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(54) **Title**: AN EDIBLE PRODUCT

(57) **Abstract**: The present invention relates to an edible product for enhancing immunity. Some efforts have been made to make food/medicament composition from natural sources. Still there is a need for an edible product or food supplement which provides for improving immunity within the people to fight against these common diseases and develop a 'self prevention' and self treatment system within them. It is an object of the present invention to provide an edible product which enhances immunity by using a polysaccharide other than what has been disclosed in the art. The present inventors have surprisingly found that an edible product comprising a polysaccharide (derived from one or more plants belonging to the family of Camellia sinensis var. sinensis and/or Camellia sinensis var. assamica) other than what has been disclosed in the art which is comprised of galacturonic acid is particularly useful in modulating immune response.

AN EDIBLE PRODUCT

Technical field

The present invention relates to an edible product. More particularly the present invention relates to an edible product for enhancing immunity.

Background

Most of the people (including children) in the world suffer from common diseases like flu, cold etc together with gastrointestinal infections. Sometimes these diseases occur so frequently that it can cause depression. High coughing and sneezing can create several health related problems. The root cause of these diseases is a relatively lower immunity among the population.

There are a number of medicines available in the pharmaceutical market for improving the immunity. Even there are some herbs which have been used from ancient times for improving immunity. The medicines that are available in the market are either expensive or they do not exists in the form of a readily available food which is consumed by the masses on a regular basis. Moreover consumption of medicines is also not desirable on daily basis. Though herbs do not have the above said problems but, the availability of these herbs are really geography dependent and supply is also not enough. Furthermore herbal notes are also not liked by a large number of consumers.

Some efforts have been made to make food/medicament composition from natural sources.

US 2008/0113044 relates to extracts of green tea species plant material prepared by supercritical CO_2 extraction methods.

WO 2011/069781 relates to polysaccharides obtained from the species *Camellia sinensis*, which comprise a rhamnogalacturonan-I core, and wherein the molar ratio of

galacturonyl acid residues to rhamnosyl residues in the backbone of the polysaccharide is close to 1:1. WO 2011/069781also provides edible products or pharmaceutical compositions containing such polysaccharides, in order to modulate immune response. The molecular weight of the polysaccharides as disclosed is at least 70 kDa.

Though prior art describes a polysaccharide in order to modulate immune response there are other polysaccharides and other substances that yet to be explored which can also enhance immunity.

Therefore still there is a need for an edible product or food supplement which comprises polysaccharides other than what has been disclosed in the prior art and which improves immunity within the people to fight against these common diseases and develop a 'self prevention' and 'self treatment' system within them.

Objects of the invention

It is therefore an object of the present invention to provide for a suitable alternative.

It is another object of the present invention to provide an edible product which enhances immunity by using a polysaccharide other than what has been disclosed in the art.

It is yet another object of the present invention to provide a process for producing a plant extract to eventually provide an edible product.

The present inventors have surprisingly found that an edible product comprising a polysaccharide (derived from one or more plants belonging to the family of *Camellia sinensis var. sinensis* and/or *Camellia sinensis var. assamica*) other than what has been disclosed in the art which is comprised of galacturonic acid is particularly useful in modulating immune response and satisfies one or more of the aforesaid objects.

Summary of the invention

An edible product comprising from 1 to 20% by weight of a plant extract from one or more plants belonging to the family of *Camellia sinensis var. sinensis* and/or *Camellia sinensis var. assamica*, characterized in that the extract comprises 1 to 10% of a polysaccharide comprised of galacturonic acid and wherein the molecular weight of the polysaccharide is in the range of 18 – 35 KDa.

A process for preparation of an edible product of the first aspect comprising the steps of:

a) extracting raw plant material in water in a weight ratio of 1:1 to 1:20 at a temperature of 85 to 100°C for about 60 to 180 minutes,
b) filtering the solution of step (a) thereby producing a soluble plant extract,
c) adding 30 to 70% ethanol solution in the soluble extract of step (b),
d) centrifuging the solution of step (c) thereby producing sediment and supernatant,
e) washing the sediment of step (d) with 30-50 % ethanol,
f) centrifuging the solution of step (e),
g) drying the sediment to obtain the plant extract; and
h) mixing the plant extract obtained in step (g) with the other food ingredients.

According to a third aspect of the present invention there is provided the use of a composition of the first aspect for improving immunity.

These and other aspects, features and advantages will become apparent to those of ordinary skill in the art from a reading of the following detailed description. For the avoidance of doubt, any feature of one aspect of the present invention may be utilised in any other aspect of the invention. The word “comprising” is intended to mean “including” but not necessarily “consisting of” or “composed of.” In other words, the listed steps or options need not be exhaustive. It is noted that the examples given in the description below are intended to clarify the invention and are not intended to limit the invention to those examples per se. Similarly, all percentages are weight/weight percentages unless otherwise indicated. Except in the operating and comparative

examples, or where otherwise explicitly indicated, all numbers in this description indicating amounts of material or conditions of reaction, physical properties of materials and/or use are to be understood as modified by the word “about”. Numerical ranges expressed in the format "from x to y" are understood to include x and y. When for a specific feature multiple preferred ranges are described in the format "from x to y", it is understood that all ranges combining the different endpoints are also contemplated.

Detailed description of the invention

According to the present invention there is provided an edible product comprising from 1 to 20% by weight of a plant extract characterized in that the extract comprises 1 to 10% of a polysaccharide. The percentages of the plant extract in the composition as mentioned above are on solid weight basis. If the composition contains high amount of water then the meaning of the weight percent has to construe accordingly.

The edible product of the present invention preferably comprises 2 to 20 %, more preferably the edible product of the present invention comprises 5 to 20 % by weight of a plant extract. The extract preferably comprises 3 to 10 %, more preferably comprises 5 to 10 % of a polysaccharide.

Polysaccharides are long carbohydrate molecules which consist of several numbers of monosaccharide either in straight chain or in branch chain. It generally composed of monosaccharide like arabinose, rhamnose, glucose, galacturonic acid, glucuronic acid and many more. It has a molecular weight generally greater than 5 kDa.

The plant extract:

The plant extract used for the purpose of the present invention comprises a polysaccharide. The polysaccharide is comprised of galacturonic acid. Preferably the polysaccharides is comprised of more than 80% of galacturonic acid, more preferably more than 90% of galacturonic acid. Most preferably the polysaccharide is comprised of only galacturonic acid. Preferably the polysaccharide does not contain any neutral sugars like rhamnose, arabinose etc and/or their derivatives.

For the purpose of getting the plant extract. Any part of the plant may preferably be selected for this purpose. It may preferably include stem, fruit, leaf or any other part of a particular plant.

The plant extract is obtained from one or more plants belonging to the family of *Camellia sinensis var. sinensis* and/or *Camellia sinensis var. assamica*. There is no particular preference for the part of the plant that has to be taken for the extraction purpose. Leaf, buds stem or any other part of the *Camellia sinensis* may be used for the purpose of the extraction. Even a mixture of different parts of the *Camellia sinensis* may be used for the extraction.

The plant extract preferably means but not limited to freeze dried alcohol extract of the selected plant. Though freeze dried alcohol extract of the selected plant in powder form is preferred but, the above extract in diluted form may also be preferably used.

The edible product of the present invention comprises a plant extract which further comprises a polysaccharide which is comprised of galacturonic acid. The molecular weight of the polysaccharide is preferably in the range of 18 – 35 KDa, more preferably in the range of 19 – 30 KDa, most preferably 20 – 25 KDa.

The polysaccharide preferably has a 1-4 carbon linkage.

The polysaccharide preferably has no branch chain.

The rigidity of a compound may be of importance for characterizing a compound. The Mark-Houwink rigidity parameter may be estimated by using the well known Mark-Houwink equation.

The Mark–Houwink equation gives a relation between intrinsic viscosity $[\eta]$ and molecular weight M.

$$\eta = KM^{\alpha}$$

From this equation the molecular weight of a polymer can be determined from data on the intrinsic viscosity and vice versa.

The values of the Mark–Houwink parameters, α and K, depend on the particular polymer-solvent system. For most flexible polymers: $0.5 \leq \alpha \leq 0.8$, for semi-flexible polymers: $\alpha \geq 0.8$ and for polymers with an absolute rigid rod α = 2.0.

In size exclusion chromatography, such as gel permeation chromatography, the intrinsic viscosity of a polymer is directly related to the elution volume of the polymer. Therefore, by running several monodisperse samples of polymer in a gel permeation chromatograph (GPC), the values of K and α can be determined graphically using a line of best fit. Then the molecular weight and intrinsic viscosity relationship is defined.

Also, the molecular weights of two different polymers in a particular solvent can be related using the Mark–Houwink equation when the polymer-solvent systems have the same intrinsic viscosity:

$$K_1 M_1^{\alpha 1} = K_2 M_2^{\alpha 2}$$

Knowing the Mark–Houwink parameters and the molecular weight of one of the polymers allows one to find the molecular weight of the other polymer using a GPC. The GPC sorts the polymer chains by volume and as intrinsic viscosity is related to the volume of the polymer chain, the GPC data is the same for the two different polymers.

The polysaccharide preferably has Mark-Houwink rigidity parameter greater than 0.8, more preferably greater than 0.9, further more preferably greater than 1 and most preferably greater than 1.1. The most preferable solvent for measuring this is ethanol for the current system.

The edible product of the present invention is preferably in the form of a liquid, solid or semi-solid products. Some examples of such edible product are in the form of a soup, a beverage, a spread, a dressing, a dessert or bread.
Preferably the beverage is in the form of a tea based beverage.

The edible product also may preferably in the form of a solid or powdered food supplement.

Process of extraction of the plant material:

The present invention also provides for a process for extraction of a plant material comprising the steps of:

The step (a)

The raw plant material is extracted in water in a weight ratio of 1:1 to 1:20 at a temperature of 85 to 100°C for about 60 to 180 minutes. The preferable ratio of the raw plant to water is in the range of 1:1 to 1:15 and most preferably from 1:1 to 1:10. The temperature of the extraction is preferably in the range of 90 to 100°C and more preferably in between 95 to 100°C. The time for extraction is preferably in between 80 to 180 minutes, more preferably in between 100 to 180 minutes.

The step (b)

The solution produce after the extraction is filtered. After the filtration the solution (filtrate) comprising soluble plant extract,

The step (c)

After that, to the solution comprising the soluble plant extract (filtrate), 30 to 70% ethanol solution being added. The alcohol percentage as mentioned above means that the alcohol is added to the filtrate until the concentration reach 30 to 70%.The preferable concentration of ethanol solution is in the range of 35 to 60

The step (d)

After adding the ethanol in step (c), the solution is subjected to centrifugation thereby producing sediment and supernatant.

The step (e)

After the centrifugation the sediment is subjected to a washing step. The sediment is washed with 30 to 50 % ethanol, preferably with 30 to 45% and more preferably with 35 to 45%.

The step (f) and (g)

After the above washing step, the washed sediment (sediment + solvent) is again subjected to centrifugation followed by drying the sediment produced after the centrifuging to obtain the plant extract. The drying preferably carried out in a vacuum dryer.

The supernatant that produced as mentioned above at step (d) may further preferably be treated by adding 60 to 85% ethanol solution. The alcohol percentage as mentioned above means that the alcohol is added to the supernatant until the concentration reach 60 to 85%.The preferable concentration of the ethanol solution is in between 65 to 85% more preferably in between 70 to 80%. After this step the solution is centrifuged thereby producing further sediment. This sediment is then further washed with 30-50 % ethanol, preferably with 35 to 45% of ethanol. The solution is then followed by centrifugation and further followed by drying the sediment produced by the centrifuging. The drying preferably carried out in a vacuum dryer.

Process of preparation of the edible product of the invention:

The step (h)

The plant extract obtained may then be mixed with other food ingredients to obtain the edible product. The other food ingredients as mentioned herein preferably means, but not limited to, any base food formulation like flour (in case of noodles or bread), other power-mix (in case of soup) etc. to obtain the edible product.

The composition as claimed in any one of the preceding claims can be used for improving immunity. For example, the composition may be for use as a medicament for improving immunity. Additionally or alternatively the composition may be used in the manufacture of a medicament for improving immunity.

Now the invention will be demonstrated by means of following non-limiting examples. The examples given below are only for illustrations and in no way limits the scope of the invention.

Examples

Preparation of the plant extract:

For this experiment the plant selected was tea plant. The commercial sample of Wufeng green tea leaves was purchased from market in city of Wuhan, Hubei province in China.

Green tea leaves (2.5 kg) were first extracted with 25 L water at 100° C for 2 hours. The residue was then removed by filtration. The spent leaves (residue) were re-extracted under the same conditions. The supernatants (from the first and the second extraction) were combined and concentrated. This supernatant was divided into two batches. In the first batch ethanol was added until the ethanol concentration reached 40% (40% ethanol precipitation fraction, Extract 1) and in the second batch ethanol was added until the ethanol concentration reached 70% (70% ethanol precipitation fraction, Extract 2) respectively. In both the cases the precipitates were collected by centrifugation at ~9829g for about 10 minutes followed by washing twice with 95% ethanol, and then freeze dried to obtain the plant extract.

Invitro - Phagocytosis activity of the plant extract:

The human promyelocytic leukemia cell line HL60 (Supplier: ATCC, Number: CCL – 240TM) was used to evaluate the phagocytosis-enhancing capacity of the plant extract. The HL-60 cells (5.0 × 105 cells/ml) were differentiated along monocytic lineage by the addition of 1α, 25-dihydroxyvitamin D3 (VD3, Supplier: Sigma, Number: L4130) and incubated (CO_2 incubator, at 37°C in 5% CO_2) in complete medium for 48h. The complete medium was prepared in RPMI medium. The RPMI medium comprised of RPMI 1640, HEPES and L-Glutamine which was obtained from Gibco as a total

mixture (No: 22400-089). The complete medium was then prepared in RPMI 1640 medium with 10% (v/v) of Fetal bovine serum (United States origin, Supplier: Gibco, Number: 16000-044) and 1% of Penicillin / Streptomycin solution (100U/mL of Penicillin and 100μg/mL of Streptomycin, Supplier: Gibco, Number: 15140-122).

After differentiation, 200 μL/well of HL-60 cells (8×105 cells/ml) were transferred into a 96-wells flat-bottom plate (Supplier: Corning, No: CLS3599). The non-differentiated HL60 cells were incubated at the same concentration (in complete medium without VD3) as a background control.

The cells were treated with the test samples (Plant extract, final concentration at 1.89 μg/ml and 18.9 μg/ml, respectively) and was added in 60 μl of a 0.0033% suspension of Yellow Green labeled microspheres (Latex beads from Polysciences Inc, USA. Cat No.17155)and was incubated at 37°C in 5% CO2 for 24 hr in a incubator. To set the basal level, the differentiated HL60 cells in complete medium were used and 50 μg/ml LPS (Lipopolysaccharides from Escherichia coli, Supplier: Sigma, Cat. No. L-4130) was used as a positive control.

After the incubation period, the cells were transferred to a 96-wells V-bottom plate (Supplier: Corning, No: CLS3894) and washed with phosphate buffered saline (0.05M, pH 7.2) three times. For analysis, the cells were transferred to a 96-wells clear-bottom black fluorescence plate (Supplier: Corning, No: CLS3603) and fixed with 37% formaldehyde solution (From Sigma) containing 10-15% methyl alcohol for 30 min at room temperature in the dark. The ratio of phagocytosis was measured by using Beckman Z2 coulter counter (Beckman Coulter, Fullerton, CA). The data were normalized using the positive control and expressed as a relative fluorescence unit (RFU).

The phagocytosis stimulating effect of an ingredient at a specific test-concentration is normalized to the stimulating effect the E. coli-LPS positive control according to the formula below. In this formula, “basal” stands for the constitutive level of phagocytosis as obtained with differentiated HL60 cells to which no stimulating ingredients are added.

$$normalised\ \ phagocytosis\ (\%) = \frac{\left(RFU_{ingredient} - RFU_{basal}\right)}{\left(RFU_{LPS} - RFU_{basal}\right)} \cdot 100\%$$

Samples are typically analyzed in duplicate (ingredient dilutions) or triplicate (basal and LPS-stimulated phagocytosis). A normalized value is calculated from each RFU value and then all normalized values for each sample are averaged and expressed with their standard deviation.

The signal from the non-differentiated HL60 cells is the background control signal. The non-differentiated and non-stimulated HL60 cells should always give a lower signal than the basal signal from the differentiated non-stimulated HL60 cells. The reason for this is that HL60 cells that are not differentiated along the monocyte or macrophage lineage have limited phagocytosis ability.

An ingredient is considered an active stimulator of phagocytosis when its normalized phagocytosis value is equal to or greater than 40% (arbitrary threshold) of the LPS-effect.

The results of this experiment are shown below in Table 1:

Table 1

Sample	Concentration (µg/mL)	Phagocytosis activity	
		Normalized Value	SD
Extract 1	18.9	108	26
Extract 2	18.9	66	3
Extract 1	1.89	69	21
Extract 2	1.89	14	30

The results indicated in the above table shows the dose dependent efficacy of both the extracts on the phagocytosis activity. Both of the extracts were subjected to two different concentration levels wherein one concentration is 10 orders higher than the other. It is evident from the above table that Extract 1 works well at both (low and high) concentrations. Extract 2 works better at higher concentration level. At lower concentration Extract 2 does not provides any conclusive result (as the deviation is much higher than the measured value). Therefore it can be concluded from the above table that both of the plant extract is providing improved immunity (one at high concentration and one at both low and high concentration).

Invitro measurement of γδ T cells activity of the plant extract:

The γδ T cell is one of the important members of immunity network. Activation of this cell type is implicated in better immune response. It has been well documented that tea is a potent activator of γδ T cells through theanine (Kamath, A.B., Wang, L and Das, H et al. Proc .Natl. Acad.Sci, USA. 10, 6009-14, 2003). Theanine, when metabolized converts to ethyl amine which is the ligand for γδ T cells (Bukowski, J.F., Morita, C.T and Brenner, M.B. Immunity, 11, 57-65, 1999). It has been also shown that in vivo activation of γδ T cells can reduce common cold/ cough in human volunteers (Journal of the American College of Nutrition, Vol. 26 No. 5, 445–452 (2007). Evaluation of one cell surface protein i.e. Vγ 2δ 2 indicate the activation of γδ T cells. Hence induction of higher amount of vγ 2δ 2 cells by an inducer indicates higher potency as an immunomodulator.

Protocol:

a) Cytotoxicity evaluation: The extracts were incubated with lymphocytes isolated from three donors using a range of concentrations in between 0.1 – 0.0000001% for 24 hrs. Post incubation, MTT assay was performed to identify the maximum non-cytotoxic concentration (For the experiment the concentrations were varied from 0.1 to 0.001). MTT is Thiazolyl Blue Tetrazolium Bromide dye and yellow in colour. It is generally used to check the viability/cytotoxicityof the cells. After the incubation the MTT changed

its colour to purple if the cells are viable. The purple product was insoluble. The insoluble precipitates were dissolved in acidic isopropanol and optical density (OD) was measured at 540 nm. More OD indicates more viability. The highest concentration of the extracts that showed more than 95 % cell viability was considered non cytotoxic. Subsequent experiments were performed using several concentrations lower than maximum non cytotoxic concentration according to the following Table 2.

b) Isolation of purified γδ T lymphocytes from human peripheral blood: About 30 mL of blood was withdrawn from normal human donors by vein puncture method. Lymphocytes were isolated using Ficoll density gradient (used to separate the cells according to the density, Supplier: Sigma USA). Equal volumes (5 mL) of blood and Ficoll density gradient were added in a tube. It was then centrifuged at 400g. After the centrifugation, 3 layers were separated viz. RBC as pellet, serum as interface and lymphocytes as the top layer. The lymphocytes fraction was then picked up using syringe. Then the lymphocytes were stimulated with human rIL-2 (100 IU/ml) (Supplier: R & D Systems, USA) and ascites 1:50 diluted (anti-CD3 antibody, (Supplier: R & D Systems, USA) and incubated (culture continued) for 12 days under ambient condition (at ~25°C). Post 12 days of culture, the cells were separated using magnetic columns (Invitrogen, USA , MAC 130-041-301) and specific antibodies (anti Pan γδ hapten, supplier: R & D Systems, USA) conjugated and anti-hapten FITC (Supplier: R & D Systems, USA) labeled magnetic beads).

3) Immunophenotyping assay: Lymphocytes from human blood, isolated as described above were incubated with rIL-2 (30 IU/ml) and active (one concentration each). The cells were fed rIL-2 on every third day till day 9. On Day 12 the cells were harvested and analyzed for immunophenotyped. PBMCs incubated with rIL-2 alone were used as control. PBMC incubated with rIL-2 and ethylamine (Sigma) served as positive control. Lymphocytes were incubated with specific antibodies for the following phenotype as described - Vδ2 FITC-CD3PE (Fluorescence labeled antibody to detect specifically γδT cells, supplier: R & D Systems, USA). The data was analyzed using dual color flow cytometer (Becton Dickinson, FACS caliber).

The results of the experiment are shown below in Table 2.

Table 2

Sample	Concentration (wt%)	Mean value	SD	SE
Control (blood)	-	6.75	1.77	0.88
Extract 1	0.1	19.50	10.61	5.30
	0.05	13.00	1.41	0.71
	0.01	11.50	4.95	2.47
	0.001	8.50	0.71	0.35
Extract 2	0.1	29.50	4.95	2.47
	0.05	34.50	2.12	1.06
	0.01	9.00	4.24	2.12
	0.001	9.00	1.41	0.71

From the above Table it is evident that the both of the extract are providing better results on blood compared with a sample that has only blood and not the plant extract. With increasing the concentration the effect is more pronounced. Table 2 also shows that Extract 2 behaves much better at higher concentration than Extract 1 in this assay.

Characterization of the plant extract:

Two extracts viz. Extract1 and Extract2, were prepared using extraction of green tea with hot water and subsequent precipitation with 40% and 70% ethanol, respectively. The extraction yield for Extract1 and Extract2 was 4.08% and 2.13% by weight of the stating tea leaf, respectively.

The phagocytosis experiments (as disclosed above) shows that Extract1 is having higher activity than Extract2; therefore, Extract 1 was selected for the purpose of the characterization experiments.

Extract1 (5 g) was dissolved in 40 ml distilled water and centrifuged (43540g for 10 min). The sediment was dissolved in 20 mL distilled water again and centrifuged (43540g, 10 min) to remove the residue. The supernatant was combined and loaded on Sigma supplied DEAE-Sepharose Fast Flow column (45×5 cm) previously treated with 2.0 M NaCl and equilibrated with distilled water. The Extract1 were first eluted with distilled water and then with increasing molarity (0.1 M, 0.2 M, 0.4 M and 2.0 M) of NaCl, respectively. Thus 5 different Extracts were obtained from Extract 1 viz. Extract1-0 (with distilled water), Extract1-1(with 0.1 M NaCl), Extract1-2 (with 0.2 M NaCl), Extract1-4 (with 0.4 M NaCl) and Extract1-20 (with 2 M NaCl).

As the yield of Extract1-2 was higher, it was chosen for further investigation. Extract1-2 was purified further using Sephacryl S-300 high resolution column (Supplier: Pharmacia) and two fractions viz. Extract1-2a and Extract1-2b were obtained.

Homogeneity and molecular weight determination:

Different Dextran P (standard samples supplier by Pharmacia (P-5, P-10, P-20, P-50, P-100, P-200, P-400 and P-800) solutions were prepared weighing different Dextran P standard samples (2mg) and dissolved them in 1ml 0.2M NaCl solution, respectively, and then centrifuged at 10884 g for 10min.

The standard Dextran P samples (20µl) are injected to high performance gel permeation chromatography (KS-804 and KS-805 column supplied by Pharmacia in serials, ID 8 mm, and length 300 mm, Shodex). The mobile phase was 0.2M NaCl solution and the flow rate was 0.8ml/minute. The retention time was recorded for each sample. The standard curve was established and plotted wherein the ordinate represents the logarithm of molecular weight and the abscissa stands for the retention time.

After that experiment was performed with the extracts samples following the same procedure of standard curve establishment. According to the retention time of samples, the molecular weight was calculated from the standard curve.

The above analysis showed that Extract1-2a and Exatrct1-2b were homogeneous polysaccharides with average molecular weight of $2.2x10^4$ D and $2.0x10^4$ D respectively.

Monosaccharide analysis:

Determination of neutral sugars in the samples: 100mg of samples were hydrolyzed by treatment with 4mL 2M trifluoroacetic acid (TFA, Sinopharm Chemical Reagent Company Limited) at 120°C for 2hours. After the hydrolysis, the solution was dried at 40°C under reduced pressure (20 mbar). Then 3mL of methanol was added followed by drying to remove TFA. This step was repeated for 5 times. The released sugars were then dissolved in 3ml of distilled water and ~25mg of NaBH4 (Sinopharm Chemical Reagent Company Limited) was added and it was thoroughly mixed. After that acetic acid was added drop-wise to neutralize (until no bubble emerged) excess NaBH4 that may present in the system. 10mL methanol was then added and dried under reduced pressure (20 mbar) and this step was repeated for 4-5 times. After that 4ml of acetic anhydride (Sinopharm Chemical Reagent Company Limited) was added and the reaction was initiated by heating it at 100°C for 1hours followed by drying it under reduced pressure (20 mbar). The alditol acetates thus formed were extracted with chloroform and transferred to a separating funnel. After that 10 mL of water was added in the separating funnel and after shaking the water was removed. This step was repeated for 3 times. Then the chloroform layer was dried by anhydrous Na_2SO_4 and then fresh chloroform was added to settle the volume at 10 ml. The samples were analyzed by gas chromatography-mass (GC-MS).

Determination of acidic sugars in the samples: 100mg of samples were taken and dissolved it in 20mL of distilled water. Then 200mg of carbodiimide (Fluka) was added

to activate carboxyl group of the sugar and kept it for 3 hours. The pH value was controlled at 4.75 during the reaction. After that 200mL of 2M NaBH4 was slowly added to reduce sugar to alditol for 2hours. The samples were dialyzed using dialysis bag of molecular cut off 3000 D for 48hours and then freeze dried. The above steps were repeated twice.

Then samples were then hydrolyzed by treatment with 4mL 2M TFA at 120°C for 2hours. After the hydrolysis, the solution was dried at 40°C under reduced pressure (20mbar). Then 3mL of methanol was added followed by drying to remove TFA. This step was repeated for 5 times. The released sugars were then dissolved in 3ml of distilled water and ~25mg of NaBH4 was added and it was thoroughly mixed. After that acetic acid was added to neutralize excess NaBH4 that may present in the system. 10mL methanol was then added and dried under reduced pressure (20mbar) and this step was repeated for 4-5 times. After that 4ml of acetic anhydride was added and the reaction was initiated by heating it at 100°C for 1hours followed by drying it under reduced pressure (20mbar). The alditol acetates thus formed were extracted with chloroform and transferred to a separating funnel. After that 10 mL of water was added in the separating funnel and after shaking the water was removed. This step was repeated for 3 times. Then the chloroform layer was dried by anhydrous Na_2SO_4 and then fresh chloroform was added to settle the volume at 10 ml. The samples were analyzed by gas chromatography-mass (GC-MS).

GC-MS analysis revealed that Extract1-2a and Exatrct1-2b contained no neutral sugars and the only acidic sugar found was galacturonic acid, indicating that Extract1-2a and Exatrct1-2b were composed of galacturonic acid.

Methylation analysis:

The extracts sample in which the carboxyl groups had already been reduced to hydroxyl group (5 mg) was dissolved in 3 ml of dimethylsulfoxide (Sigma). After that 500mg of NaOH was added to this solution. The mixture was then sealed and stirred for 1hours to mix well after air removal by nitrogen. After that 0.2 mL of methyl iodide

(Sinopharm Chemical Reagent Company Limited) was added to the mixture for methylation for 20 minutes. Again 0.5 mL of methyl iodide was added again to the mixture for methylation for 1hours. 2ml of water was then added after the reaction. This solution was extracted by 3mL chloroform (Sinopharm Chemical Reagent Company Limited). After that the chloroform part was washed 3 times with water and then dried. The methylated polysaccharide was retreated twice as described above. The methylated polysaccharide was then hydrolyzed by treatment with 4ml 2M TFA at 120°C for 2h. After the hydrolysis, the solution was dried at 40°C under reduced pressure (20 mbar). 3mL of methanol was added and then evaporated to remove TFA. This step was repeated for 5 times. The released sugars were dissolved in 3mL of distilled water. After that ~25mg of NaBH4 was added to the sugar solution and mixed it well. The released sugars were reduced by NaBH4 for 2hours at room temperature to alditols. After that the excess NaBH4 was neutralized by acetic acid. Then 10mL of methanol was added and dried under reduced pressure (20 mbar) and this step was repeated for 4-5 times. 4ml acetic anhydride (Sinopharm Chemical Reagent Company Limited) was then added to have a reaction at 100°C for 1h and then dried it under reduced pressure (20 mbar). The alditol acetates were extracted with chloroform and transferred to a separating funnel. After that 10 mL of water was added in the separating funnel and after shaking the water was removed. This step was repeated for 3 times. Then the chloroform layer was dried by anhydrous Na_2SO_4 and then fresh chloroform was added to settle the volume at 10 ml. The samples were analyzed by gas chromatography-mass (GC-MS).

Methylation analysis of Extract1-2a and EXtract1-2b by GC-MS showed the presence of two components viz. 2, 3, 4, 6-tetra-O-Me-Gal and 2, 3, 6-tri-O-Me-Gal. The backbone therefore was deduced to be just composed of 1→4 linked galacturonic acid residues.

Chain rigidity analysis:

10 mg of sample were taken (equal mixture of both Extract1-2a and Extract1-2b) and dissolved in 10 mL of 0.05 M sodium nitrate (Sigma). The sample solution was then

filtered through a 0.2 µm nylon filter (supplier: Millipore) before Gas Permeation Chromatography (GPC) analysis.

Viscotek TDA SEC/GPC system was used to analyze the samples. The unit is equipped with a differential refractometer, four-capillary viscometer, right angle light scattering (RALS), and low angle light scattering detectors (LALS). Each sample was injected twice at a volume of 120 µL and eluted with 0.05 M $NaNO_3$ at 0.7 ml/min. Separation was performed on one ViscoGEL aqueous columns (Bentec, Italy). The temperature was maintained at ~30°C during the chromatography and detection.

Mark-Houwink character constant α is the key parameter to show the chain conformation. (α>0.8 means rigid polymer chains)

The measured value of α was found to be 1.2.

Preparation of the food compositions with the plant extract of the invention

Soup Composition:

The soup composition was made by just mixing the dry ingredient according to the following Table:

Table 3:

Ingredient	Wt %
Corn Starch	45
NaCl	10
Sugar (commonly available sucrose)	11
Yeast Extract	2
Fat Powder (Lecithin)	4
Liquid Fat (Lecithin)	2
Dried vegetable pieces	13
Flavour	2.5

Colour	0.5
Extract 1 + Extract 2 (1:1)	10

The soup was then made using the above composition in hot water (~90°C) and tasted by a group of professional taster. It was found that the plant extract is not producing any taste which is not desirable in a soup.

Noodles Composition:

The noodles composition was made using the following table. First the flour and the salt and all the other ingredients were added in bowl. Water was then added slowly and kneaded until become smooth. After that the dough was covered with damp cloth for 20 minutes. It was then kneaded again. After that the required amount of (as indicated in the following table) corn flour was sprinkled on a board and the dough was rolled until becomes very thin. This was folded into 4 or 5 layers and cut across the layers as thin as possible.
The thin strips were then dried.

Table 4:

Ingredient	Wt %
Wheat Flour	50
Water	19
Corn Powder (gluten)	1.5
NaCl	1.3
Guar Gum (Supplier: Rama Industries, Gujarat, India))	0.1
Vegetable oil	18
Sodium Bicarbonate	0.1
Extract 1 + Extract 2 (1:1)	10

The noodles was then made using the above composition and tasted by a group of professional taster. It was found that the plant extract is not producing any taste which is not desirable in noodles.

From the above description and illustration it is evident that by way of present invention it is now possible to provide to an edible product which enhances immunity benefit and thereby satisfying the objects of the invention.

Claims

1. An edible product comprising from 1 to 20% by weight of a plant extract from one or more plants belonging to the family of *Camellia sinensis var. sinensis* and/or *Camellia sinensis var. assamica*, characterized in that the extract comprises 1 to 10% of a polysaccharide which is comprised of galacturonic acid and wherein the molecular weight of the polysaccharide is in the range of 18 – 35 KDa.

2. An edible product as claimed claim 1 wherein the polysaccharide has a 1-4 carbon linkage.

3. An edible product as claimed in any one of the preceding claims wherein the polysaccharide has no branch chain.

4. An edible product as claimed in any one of the preceding claims wherein the polysaccharide has Mark-Houwink rigidity parameter greater than 0.8.

5. An edible product as claimed in any one of the preceding claims in the form of a liquid such as a soup or a beverage, a spread, a dressing, a dessert or bread.

6. An edible product as claimed is claim 8 wherein the beverage is a tea based beverage.

7. An edible product as claimed in any one of claims in the form of a solid or powdered food supplement.

8. A process for preparation of an edible product as claimed in any one of the preceding claims comprising the steps of:
    a) extracting raw plant material in water in a ratio of 1:1 to 1:20 at a temperature of 85 to 100°C for about 60 to 180 minutes,
    b) filtering the solution of step (a) thereby producing a soluble plant extract,
    c) adding 30 to 70% ethanol solution in the soluble extract of step (b),

d) centrifuging the solution of step (c) thereby producing sediment and supernatant,
e) washing the sediment of step (d) with 30-50 % ethanol,
f) centrifuging the solution of step (e),
g) drying the sediment to obtain the plant extract; and
h) mixing the plant extract obtained in step (g) with other food ingredients.

9. A process as claimed in claim 11 wherein the supernatant produced at step (d) further treated which comprises the steps of:
a) adding 60 to 85% ethanol solution in the supernatant ,
b) centrifuging the solution of step (a) thereby producing further sediment,
c) washing the sediment of step (f) with 30-50 % ethanol,
d) centrifuging the solution of step (g),
e) drying the sediment to obtain the plant extract.

10. The use of an edible product as claimed in any one of the preceding claims for improving immunity.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2013/063393

A. CLASSIFICATION OF SUBJECT MATTER
INV. A23F3/16 A23F3/18 A23L1/09 A61K36/82
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A23F A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, FSTA, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2011/069781 A1 (UNILEVER NV [NL]; UNILEVER PLC [GB]; UNILEVER HINDUSTAN [IN]; ALBERS R) 16 June 2011 (2011-06-16) cited in the application the whole document -----	1-10
A	XINLIN WEI ET AL: "Composition and bioactivity of polysaccharides from tea seeds obtained by water extraction", INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES, vol. 49, no. 4, 1 November 2011 (2011-11-01), pages 587-590, XP055062474, ISSN: 0141-8130, DOI: 10.1016/j.ijbiomac.2011.06.016 the whole document -----	1-10

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 October 2013	11/11/2013

Name and mailing address of the ISA/	Authorized officer
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1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RENJIE XU ET AL: "Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant ()", FOOD AND CHEMICAL TOXICOLOGY, PERGAMON, GB, vol. 50, no. 7, 12 October 2011 (2011-10-12), pages 2473-2480, XP028429987, ISSN: 0278-6915, DOI: 10.1016/J.FCT.2011.10.047 [retrieved on 2011-10-19] the whole document	1-10
A	US 2005/186314 A1 (SASAME MASAMI [JP] ET AL) 25 August 2005 (2005-08-25) paragraphs [0214] - [0231]; claims 1-11	1-10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/063393

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