time; (e) drying and grinding the fermented mung bean to a fine powder; and (f) extracting the fermented mung bean extract rich in bioactive from the fine powder of step (e).

In step (b) the *Rhizopus* sp. may comprise *Rhizopus microsporus*.

Step (f) further may comprise the steps of: (a) dissolving the fine powder of fermented mung bean in a solvent; (b) separating a layer containing solubles; (c) freeze drying the layer containing soluble to obtain the fermented mung bean extract rich in bioactive.

The solvent may comprise water.

Step (a) may be by soaking in distilled water for mung bean hull removal, washing the dehulled mung bean; boiling the dehulled mung bean in water, draining and cooling to room temperature and drying the dehulled mung bean to a moisture content ranging from 45% to 55%.

We further describe a method for preparing a germinated mung bean extract comprises the steps of: (a) soaking mung bean in water; (b) incubating the mung bean anaerobically for a period of time to obtain germinated mung bean,(c) drying and grinding the germinated mung bean to obtain fine powder of germinated mung bean; and (d) extracting the germinated mung bean extract rich in bioactive from the fine powder of step (c).

Step (d) may further comprise the steps of (a) dissolving the fine powder of germinated mung bean in a solvent or water; (b) separating a layer containing solubles; (c) freeze drying the layer containing soluble to obtain the germinated mung bean extract rich in bioactive.

Freeze drying method may be used to produce a dried powder composition.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is illustrated, although not limited, by the following description of embodiments made with reference to the accompanying drawings in which:

FIGURE 1: illustrates process flow for preparation of starter culture for fermentation process of the production of bioactive fermented bean extract in a preferred embodiment of the invention.

FIGURE 2: illustrates process flow of fermented mung bean in a preferred embodiment of the invention.

FIGURE 3: illustrates process flow of fermented soybean in a preferred embodiment of the invention.

FIGURE 4: illustrates process flow of anaerobic germination for mung bean in a preferred embodiment of the invention.

FIGURE 5: illustrates total phenolic content of different types of bean extracts in a preferred embodiment of the invention.

FIGURE 6: illustrates IC_{50} value of different types of bean extracts to scavenge free radical in 1,1-diphenyl-2-picryl-hydrazil (DPPH) assay in a preferred embodiment of the invention.

FIGURE 7: illustrates ferric reducing antioxidant power (FRAP) value of different types of bean extracts in a preferred embodiment of the invention.

FIGURE 8: illustrates total soluble phenolic acids content of different types of bean extracts in a preferred embodiment of the invention.

FIGURE 9: illustrates immunoproliferative responses of splenocytes after treated with different types of bean extracts in a preferred embodiment of the invention.

FIGURE 10: illustrates immunoproliferative responses of thymocytes after treated with different types of bean extracts in a preferred embodiment of the invention.

FIGURE 11: illustrates picture of blood glucose changes of normaglycemic mice treated with different types of bean extracts prior to 8 hours fasting in a preferred embodiment of the invention.

FIGURE 12: illustrates of blood glucose changes of glucose induce hyperglycemic mice treated with different types of bean extracts concurrently feed with glucose solution in a preferred embodiment of the invention.

FIGURE 13: illustrates of blood glucose changes of alloxan induced diabetic mice treated with different types of bean extracts concurrently feed with glucose solution in a preferred embodiment of the invention.

FIGURE 14: illustrates of blood glucose changes of alloxan induced diabetic mice treated with different types of bean extracts continuously for 10 days in a preferred embodiment of the invention.

FIGURE 15: illustrates swimming time of mice for antistress study treated with different types of bean extracts in a preferred embodiment of the invention.

DETAILED DESCRIPTION OF THE EMBODIMENTS

In order to achieve the above object of the present invention, the present invention provides a composition for promoting health comprising a fermented or a germinated mung bean extract wherein the extract having activities of antioxidant, anti-pigmentation, anti-diabetic, antistress, and liver protection effects. Besides, these extracts also possess immunostimulatory effect on immune cells proliferation and cytotoxic effects on human cancerous cells.

In one embodiment of the present invention, the composition for promoting health comprising the fermented mung bean extract; wherein the extract is a product of fermentation of mung bean with *Rhizopus* sp. Most preferably, the *Rhizopus* sp. is *Rhizopus microsporus*. *Rhizopus* sp. 5351 from MARDI's collection was used for fermenting. The *Rhizopus* sp. 5351 was identified by CABI (IMI Number 500245) as *Rhizopus microsporus*. *Rhizopus microsporus* is readily available and accessible from MARDI as starter culture.

In order to achieve the composition of the present invention, studies were conducted on the content of the fermented mung bean prior to extraction. The fermented mung bean preferably comprises moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 4.0%, protein content ranges from 27 to 37%, crude fibre content ranges from 1.5 to 3.0%; and carbohydrate content ranges from 45 to 60% as indicated in Table 3.

The present invention also provides a composition for promoting health comprising extract of germinated mung bean. A content studies prior to extraction step shows that the germinated mung bean preferably comprises moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 2.5%, protein content ranges from 27 to 33%, crude fibre content ranges from 1.5 to 6.0%; and carbohydrate content ranges from 50 to 60%.

The composition for promoting health of the present invention is usable as an agent for the treatment, co-treatment or prevention of diabetic, immunodeficiency or suppression of immune cell proliferation, skin pigmentation, liver damage, and stress.

In one embodiment, a nutraceutical may be formulated comprising a composition and a nutraceutical acceptable carrier. The nutraceutical may take the form of a food product, foodstuff, dietary supplement, nutritional supplement or a supplement composition for a food product or a foodstuff.

The composition for promoting health of the present invention is for use as a medicament.

In another embodiment, a cosmetic composition comprising a composition of the present invention and a cosmetically acceptable carrier. The cosmetic composition which is a skin care preparation.

The composition of the present invention which when consumed by an animal or human, has an effect on the animal or human selected from the group consisting of; as antioxidant, anti-pigmentation, immunostimulation of immune cells proliferation, antistress, anti-diabetic and liver protective substances.

The present invention also provides a method for preparing the above extracts of the invention. FIGURE 1 illustrates process flow for preparation of starter culture used for preparing fermented mung bean/soy bean in Example 1 and Example 2.

Describe herein generally is a method for preparing a fermented mung bean extract comprises the steps of: (a) preparing the mung bean; (b) inoculating the mung bean with *Rhizopus* sp.; (c) incubating the mung bean aerobically; (d) fermenting the mung bean anaerobically for a period of time; (e) drying and grinding the fermented mung bean to a fine powder; and (f) extracting the fermented mung bean extract rich in bioactive from the fine powder of step (e). Most preferably in step (b) the *Rhizopus* sp. is *Rhizopus microsporus*.

FIGURE 2 illustrates detail steps of preparing a fermented mung bean as in Example 1 prior to extraction. Whereas, FIGURE 3 illustrates detail steps of preparing a fermented soybean as in Example 2 prior to extraction.

Non-fermented soybean extract, non-fermented mung bean extract and fermented soybean extract were also studied and used for comparative purpose.

The method for preparing a fermented mung bean extract wherein the extraction step (f) further comprises the steps of; dissolving the fine powder of fermented mung bean in a solvent or water; separating a layer containing solubles; freeze drying the layer containing soluble to obtain the fermented mung bean extract rich in bioactive.

In one embodiment, the preparing step (a) is by soaking in distilled water for mung bean hull removal, washing the dehulled mung bean; boiling the dehulled mung bean in water, draining and cooling to room temperature and drying the dehulled mung bean to a moisture content ranging from 45% to 55%.

Describe herein generally is a method for preparing a germinated mung bean extract comprises the steps of: (a) soaking mung bean in water; (b) incubating the mung bean anaerobically for a period of time to obtain germinated mung bean, (c) drying and grinding the germinated mung bean to obtain fine powder of germinated mung bean; and (d) extracting the germinated mung bean extract rich in bioactive from the fine powder of step (c). FIGURE 4 illustrates detail steps of germinated mung bean as in Example 3 prior to extraction. The extraction step (d) further comprises the steps of: (a) dissolving the fine powder of germinated mung bean in a solvent or water; (b) separating a layer containing solubles; (c) freeze drying the layer containing soluble to obtain the germinated mung bean extract rich in bioactive.

The fermented mung bean extract and germinated mung bean extract were tested for GABA and amino acid content. The results were compared with non-fermented soybean extract, non-fermented mung bean extract, and fermented soybean extract as summarized in Table 1. The germinated and fermented mung bean extract contain the highest concentration of GABA and free amino acids, respectively.

Table 1. Changes in the levels of GABA and free amino acids (AA) profile of bioactive extracts obtained from non-fermented mung bean, anaerobically germinated mung bean, non-fermented soybean, fermented mung bean and fermented soybean inoculated with MARDI's *Rhizopus* sp. 5351 strain under different fermentation condition.

AA	MB	GMB	FMB	SB	FSB
			(g/100g Dry Weight)		
His	0.010	0.125	0.157	0.005	0.117
Ser	0.011	0.044	0.093	0.022	0.145
Arg	0.046	0.296	0.334	0.012	0.395
Gly	0.008	0.152	0.100	0.006	0.123
Asp	0.043	0.046	0.160	0.016	0.112
Glu	0.047	0.578	0.276	0.019	0.234
Thr*	0.008	0.090	0.112	0.000	0.140
Ala	0.020	0.325	0.513	0.009	0.277
GABA	**0.016**	**0.539**	**0.122**	**0.001**	**0.328**
Pro	0.007	0.125	0.110	0.000	0.181
Lys*	0.001	0.086	0.134	0.000	0.201
Tyr	0.008	0.141	0.421	0.003	0.196
Met*	0.001	0.019	0.019	0.000	0.039
Val*	0.007	0.150	0.183	0.000	0.168
Ile*	0.000	0.075	0.114	0.000	0.137
Leu*	0.004	0.122	0.195	0.002	0.216
Phe*	0.019	0.160	0.283	0.005	0.203
FAAs	0.256	3.073	3.326	0.100	3.212
EAAs	0.040	0.702	1.040	0.007	1.104

Each value in the table represents the mean ± standard deviation from triplicate analyses
Abbreviations: * indicated essential amino acid; AA = amino acids; FAAs = total free amino acids content; EAAs = total essential amino acids content; MB = mung bean; GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h; SB = soybean; FSB = aerobic fermentation of soybean for 30 h, followed by anaerobic fermentation for 20 h.

The phenolic content of the fermented mung bean extract and germinated mung bean extract were determined. FIGURE 5 illustrates total phenolic content of different types of bean extracts. Surprisingly, the fermented mung bean extract shows substantially high total phenolic content in comparison with non-fermented mung bean extract, germinated mung bean extract, non-fermented soybean extract and fermented soybean extract.

FIGURE 6 illustrates IC_{50} value of different types of bean extracts to scavenge free radical in 1,1-diphenyl-2-picryl-hydrazil (DPPH). The result shows that the fermented mung bean extract able to scavenge the free radical at lower concentration as compared to non-fermented mung bean extract, germinated mung bean extract, non-fermented soybean extract and fermented soybean extract.

The composition comprising extract of fermented mung bean or germinated mung bean extract of the present invention exhibits antioxidant property as illustrated in FIGURE 7. FIGURE 7 illustrates ferric reducing antioxidant power (FRAP) value on different types of bean extracts. The result indicates that fermented mung bean extract shows the highest FRAP value. The composition comprising extract of fermented mung bean of the present invention are also rich in phenolic acids as illustrated in FIGURE 8. FIGURE 8 illustrates total soluble phenolic acids content on different types of bean extracts. The soluble phenolic acids profile of each extract is summarized in Table 2.

Table 2. Soluble Phenolic Acids Profile of Different Bean Extracts

Bioactive Extract	Protocatechuic	β-Hydrobenzoic	Vanillic Acid	Caffeic Acid	Syringic Acid	p-Coumaric Acid	Ferullic Acid
			(μg/g extract)				
MB	2.93	4.59	0	0	0	8.97	0
GMB	12.94	4.63	3.74	14.04	0	7.02	4.70
FMB	201.32	0	16.30	7.05	15.26	7.50	6.17
SB	0	0	0	0	10.49	0	7.76
FSB	15.22	6.19	4.64	0	3.36	7.85	5.38

Abbreviations: MB = mung bean; GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h; SB = soybean; FSB = aerobic fermentation of soybean for 30 h, followed by anaerobic fermentation for 20 h.

The composition comprising extract of fermented mung bean or germinated mung bean of the present invention exhibits immunoproliferative effect as illustrated in FIGURE 9 and FIGURE 10. FIGURE 9 illustrates immunoproliferative responses of splenocytes after treated with different types of bean extracts. FIGURE 10 illustrates immunoproliferative responses of thymocytes after treated with different types of bean extracts.

The composition comprising extract of fermented mung bean or germinated mung bean of the present invention exhibits anti-diabetic property as illustrated in FIGURE 11, FIGURE 12, FIGURE 13 and FIGURE 14. FIGURE 11 illustrates picture of blood glucose changes of normaglycemic mice treated with different types of bean extracts prior to 8 hours fasting. Whereas, FIGURE 12 illustrates the blood glucose changes of glucose induce hyperglycemic mice treated with different types of bean extracts concurrently feed with glucose solution. FIGURE 13 and FIGURE 14 illustrate the blood glucose changes of alloxan induced diabetic mice treated with different types of bean extracts for 3 hours and 10 days, respectively. None of the fermented and germinated mung bean extracts reduced the blood sugar of normaglycemic mice lower than their initial blood sugar. On the other hand, feeding of the fermented mung bean extract and germinated mung bean extract to glucose induced hyperglycemic and alloxan induced diabetic mice was able to regulate the blood glucose level.

The extracts were used in antistress study, the result shows that fermented mung bean extract and germinated mung bean extract exhibit antistress effect comparable to diazepam. FIGURE 15 illustrates swimming time of mice for antistress study treated with different types of bean extracts in a preferred embodiment of the invention.

The composition comprising fermented mung bean extract of the present invention inhibits breast cancer cell, K562 leukemia cell and exhibits immunoproliferation effect.

The composition comprising fermented and germinated mung bean extract of the present invention were able to recover the liver profile of mice under the acute injury induced by ethanol.

The resultant product from fermented or germinated mung bean may preferably dry to obtain concentrated bioactive extract. Drying may be achieved by spray drying or freeze drying. Preferably, freeze drying method is use to produce a dried powder composition.

The composition of the present invention may be further processed to form nutraceuticals suitable for oral administration. One example of the composition may be used in the preparation of healthful functional drink comprises composition of the present invention which comprises fermented mung bean extract or germinated mung bean extract with citric acid, high fructose syrup and water.

Another example as capsule for health supplement comprises composition of the present invention which comprises fermented mung bean extract or germinated mung bean extract with water and maltodextrin.

The compositions of this invention may be used in the manufacture of a medicament for the prevention of human health complications which may comprise diabetes, immunodeficiency of immune cells proliferation, liver damage and stress.

According to another embodiment, the extract may be used as an immunostimulator.

According to a further embodiment, the extract may be used as antioxidant and whitening agent. One example of use is as whitening cream comprises composition of the present invention which comprises fermented mung bean extract or germinated mung bean extract with vegetable oils, Tween 20, cetyl alcohol, water, xantham gum and sorbitol.

The composition of the present invention also provides a cosmetic composition for anti-pigmentation, promotion of skin whitening. The fermented and germinated mung bean extracts show significant inhibition effect against the tyrosinase enzyme for pigmentation process.

Hereinafter, the present invention is described in detail using mung bean and soybean as examples.

Example 1

Mung Bean fermentation with *Rhizopus* sp. 5351 (*Rhizopus microsporus*)

For the solid state fermentation of mung bean, mung bean was soaked overnight in distilled water (1:3 w/v) for 18 hours. The dehulled mung bean was washed few times and were boiled in water (1:6 w/v) for 60 minutes, drained and cooled to room temperature. The cooked mung bean was dried to the moisture content of 45 - 55% before inoculated with MARDI's *Rhizopus* sp. 5351 strain at the level of 2 g/kg mung bean, packed in perforated polyethylene bags (thickness: 15 – 20 mm, perforation distance: 1 cm apart, 100 g cooked mung bean per pack). The inoculated mung bean was aerobically incubated at room temperature (30°C) for 24 hours, followed by anaerobic fermentation for 24 hours before dry the fermented mung bean at 65°C for 1 day and lastly, ground to fine powder as illustrated in FIGURE 2.

Example 2

Soybean fermentation *Rhizopus* sp. 5351 (*Rhizopus microsporus*)

Solid state fermentation of soybean was carried out in another example where soybean was soaked overnight in distilled water (1:3 w/v) for 18 hours. The dehulled soybean was washed few times and steamed for 30 minutes. The cooked soybean was dried to the moisture content of 45 - 55% before inoculated with MARDI's *Rhizopus* sp. 5351 strain at the level of 2 g/kg soybean, packed in perforated polyethylene bags (thickness: 15 – 20 mm, perforation distance: 1 cm apart, 100 g cooked soybean per pack). The inoculated soybean was initially incubated aerobically at 30°C for 30 hours and then successively subjected to anaerobic fermentation for 20 hours. Finally, the fermented soybean was dried at 70°C for 2 days before ground to fine powder as illustrated in FIGURE 3.

Example 3

Mung bean anaerobic germination

Mung bean was also subjected to anaerobic germination in another example. A 100 g of mung bean was weighed and washed few times to remove any dirt. The cleaned mung bean was soaked with 500 mL distilled water with adjusted pH to 4 at 40°C for 4 h. Then, the bean was transferred to anaerobic jar and incubated under anaerobic condition for 72 hours in the dark condition at room temperature. Finally, the germinated mung bean was dried at 75°C for 2 days before ground to fine powder (FIGURE 4).

Example 4

Proximate analysis of fermented and germinated beans prior to extraction

The proximate analysis was done on both fermented and germinated beans and the results are summarized in Table 3. The crude fibre, fat and moisture content were determined by AOAC (1984) official methods and crude protein content was analyzed using Kjeldahl method. The carbohydrate content was calculated as follows:

Carbohydrate content = 100 – (Moisture + Fat + Ash + Protein + Crude fibre)

Table 3. Proximate Composition of Fermented and Germinated Mung Bean

	Proximate Composition (%)				
	Moisture Content	**Fat Content**	**Protein Content**	**Crude Fibre Content**	**Carbohydrate Content**
MB	**3 – 7**	**1.0 – 1.5**	**25 – 27**	**1.0 – 1.5**	**60 – 65**
GMB	**3 – 7**	**1.5 – 2.5**	**27 – 33**	**1.5 – 6.0**	**50 – 60**
FMB	**3 – 7**	**1.5 – 4.0**	**27 – 37**	**1.5 – 3.0**	**45 – 60**

Abbreviations: MB = mung bean; GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h.

Example 5

Extraction of Example 1 and 2

Powderized fermented beans from Example 1 and 2 were subjected to extraction for the following application. The water soluble bioactive extract was extracted from fermented beans using distilled water. A total of 1 g powderized fermented bean with the moisture content of 2.5 – 3.0% was mixed with 20 mL distilled water. The mixture was vigorously shaken at 300 rpm (30°C) for 30 minutes, followed by centrifugation at 10,000 rpm for 5 minutes. The supernatant obtained was stored overnight at -40°C prior to freeze drying to obtain a concentrated bioactive extract.

Example 6

Extraction of Example 3

Powderized germinated mung bean from Example 3 was also underwent extraction. The water soluble bioactive extract was extracted from germinated mung bean using distilled water. A total of 1 g fine germinated mung bean powder with the moisture content of 2.0 – 2.5 % was mixed with 20 mL distilled water. The mixture was vigorously shaken at 300 rpm

(30°C) for 30 minutes, followed by centrifugation at 10,000 rpm for 5 minutes. The supernatant obtained was stored overnight at -40°C prior to freeze drying to obtain a concentrated bioactive extract.

Example 7

GABA and amino acid profiling

The extract from Example 1, 2 and 3 were tested to evaluate their GABA and amino acids profile of water soluble fraction according to Waters UPLC™ amino acids analysis solution. The water soluble bioactive extract from germinated mung bean from Example 6, fermented mung bean and fermented soybean from Example 5 were derivatized with 70 µL of AccQ-Tag™ Ultra borate buffer and 20 µL of AccQ™ Fluor reagent before subjected to UPLC analysis. The GABA content and amino acids profile of germinated or fermented bean extracts was separated using AccQ-Tag™ Ultra column (2.1 mm x 100 mm, 1.7 µm) at the flow rate of 0.7 mL/min with column temperature controlled at 55°C under the UV spectra of 260 nm. The gradient elution consisting of AccQ-Tag™ Ultra Eluent A and AccQ-Tag™ Ultra Eluent B with the injection volume of 1 µL. Gradient elution was conducted as follows: from 0 to 0.54 min, maintained at 99.9% A; from 0.54 to 5.74 min, linear gradient from 99.9 to 90.9% A; from 5.74 to 7.74 min, linear gradient from 90.9 to 78.8% A; from 7.74 to 8.50 min, linear gradient from 78.8 to 40.4% A and then hold for 0.3 min at 40.4% A; from 8.80 to 8.90 min, linear gradients from 40.4 to 99.9% A and then maintained at 99.9% for another 2.1 min. Quantification was made using calibration curves obtained by injecting known amounts of amino acids standard and GABA as external standards with known retention times. The total essential amino acids were calculated based on the sum of phenylalanine, threonine, methionine, leucine, isoleucine, lysine and valine. The levels of GABA and free amino acids (AA) profile of anaerobically germinated mung bean, fermented mung bean and fermented soybean are summarized in Table 1.

Example 8

Determination of phenolic content

The total phenolic content of bioactive extracts of the different bean extracts was determined according to Marina et al. (2009) with some modifications. Aliquots of the 1 mL water extract (2 mg/mL) were taken in a test tube and mixed with 5 mL Folin-Ciocalteu reagent (1:1 with water) and allow to stand at room temperature for 5 minutes. A total of 4 mL of sodium carbonate solution (7.5%, w/v) were added sequentially and vortex the reaction mixture. The tubes were placed in the dark for 2 hours and the absorbance was recorded at 765 nm against the reagent blank. The amount of total phenolic content was calculated as gallic acid equivalents (mg GAE/g extract) from a gallic acid calibration curve as shown in FIGURE 5. As can be observed, the fermented mung bean extract have substantially high total phenolic content.

Example 9

Determination of scavenging activity

The free radical scavenging activity of the different bean extracts was tested using 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to Thaipong et al.(2006) procedure with minor modifications. The germinated or fermented beans were extracted by boiling with distilled water at 100°C for 10 minutes. The DPPH stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and then stored at -20°C. The working solution was obtained by mixing 10 mL stock solution with 45 mL methanol to obtain an absorbance of 1.1 ± 0.02 units at 517 nm using spectrophotometer. To 150 µL of the water extract sample, 2850 µL of freshly prepared methanolic DPPH solution was added and shaken vigorously. The decolorizing process was recorded after left stand for 30 minutes in the dark condition. The absorbance (Abs) was measured at 517 nm and compared with a blank control. IC_{50} (Inhibitory concentration of 50%) was determined from the DPPH calibration curve, denoted the concentration of sample required to scavenge the 50% of the DPPH free radicals and the result was presented in IC_{50} value as shown in FIGURE 6. The scavenging ability was calculated as follows:

Scavenging activity (%) = $[1 - (Abs_{sample}/Abs_{control})] \times 100$

Example 10

Determination of antioxidant activity using FRAP Assay

The FRAP assay was done according to method of Thaipong et al. (2006). The stock solutions including 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-*s*-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution,

and 2.5 mL $FeCl_3.6H_2O$ solution and then warmed at 37°C before using. A total of 150 µL of bioactive extract (5 mg/mL) from germinated or fermented beans was allowed to react with 2850 µL of FRAP solution and shaken vigorously before left to stand for 30 minutes in the dark. The reading of the colored product (ferrous tripyridyltriazine complex) was taken at 593 nm. The ascorbic acid was used as a standard and was linear in the range fall between 20 to 200 ppm. The result was expressed as ascorbic acid equivalent (mg AAE/ g extract) as summarized in FIGURE 7. The result indicates that germinated/fermented bean extracts show higher FRAP value.

Example 11

Determination of soluble phenolic acids compounds

Soluble phenolic acids compounds in germinated or fermented beans (5 g each) were extracted with 70% ethanol (4 x 50 mL, 10 min each) according to Tian et al. (2004). Each extract was pooled and evaporated to 10 mL at 30°C under reduced pressure and then was lyophilized to dryness. Dry samples were dissolved in 2 mL of 2.5% methanol and analyses of soluble phenolic acids compounds were carried out with a HPLC, Alliance Separation Module (Waters, 2695), equipped with a diode array detector (Waters, 2996). A 10 µL aliquot of sample solution was separated using a reverse-phase analytical column (100 mm x 34.6 mm Chromolith Performance RP-18e, Merck) with the temperature controlled at 30°C. The mobile phase consisted of mobile phase A (methanol and distilled water mixtures at 2.5:97.5, v/v) and mobile phase B (methanol and distilled water mixtures at 50:50, v/v) with a flow rate of 2.1 mL/min. Both mobile phase A and B were adjusted to pH 3 with H_3PO_4. Gradient elution was performed as follows: from 0 to 5 min, 100% A; from 5 to 6 min, linear gradients from 100 to 90% A; from 6 to 10 min, linear gradient from 90 to 82% A; from 10 to 15 min, linear gradient from 82 to 75% A; from 15 to 20 min, linear gradient from 75 to 65% A; from 20 to 22 min, linear gradient from 65 to 0% A; and from 22 to 24 min, linear gradient from 0 to 100% A. Peak identification was made by comparing retention times and UV spectra (280 nm) with authentic compounds. Quantification was made using calibration curves obtained by injecting known amounts of pure compounds as external standards. The total soluble phenolic acids content was calculated based on the sum of protocatechuic, β-hydroxybenzoic, vanillic acid, syringic acid, caffeic acid, p-coumaric acid and ferulic acid and were shown in FIGURE 8.

Example 12

Anti-pigmentation activity

Anti-pigmentation effect of germinated and fermented beans was studied using tyrosinase inhibition assay using L-tyrosine and L-DOPA as substrates (Lee et al., 1996). Extracts compounds were dissolved in distilled water to a final concentration of 40 mg/mL. Kojic acid was used as positive control. 70 µL of each sample solution of different concentrations was combined with 30 µL of tyrosinase (100 Units/mL in 50mM phosphate buffer, pH 6.8) in triplicate in a 96-well microtitre plates. After incubation at room temperature for 5 minutes, 110 µL of substrate (2mM L-tyrosine or 0.4mM L-DOPA) were added to each well. Microtitre plates were incubated for 30 minutes at room temperature. Optical densities of the wells were then determined at 492 nm with the BIO-TEK Power Wave XS multi-well plate reader (A.D.P., Weltevreden Park, South Africa). Tables 4 and 5 summarize the percentage inhibition of tyrosinase on L-tyrosine substrate and L-DOPA substrate treated with different bean extracts, respectively. The results obtained suggest that the both germinated and fermented bean extracts show significant inhibition effect against the tyrosinase enzyme for pigmentation process.

Table 4. Percentage Inhibition of Tyrosinase (L-Tyrosine Substrate) treated with different Bean Extracts

Extract Concentration (50 mg/mL)	**Percentage of Inhibition**
MB	40.06
GMB	51.16
FMB	85.71
SB	26.8
FSB	84.16

Abbreviations: MB = mung bean; GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h; SB= soybean; FSB = aerobic fermentation of soybean for 30 h, followed by anaerobic fermentation for 20 h.

Table 5. Percentage Inhibition of Tyrosinase (L-DOPA Substrate) treated with different Bean Extracts

Extract Concentration (50 mg/mL)	**Percentage of Inhibition**
MB	**-**
GMB	17.06
FMB	39.12
SB	**-**
FSB	**-**

Abbreviations: MB = mung bean; GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h; SB= soybean; FSB = aerobic fermentation of soybean for 30 h, followed by anaerobic fermentation for 20 h.

Example 13

Cytotoxic activity on human breast adenocarcinoma cell line MCF-7

Cytotoxic effects of the germinated and fermented bean extracts on human breast adenocarcinoma cell line MCF-7 was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. A total of 2 X 10^5 cells was treated with the bioactive extract from germinated or fermented beans and incubated in a 96 well plate at 37°C under 5% CO_2 incubator for selected periods (24 h, 48 h, 72 h). After incubation, 20 µL of MTT at 5 mg/mL concentration was added into each well and incubated for 4 hours at 37°C under 5% CO_2 incubator. Thereafter, medium consisting of MTT (170 µL) was removed from each well and DMSO (100 µL) extraction buffer was added to each well to extract and solubilize the formazan crystal. The solution was vigorously mixed to dissolve the reacted dye and incubated for 20 minutes at 37°C under 5% CO_2 incubator. Finally, the plate was read on a Powervex 340 Microtiter-Plate ELISA Reader using 570 nm wavelength. Table 6 indicates the IC_{50} value of MCF 7 cell treated with different bean extracts at different incubation time. Only fermented mung bean and soybean extracts were able to inhibit 50% of breast cancer cell viability at all the tested time points.

Table 6. IC_{50} Value of MCF 7 Cell Treated with Different Bean Extracts at Different Incubation Time

Bean Extract	IC_{50} value (mg/mL)		
	24h	48h	72h
GMB	>10	>10	>10
FMB	8.30	4.80	2.30
FSB	8.47	7.80	3.60

Abbreviations: GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h; FSB = aerobic fermentation of soybean for 30 h, followed by anaerobic fermentation for 20 h.

Example 14

Cytotoxic activity on human erythromyeloblastoid leukemia cell line K562

Cytotoxic effects of the germinated and fermented bean extracts on human erythromyeloblastoid leukemia cell line K562 was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. A total of 2 X 10^5 cells was treated with the bioactive extract from germinated or fermented bean and incubated in a 96 well plate at 37°C under 5% CO_2 incubator for selected periods (24 h, 48 h, 72 h). After incubation, 20 µL of MTT at 5 mg/mL concentration was added into each well and incubated for 4 hours at 37°C under 5% CO_2 incubator. Thereafter, medium consisting of MTT (170 µL) was removed from each well and DMSO (100 µL) extraction buffer was added to each well to extract and solubilize the formazan crystal. The solution was vigorously mixed to dissolve the reacted dye and incubated for 20 minutes at 37°C under 5% CO_2 incubator. Finally, the plate was read on a Powervex 340 Microtiter-Plate ELISA Reader using 570 nm wavelength. Table

7 indicates the IC_{50} value of K562 cell treated with different beans extract at different incubation time. Only fermented mung bean extract was able to inhibit 50% of K562 leukemia cell viability particularly at 48 hours and above.

Table 7. IC_{50} Value of K562 Cell Treated with Different Bean Extracts at Different Incubation Time

Bean Extract	IC_{50} value (mg/mL)		
	24 h	48 h	72 h
GMB	>10	>10	>10
FMB	>10	9.80	4.87
FSB	>10	>10	7.33

Abbreviations: GMB = anaerobic germination of mung bean for 72 h; FMB = aerobic fermentation of mung bean for 24 h, followed by anaerobic fermentation for 24 h; FSB = aerobic fermentation of soybean for 30 h, followed by anaerobic fermentation for 20 h.

Example 15

Immunoproliferation effects

Immunoproliferation effects of the germinated and fermented bean extracts on lymphocytes from spleen and thymus were tested using MTT assay. Splenocytes and thymocytes from mice were isolated as stated below. Mice were deeply anaesthetised by placing the tissue paper with chloroform on the mouth and were sacrificed by cervical dislocation in a sterile condition. Then, thymus and spleen were removed and washed with HANK'S Balance Salt (HBSS) (Sigma, USA). The spleen or thymus was pressed with a rubber syringe plunger and pushed through a sterile wire mesh screen with Phosphate buffer saline (PBS- 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) -0.1% Bovine serum albumin (BSA)- 2 mg/mL Ethylenediaminetetraacetic acid (EDTA) buffer. Red blood cells in the spleen cell suspension were removed using lysis buffer (8 g NH_4Cl, 1 g Na_2EDTA, 0.1 g KH_2PO_4, pH 7.4). The cell suspension was washed three times in PBS-BSA-EDTA buffer and resuspended in RPMI-1640 media (Flowlab, Australia) with 100 IU/mL of penicillin, 100 µg/mL of streptomycin (Flowlab, Australia), 10% v/v Foetal Bovine Serum (FBS) (PAA, Austria). Cell counting was performed to determine the splenocytes and thymocytes cell number and cell viability with using MTT assay. FIGUREs 9 & 10 summarize the percentage of proliferation effect of different bean extracts on splenocytes and thymocytes cell, respectively. MTT assay was performed as stated in Example 13. Fermented mung bean extract was able to stimulate proliferation of both splenocytes and thymocytes at all the tested time points. On the other hand, germinated mung bean extract only showed immunoproliferation effect on thymocytes at 24 hours while fermented soybean extract was not able to stimulate immunoproliferation on both cells at all-time points.

Example 16

Hypoglycemic and hyperglycemic effects

Hypoglycemic and glucose induce hyperglycemic effects of germinated and fermented bean extracts were also evaluated to determine whether they would induce hypoglycemia besides reducing blood sugar by feeding the extracts to mice (Aderibigbe et al., 2001). In accordance with the study, no significant changes of glucose level on the normaglycemic mice were noted as shown in FIGURE 11. None of the bean extracts reduced the blood sugar lower than the initial blood sugar of the mice. On the other hand, feeding of the bean extracts to glucose induced hyperglycemic mice was able to regulate the blood glucose level more comparably to *Momordica charantia* with minimal changes of blood glucose level as compared to initial blood glucose level as shown in FIGURE 12. Furthermore, bean extracts was able to reduce the postprandial glucose level of alloxan induced diabetic mice as shown in FIGURE 13. Unlike *Momordica charantia*, 10 days continuous feeding of fermented mung bean extract was able to reduce the fasting glucose level of alloxan induced diabetic mice as shown in FIGURE 14.

Example 17

Toxicity test

The different bean extracts were also evaluated to determine on their acute toxicity by feeding the extracts to mice. In accordance with the OECD guideline 423: acute oral toxicity, mice were feed with 5g/kg body weight of extracts and observed up to 14 days. All mice survive and no negative effect upon the mice behavior up to 14 days.

Example 18

Antistress - swimming endurance test

Antistress effect of germinated and fermented bean extracts was also evaluated by swimming endurance test on mice according to Kulkarni and Juvekar (2008). Mice were divided into 4 groups with each group of 6. Control group was treated with 0.2% sodium carboxy methyl cellulose in saline; while positive control group received diazepam (2 mg/kg, p.o.). Group 3 and group 4, mice were feed with either fermented or germinated mung bean extract in 1000 mg/kg, p.o. for 21 days by oral route. Swimming test was carried out on 21st days and immobility time was recorded as shown in FIGURE 15. Lipid profile of total cholesterol and triglyceride (TG), total protein and liver profile of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) of the blood samples from the tested mice were evaluated simultaneously. Both germinated and fermented mung bean extracts were able to reduce the immobility time, total cholesterol, TG, total protein, ALT, ALP and AST of the mice as comparable to the drug diazepam as summarized in Table 8.

Table 8. Effect of fermented and germinated mung bean extracts on different biochemical parameters in plasma of swimming endurance test mice.

	Total cholesterol mmol/L	**Triglyceride** mmol/L	**Total Protein** g/L	**ALT** U/L	**ALP** U/L	**AST** U/L
Control	5.26±0.42	3.83±0.98	93.33±9.24	134.85±8.34	176.25±13.44	1089.40±62.79
Diazepam	4.57±0.30	3.40±0.55	80.63±21.21	101.13±8.34	156.67±5.66	826.93±72.97
Fermented 1000mg/kg	4.01±0.66	2.65±0.57	92.58±8.24	91.14±5.52	108.20±1.41	806.90±79.90
Germinated 1000mg/kg	4.56±0.66	2.15±0.74	68.20±12.22	70.83±16.55	92.50±2.12	695.70±72.83

Example 19

Antistress – acute restrain test

Antistress effect of germinated and fermented bean extracts was also evaluated by acute restrain stress test on mice according to Kulkarni and Juvekar (2008). Mice were divided randomly into 5 groups, each group containing 6 mice. Group I mice received 0.2% sodium carboxy methyl cellulose in saline; (vehicle control). Group II mice received 0.2% sodium carboxy methyl cellulose in saline and stress; (negative stress control). Group III mice were treated with diazepam (2 mg/kg, p.o.) and stress; (positive control). Group IV mice were treated with fermented mung bean extract 1000 mg/kg, p.o. and stress. Group V mice were treated with germinated extract 1000 mg/kg, p.o. and stress.

After pretreatment with extracts for 7 days, the fore limbs and hind limbs of mice were tied separately and then together securing them with adhesive tape thereby immobilizing them for 2 hours on last day. After the induction of stress for 2 hours, blood was collected and lipid profile of total cholesterol and triglyceride (TG), total protein and liver profile of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) of the blood samples from the tested mice were evaluated simultaneously. Both germinated and fermented mung bean extracts were able to reduce the total cholesterol, TG, total protein, ALT, ALP and AST of the mice as comparable to the drug diazepam as summarized in Table 9.

Table 9. Effect of fermented and germinated mung bean extracts on different biochemical parameters in plasma of acute restrain induced mice.

	Total cholesterol mmol/L	**Triglyceride** mmol/L	**Total Protein** g/L	**ALT** U/L	**ALP** U/L	**AST** U/L
Control	6.23±0.27	3.96±0.78	138.78±6.70	148.90±12.73	186.33±16.97	1622.93±127.56
Stress control	8.32±0.87	5.43±0.30	157.88±13.15	216.00±26.73	482.50±38.18	2159.25±15.91
Diazepam	5.32±0.86	2.55±0.49	104.78±25.03	124.62±17.47	113.80±7.78	1541.08±164.05
Fermented 1000mg/kg	5.27±0.92	3.14±0.81	108.48±10.82	153.68±25.10	219.20±13.44	1294.33±54.59
Germinated 1000mg/kg	6.26±0.47	3.29±0.44	140.24±3.96	158.42±19.02	130.80±24.75	1762.40±121.98

Example 20

Antistress – cold restrain test

Antistress effect of germinated and fermented bean extracts were also evaluated by chronic cold restrain stress test on mice according to Kulkarni and Juvekar (2008). Mice were divided randomly into 5 groups, each group containing 6 mice. Group I mice received 0.2% sodium carboxy methyl cellulose in saline; (vehicle control). Group II mice received 0.2% sodium carboxy methyl cellulose in saline and stress; (negative stress control). Group III mice were treated with diazepam (2 mg/kg, p.o.) and stress; (positive control). Group IV mice were treated with fermented mung bean extract 1000 mg/kg, p.o. and stress. Group V mice were treated with germinated extract 1000 mg/kg, p.o. and stress. After 21 days of pretreatment with extracts, the same treatment was continued for 7 days and all animals except for vehicle control group were concomitantly exposed to cold restraint stress (4°C for 1 hour) during those 7 days. On the last day, animals were treated as usual, exposed to cold restraint stress and sacrificed. Blood samples were collected, pelleted (5000 *g,* 10 minutes) to obtain the plasma. Glucose, corticosterone, lipid profile of total cholesterol and triglyceride (TG), total protein and liver profile of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) of the blood samples from the tested mice were evaluated simultaneously. The animals were dissected and the organs adrenal glands and spleen were removed and their wet weights were recorded. The brains were immediately frozen in liquid nitrogen and preserved at -70°C until analyzed for biochemical parameters of norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT). Both germinated and fermented mung bean extracts were able to reduce the corticosterone, glucose, total cholesterol, triglyceride, total protein, ALT, ALP and AST of the mice as comparable to the drug diazepam (Table 10). Besides, the germinated and fermented mung bean extracts were able to reduce the adrenal gland weight and increase the spleen weight (Table 11). Added to these, both germinated mung bean and fermented mung bean extracts were able to

increase the brain Norepinephrine and Dopamine level, but showed a decrease in brain 5-Hydroxytryptamine level as compared to stress control (Table 12).

Table 10. Effect of fermented and germinated mung bean extracts on different biochemical parameters in plasma of chronic cold restrain induced mice.

	Corticosterone (ng/100 ml)	**Glucose mmol/L**	**Total cholesterol** mmol/L	**Triglyceride** mmol/L	**Total Protein** g/L	**ALT** U/L	**ALP** U/L	**AST** U/L
Control	8.58±0.75	9.13±1.37	5.11±0.56	2.82±0.30	97.90±8.10	85.65±6.22	117.00±7.23	838.35±33.52
Stress control	14.74±0.35	9.60±0.14	5.87±0.54	3.19±0.88	118.37±22.04	95.85±4.67	142.50±11.72	1062.40±135.48
Diazepam	8.81±0.49	5.07±0.49	4.06±0.90	1.70±0.49	92.43±12.42	87.30±2.83	127.50±13.44	753.95±144.25
Fermented 1000mg/kg	7.43±0.31	8.13±0.99	4.19±0.08	3.16±0.70	91.05±7.71	101.33±47.94	160.00±6.36	473.80±131.88
Germinated 1000mg/kg	8.90±0.14	9.04±0.61	4.77±0.13	3.33±0.99	96.17±10.61	101.68±77.98	118.40±4.95	756.74±201.10

Table 11. Effect of fermented and germinated mung bean extracts on adrenal gland and spleen organ body weight indices in chronic cold restrain induced mice.

	Spleen gland weight (mg/100g)	**Adrenal gland weight (mg/100g)**
Control	241.27±11.14	31.07±2.43
Stress control	210.33±13.61	35.13±3.09
Diazepam	430.71±7.94	30.62±3.33
Fermented 1000mg/kg	280.65±13.83	26.06±1.50
Germinated 1000mg/kg	272.68±4.97	26.97±1.49

Table 12. Effect of fermented and germinated mung bean extracts on brain neurotransmitter levels in chronic cold restrain induced mice.

	Brain NE level (µg/g)	Brain DA (µg/g)	Brain 5-HT level (µg/g)
Control	13.48±0.51	0.58±0.09	0.08±0.07
Stress control	4.17±0.06	0.51±0.02	2.20±0.07
Diazepam	10.10±0.19	0.58±0.03	0.73±0.09
Fermented 1000mg/kg	7.51±0.17	0.56±0.01	1.20±0.03
Germinated 1000mg/kg	4.63±0.08	0.54±0.04	1.65±0.06

norepinephrine (NE), dopamine (DA) and 5-hydroxytryptamine (5-HT)

Example 21

Liver protection study

Liver protection effects of germinated and fermented mung bean extracts was tested in the acute experimental liver injury induced by ethanol according to Pramyothin et al. (2007). Mice were divided randomly into 13 groups, each group containing 6 mice. Group I mice received phosphate buffer saline; (vehicle control). Mice from Group 2 to Group 13 were received ethanol for 7 days. After 7 days of ethanol induction, Group 2 mice received phosphate buffer saline for 7 days; (negative damage control). Group 3 mice were treated with silymarin (5 mg/kg, p.o.). Group 4 and 5 mice were treated with mung bean extract (200 and 1000 mg/kg, p.o.). Group 6 and 7 mice were treated with germinated mung bean extract (200 and 1000 mg/kg, p.o.). Group 8 and 9 mice were treated with fermented mung bean extract (200 and 1000 mg/kg, p.o.). Group 10 and 11 mice were treated with soybean extract (200 and 1000 mg/kg p.o.). Group 12 and 13 mice were treated with soybean extract (200 and 1000 mg/kg p.o.). After 7 days of treatment with extracts, mice from all groups were sacrificed and blood were collected, pelleted (5000 *g,* 10 min) to obtain the plasma. Albumin, cholesterol, triglyceride (TG), total protein, LDL, HDL and liver profile of Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) of the blood samples from the tested mice were evaluated simultaneously. Fermented mung bean extract was able to reduce the liver enzymes AST, ALT and ALP which were accelerated due to ethanol toxicity (Table 13). Besides, TG, LDL and cholesterol were also reduced by fermented mung bean extract as shown in Table 14.

Table 13. Effect of various bean extracts on liver profiles of liver injury mice induced by ethanol

	ALT (U/L)	ALP (U/L)	AST (U/L)
Normal uninduced	14.09±1.53	24.85±4.55	98.16±1.99
Ethanol untreated (Placebo)	48.11±1.78	25.50±5.57	367.30±1.10
Silymarin	26.72±1.20	33.80±1.14	171.70±3.79
Mung bean 200mg/kg	63.44±2.73	33.13±3.68	294.50±6.28
Mung bean 1000mg/kg	28.09±1.32	31.65±5.62	234.19±6.87
Germinated mung bean 200mg/kg	57.57±3.60	34.05±7.49	308.61±1.33
Germinated mung bean 1000mg/kg	26.34±3.50	33.70±1.90	191.93±1.51
Fermented mung bean 200mg/kg	56.26±4.71	33.90±4.79	232.48±1.52
Fermented mung bean 1000mg/kg	17.45±1.88	29.55±6.81	110.77±6.96
Soybean 200mg/kg	35.14±4.50	35.70±6.81	183.28±7.28
Soybean 1000mg/kg	25.85±6.49	33.30±4.43	174.49±1.15
Fermented soybean 200mg/kg	23.28±4.50	33.37±4.52	168.39±3.42
Fermented soybean 1000mg/kg	18.50±3.00	33.87±5.16	157.62±3.53

Table 14. Effect of various bean extracts on biochemical profiles of liver injury mice induced by ethanol

	Albumin (g/L)	Cholesterol (mmol/L)	TG (mmol/L)	Total Protein (g/L)	LDL (mmol/L)	HDL (mmol/L)
Normal uninduced	27.30±3.30	3.14±0.39	1.48±0.23	37.50±0.89	0.17±0.03	1.98±0.32
Ethanol untreated (Placebo)	27.00±2.69	3.80±0.20	2.37±0.14	43.60±0.53	0.26±0.03	2.59±0.27
Silymarin	26.40±3.31	4.20±0.36	2.77±0.16	37.90±0.80	0.17±0.01	2.14±0.25
Mung bean 200mg/kg	34.20±6.84	3.28±0.31	2.06±0.22	45.40±0.09	0.21±0.02	2.22±0.20
Mung bean 1000mg/kg	28.80±0.28	3.29±0.41	2.05±0.44	40.70±0.11	0.17±0.04	2.14±0.05
Germinated mung bean 200mg/kg	31.90±4.60	3.40±0.01	2.21±0.06	45.30±0.63	0.18±0.01	2.03±0.18
Germinated mung bean 1000mg/kg	28.45±5.80	3.13±0.06	1.84±0.35	46.00±0.68	0.16±0.05	2.22±0.23
Fermented mung bean 200mg/kg	25.15±4.14	3.18±0.21	2.26±0.12	47.90±0.40	0.16±0.01	1.80±0.41
Fermented mung bean 1000mg/kg	25.05±0.21	2.91±0.19	1.46±0.76	45.55±0.12	0.16±0.01	2.15±0.01
Soybean 200mg/kg	25.85±4.71	3.26±0.11	2.16±0.16	44.25±0.49	0.17±0.04	2.04±0.08
Soybean 1000mg/kg	25.90±5.80	2.99±0.29	2.03±0.01	40.45±0.96	0.17±0.04	1.97±0.26
Fermented soybean 200mg/kg	23.50±3.66	3.22±0.26	2.88±0.26	44.67±0.81	0.18±0.04	2.07±0.19
Fermented soybean 1000mg/kg	22.43±2.32	3.08±0.44	1.94±1.85	40.60±0.50	0.14±0.01	1.99±0.44

As will be readily evident to those skilled in the art, this invention may easily be produced in other definite forms without leaving from its scope or essential characteristics. These

embodiments are, therefore, to be considered as only illustrative and not restrictive, the scope of the invention being indicated by the claims rather than the former description.

Further Aspects

Further aspects and embodiments of the invention are now set out in the following numbered Paragraphs; it is to be understood that the invention encompasses these aspects:

Paragraph 1. A composition for promoting health comprising extract of fermented mung bean; characterized in that the extract is a product of fermentation of mung bean with *Rhizopus* sp.

Paragraph 2. A composition according to Paragraph 1, characterized in that the *Rhizopus* sp. is *Rhizopus microsporus*.

Paragraph 3. A composition according to Paragraph 1, characterized in that the fermented mung bean comprises moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 4%, protein content ranges from 27 to 37%, crude fibre content ranges from 1.5 to 3.0%; and carbohydrate content ranges from 45 to 60 %.

Paragraph 4. A composition for promoting health comprising extract of germinated mung bean.

Paragraph 5. A composition according to Paragraph 4, characterized in that the germinated mung bean further comprises moisture content ranges from 3 to 7%, fat content ranges from 1.5 to 2.5%, protein content ranges from 27 to 33%, crude fibre content ranges from 1.5 to 6.0%; and carbohydrate content ranges from 50 to 60%.

Paragraph 6. A use of a composition as in any one of claims 1 to 5 as an agent for the treatment, co-treatment or prevention of diabetic, immunodeficiency, stress, liver damage and skin pigmentation .

Paragraph 7. A nutraceutical comprising a composition as defined in any of claims 1 to 5 and a nutraceutical acceptable carrier.

Paragraph 8. The nutraceutical as in Paragraph 7 which is a food product, foodstuff, dietary supplement, nutritional supplement or a supplement composition for a food product or a foodstuff.

Paragraph 9. The composition as in any one of Claims 1 to 5 for use as a medicament.

Paragraph 10. A cosmetic composition comprising a composition as in any one of Claims 1 to 5 and a cosmetically acceptable carrier.

Paragraph 11. The cosmetic composition as in Paragraph 10 which is a skin care preparation.

Paragraph 12. The composition as in any one of Claims 1 to 5, which when consumed by an animal or human, has an effect on the animal or human selected from the group consisting of; as antioxidant, anti-pigmentation, anti-diabetic, immunostimulator of immune cell proliferation and cytotoxic effects on cancerous cells, antistress and liver protective substances.

Paragraph 13. A method for preparing a fermented mung bean extract comprises the steps of: (a) preparing the mung bean; (b) inoculating the mung bean with *Rhizopus* sp.; (c) incubating the mung bean aerobically; (d) fermenting the mung bean anaerobically for a period of time; (e) drying and grinding the fermented mung bean to a fine powder; and (f) extracting the fermented mung bean extract rich in bioactive from the fine powder of step (e).

Paragraph 14. The method for preparing a fermented mung bean extract according to Paragraph 1, characterised in that in step (b) the *Rhizopus* sp. is *Rhizopus microsporus*.

Paragraph 15. The method for preparing a fermented mung bean extract according to Paragraph 13 or 14, characterised in that step (f) further comprises the steps of: (a) dissolving the fine powder of fermented mung bean in a solvent; (b) separating a layer containing solubles; (c) freeze drying the layer containing soluble to obtain the fermented mung bean extract rich in bioactive.

Paragraph 16. A method for preparing a fermented mung bean extract according to Paragraph 13 to 15, characterized in that step (a) is by soaking in distilled water for mung

bean hull removal, washing the dehulled mung bean; boiling the dehulled mung bean in water, draining and cooling to room temperature and drying the dehulled mung bean to a moisture content ranging from 45% to 55%.

Paragraph 17. A method for preparing a germinated mung bean extract comprises the steps of: (a) soaking mung bean in water; (b) incubating the mung bean anaerobically for a period of time to obtain germinated mung bean, (c) drying and grinding the germinated mung bean to obtain fine powder of germinated mung bean; and (d) extracting the germinated mung bean extract rich in bioactive from the fine powder of step (c).

Paragraph 18. The method for preparing a germinated mung bean extract according to Paragraph 17, characterised in that step (d) further comprises the steps of: (a) dissolving the fine powder of germinated mung bean in a solvent; (b) separating a layer containing solubles; (c) freeze drying the layer containing soluble to obtain the germinated mung bean extract rich in bioactive.

CLAIMS

1. A method for preparing a germinated mung bean extract comprising the steps of:

(a) soaking mung bean in water;

(b) germinating the mung bean anaerobically in the dark for a period of time to obtain germinated mung bean;

(c) drying and grinding the germinated mung bean to obtain fine powder of germinated mung bean; and

(d) extracting the germinated mung bean extract rich in bioactive from the fine powder of step (c).

2. A method according to Claim 2, in which step (d) further comprises the steps of:

(i) dissolving the fine powder of germinated mung bean in a solvent;

(ii) separating a layer containing solubles; and

(iii) freeze drying the layer containing soluble to obtain the germinated mung bean extract rich in bioactive.

3. A method according to Claim 1 or 2, in which the germinated mung bean comprises moisture content of between 3 and 7%, fat content of between 1.5 and 2.5%, protein content of between 27 and 33%, crude fibre content of between 1.5 and 6.0%; and carbohydrate content of between 50 and 60%.

4. A method according to Claim 1, 2 or 3, in which the germinated mung bean extract comprises antioxidant activity, anti-pigmentation activity or liver protective activity.

5. Use of a mung bean extract prepared by a method according to any preceding claim in the preparation of a medicament for the treatment or co-treatment of diabetes, immunodeficiency, stress or liver damage.

6. Use of a mung bean extract prepared by a method according to any of Claims 1 to 4 as a nutraceutical.

7. Use according to Claim 6, in which the nutraceutical comprises a food product, foodstuff, dietary supplement, nutritional supplement or a supplement composition for a food product or a foodstuff.

8. Use of a mung bean extract prepared by a method according to any of Claims 1 to 4 as a cosmetic composition.

9. Use according to Claim 8, in which the cosmetic composition comprises a skin care preparation.

10. Use of a mung bean extract prepared by a method according to any of Claims 1 to 4 in the cosmetic treatment or co-treatment of skin pigmentation.