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(71) Applicant: **LAILA NUTRACEUTICALS 40-15-14, BRINDAVAN COLONY, LABBIPET VIJAYAWARDA 520 010 ANDHRA PRADESH IN**

(72) Inventor: **GOKARAJU, GANGA, RAJU 40-15-14, BRINDAVAN COLONY, LABBIPET VIJAYAWARDA 520 010 ANDHRA PRADESH IN**
GOKARAJU, RAMA, RAJU 40-15-14, BRINDAVAN COLONY, LABBIPET VIJAYAWARDA 520 010 ANDHRA PRADESH IN
GOKARAJU, VENKATA, KANAKA, RANGA, RAJU 40-15-14, BRINDAVAN COLONY, LABBIPET VIJAYAWARDA 520 010 ANDHRA PRADESH IN
GOLAKOTI, TRIMURTULU 40-15-14, BRINDAVAN COLONY, LABBIPET VIJAYAWARDA 520 010 ANDHRA PRADESH IN
BHUPATHIRAJU, KIRAN 40-15-14, BRINDAVAN COLONY, LABBIPET VIJAYAWARDA 520 010 ANDHRA PRADESH IN

(54) **Title:**
BOSWELLIA OIL, ITS FRACTIONS AND COMPOSITIONS FOR ENHANCING BRAIN FUNCTION

(57) **Abstract:**
The present invention discloses non-acidic extract/fraction selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and Boswellia oil fraction (BOIL) derived from the gum resin of Boswellia species and their compositions for improving memory/mental condition, enhancing brain/mental functions such as cognition, memory, learning, communication and brain health, for treating impaired memory, and for preventing, control or treating memory and cognition related disorders/diseases.

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(71) Applicant *(for all designated States except US)*: **LAILA NUTRACEUTICALS** [IN/IN]; 40-15-14, Brindavan Colony, Labbipet, Vijayawarda 520 010, Andhra Pradesh (IN).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **GOKARAJU, Ganga, Raju** [IN/IN]; 40-15-14, Brindavan Colony, Labbipet, Vijayawarda 520 010, Andhra Pradesh (IN). **GOKARAJU, Rama, Raju** [IN/IN]; 40-15-14, Brindavan Colony, Labbipet, Vijayawarda 520 010, Andhra Pradesh (IN). **GOKARAJU, Venkata, Kanaka, Ranga, Raju** [IN/IN]; 40-15-14, Brindavan Colony, Labbipet, Vijayawarda 520 010, Andhra Pradesh (IN). **GOLAKOTI, Trimurtulu** [IN/IN]; 40-15-14, Brindavan Colony, Labbipet, Vijayawarda 520 010, Andhra Pradesh (IN). **BHUPATHIRAJU, Kiran** [IN/IN]; 40-15-14, Brindavan Colony, Labbipet, Vijayawarda 520 010, Andhra Pradesh (IN).

(74) Agent: **ARUNA, Sree, P.**; Gopakumar Nair Associates, 3rd Floor, 'Shivmangal', Near Big Bazaar, Akurli Road, Kandivali (East), Mumbai 400 101, Maharashtra (IN).

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(54) **Title**: BOSWELLIA OIL, ITS FRACTIONS AND COMPOSITIONS FOR ENHANCING BRAIN FUNCTION

(57) **Abstract**: The present invention discloses non-acidic extract/fraction selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and Boswellia oil fraction (BOIL) derived from the gum resin of Boswellia species and their compositions for improving memory/mental condition, enhancing brain/mental functions such as cognition, memory, learning, communication and brain health, for treating impaired memory, and for preventing, control or treating memory and cognition related disorders/diseases.

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"BOSWELLIA OIL, ITS FRACTIONS AND COMPOSITIONS FOR ENHANCING BRAIN FUNCTION"

FIELD OF INVENTION:

The invention provides non-acidic Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and Boswellia oil fraction (BOIL) comprising BLPRE and BVOIL individually and their composition(s) obtained by combining with at least one biological agent or Nootropic agent for the prevention, control and treatment of brain related diseases comprising Attention-deficit Hyperactivity Disorder (ADHD) and memory deficits or to enhance brain functions such as cognition, memory, learning and communication. Importantly, the above fractions and compositions of the present invention help in improving the brain health.

BACKGROUND OF THE INVENTION:

The gum resin of *Boswellia serrata* (Burseraceae) plant has long been in use for the treatment of several diseases by the practitioners of Ayurvedic medicines in the Indian system of medicine. The extract of Boswellia was found to be a potent anti-inflammatory and anti-arthritic agent [Kimmatkar et al; *Phytomedicine*. 2003 10(1): 3-7]. The origin of anti-inflammatory actions of *Boswellia* gum resin and its extracts has been attributed to a group of triterpene acids called boswellic acids that were isolated from the gum resin of *Boswellia serrata.* Boswellic acids exert anti-inflammatory actions by inhibiting 5-lipoxygenase (5-LOX). 5-LOX is a key enzyme for the biosynthesis of leukotrienes from arachidonic acid. 3-O-Acetyl-11-keto-β-boswellic acid (AKBA) is biologically the most active component among its congeners, it being able to inhibit 5-LOX with an IC_{50} of 1.5 μM [Sailer, E. R., et. al., British J. Pharmacol., 1996: 117; p 615-618].

Boswellia gum resin and its extracts also demonstrated significant therapeutic improvements in human clinical trials confirming the anti-inflammatory effects shown in vitro and in vivo[E Ernest, BMJ, 2008; 337: a2813].

Worldwide aging of the population has increased the incidents of cognitive deficits, such as age-associated memory impairment and senile dementias, and this causes great

disruptive impact on the life of the affected individuals. The "cholinergic hypothesis of learning" played a pivotal role in the development of drugs for degenerative diseases.

A disturbance of the cortical cholinergic system accompanied by a reduction of choline acetylase (reduced acetylcholine synthesis) is inter alia detectable biochemically in case of neurological diseases. Hence, there is a demand for a medicament whose active substance can ameliorate this disturbance and highly available at the target organ (brain) and which is well tolerated, particularly in long-term therapy.

Acetylcholinesterase (AChE) is an important enzyme to hydrolyze acetylcholine, a neurotransmitter mediating the activity of parasympathetic nerve, into choline and acetate. AChE is formed in the endoplasmic reticulum, and moves and functions in the cell membrane. AChE is distributed around cholinergic nerve, particularly much at the myoneural junction, and is found in the serum, liver and other tissues.

A wide range of evidence shows that acetylcholinesterase (AChE) inhibition can improve cognitive and mental functions through enhancing cortical cholinergic neurotransmission. The acetylcholinesterase (AChE) inhibitors increase the concentration of acetylcholine and help nerve cells to communicate better. The longer acetylcholine remains in the brain, the longer those cells can call up memories. The earliest known AChE inhibitors are physostigmine and tacrine.

However, clinical studies show that physostigmine has poor oral activity, brain penetration and pharmacokinetic parameters while tacrine has hepatotoxic side effects. Studies were thus focused on finding new types of acetylcholinesterase inhibitors that would overcome the disadvantages of these two compounds.

Donepezil and Rivastigmin inaugurate a new class of AChE inhibitors with longer and more selective action with manageable adverse effects but still small improvement of cognitive impairment. Galanthamine (Reminyl), an alkaloid isolated from Galanthus nivalis, is another recently approved AChE inhibitor for the treatment of Alzheimer's. It is selective, long acting, reversible and produces beneficial effects in patients. Similarly,

huperzine A, a novel Lycopodium alkaloid discovered from the Chinese medicinal plant Huperzia serrata is a potent, reversible and selective inhibitor of AChE with a rapid absorption and penetration into the brain in animal tests. It exhibits memory-enhancing activities in animal and also in clinical trials.

Dementia with Lewy bodies (DLB) is a common cause of dementia. Changes in the acetylcholine system have been reported in brains of patients with DLB, which provides a rational basis for trials of acetylcholinesterase inhibitors in DLB.

Current treatment of dementia in Parkinson's Disease is based on the compensation of profound cholinergic deficiency, as in recent studies with the cholinesterase inhibitors galantamine, donepezil and rivastigmine. It has also been shown that cholinesterase inhibitors can improve motor function in PD [Werber EA and Rabey JM, Journal of Neural Transmission; 108 (11): 1319-1325, 2001]. The beneficial effect of cholinesterase inhibitors has been studied on patients suffering from Parkinson's disease and dementia. [Dag Aarsland, *J Geriatr Psychiatry Neurol*2004; 17; 164].

Studies show that Wernicke-Korsakoff syndrome is associated with a persisting severe anterograde amnesia in which memory is not transferred from short-to long-term storage. It is believed to be a consequence of a thiamine-deficient state often found in alcohol abusers. The memory deficit has been attributed to a number of brain lesions (corpus mamillare and dorsomedial nucleus of the thalamus), loss of cholinergic forebrain neurons and serotonin-containing neurons [Halliday G., et. al.; Alcohol Alcohol Suppl. 2 p. (245-51), 1994. Many studies and case-reports suggest efficacy of acetyicholinesterase inhibitors in the Wemicke-Korsakoff- associated memory deficit. Studies also suggest that neurons in the nucleus basalis are at risk in thiamine deficient alcoholic. [K M Cullen et al., *J NeurolNeurosurg Psychiatry* 1997;63:315–320].

The U.S. patent US5720975 relates to the use of incense (olibanum), incense extracts, substances contained in incense, their physiologically acceptable salts, their derivatives and their physiological salts, pure boswellic acid, of physiologically acceptable salts, of a

derivative, of a salt of the derivative, for production of a medicament for the prevention or treatment of Alzheimer's disease.

US publication US20060177467A1 relates to the use of the hydrogenation products of frankincense (olibanum), its hydrogenated ingredients as well as physiologically acceptable salts and derivatives thereof and hydrogenated frankincense extracts for the production of a medicament for the prophylactic and/or therapeutic treatment of cerebral ischemia, cranial/brain trauma and/or Alzheimer's disease.

There is however no prior art, to the best of inventors knowledge, relating to the use of Boswellia non-acidic oil fraction(s) and its compositions for the prevention, control and treatment of Memory and Cognition related diseases and enhancing brain functions.

SUMMARY OF THE INVENTION:

The main aspect of the present invention is to provide use of Boswellia non-acidic fraction(s) selected from Boswellia low polar gum resin extract fraction (BLPRE) having novel phytochemical composition, Boswellia volatile oil fraction (BVOIL) and Boswellia oil fraction (BOIL) comprising BLPRE and BVOIL fractions either individually or their compositions for improving brain health and brain functions , which include but not limited to cognition, memory, intelligence, motivation, attention, concentration, learning power and better communication and to alleviate disease conditions related to cognition and memory deficits and the like.

The other important aspect of the present invention is to provide use of Boswellia non-acidic fraction(s) selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and Boswellia oil fraction (BOIL) comprising BLPRE and BVOIL either individually or as compositions to prevent, control and treat brain related diseases/disorders which include but not limited to senile dementia, multi-infarct dementia, dyslexia, aphasia, organic brain syndrome, myasthenia gravis, vascular dementia, mild cognitive impairment (MCI), Lewy body dementia, Wemicke-Korsakoff-syndrome, Alzheimer's, Parkinson's disease, Attention-deficit Hyperactivity Disorder (ADHD), hypoxia, anoxia, cerebrovascular insufficiency, epilepsy, myoclonus and

hypocholinergic dysfunctions, to slowdown memory deterioration, functional loss and to treat memory impairment disorders, neurodegenerative disorders, and for controlling blood pressure and blood circulation in the brain.

In the other aspect, the invention provides compositions comprising atleast one component selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction consisting of BLPRE and BVOIL in combination with at least one component selected from biological agent(s), Nootropic agent(s).

In another aspect, the invention provides compositions comprising atleast one component selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction (BOIL) consisting of BLPRE and BVOIL in combination with at least one component selected from Boswellia extract(s), fraction(s), extracts/fractions enriched in one or more boswellic acids, their salts or derivatives thereof.

In another aspect, the invention provides compositions comprising atleast one component selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction (BOIL) in combination with one or more agents selected from natural antioxidants, anti-inflammatory agents and immune modulators.

BRIEF DESCRIPTION OF FIGURES:

Figure I: Figure shows structural formulae **1 - 9** representing prominent compounds of *Boswellia serrata*low polar gum resin extract (BsLPRE).

Figure II: Figure shows the HPLC chromatogram depicting the phytochemical profile of the *Boswellia serrata*low polar gum resin extract (BsLPRE).

Figure III: Figures IIIA, IIIB and IIIC represent bar diagrammatic representation of number of days required for learning, latency in finding feed and number of wrong entries respectively obtained during learning phase. The bars 1 to 3 represents vehicle treated control, BsLPRE (250 mg/kg) and piracetam (150 mg/kg) respectively. Each bar represent mean ± SE, n=8, * $p < 0.05$ and ** $p < 0.01$.

Figure IV: Figures IVA and IVB represent bar diagrammatic representation of latency in finding feed and number of wrong entries respectively obtained during memory retention phase. The bars 1 to 3 represents vehicle treated control, BsLPRE (250 mg/kg) and piracetam (150 mg/kg) respectively. Each bar represent mean ± SE, n=8.

DETAILED DESCRIPTION OF THE INVENTION:

1. 'Boswellia oil' or 'non-acidic Boswellia extract' or 'BOIL' used herein refers to non-acidic Boswellia gum resin extract containing non-acidic Boswellia low polar gum resin extract fraction (BLPRE) and Boswellia volatile oil fraction (BVOIL) obtained from gum resin of any of the Boswellia species.
2. '*Boswellia serrata*oil' or 'non-acidic *Boswellia serrata* extract' or 'BsOIL' used herein refers to non-acidic *Boswellia serrata* gum resin extract containing non-acidic *Boswellia serrata* low polar gum resin extract fraction (BsLPRE) and *Boswellia serrata* volatile oil fraction (BsVOIL) obtained from gum resin of the *Boswellia serrata*species.
3. '*Boswellia carterii* oil' or 'non-acidic *Boswellia carterii* extract' or 'BcOIL' used herein refers to non-acidic *Boswellia carterii*gum resin extract containing non-acidic *Boswellia carterii* low polar gum resin extract fraction (BcLPRE) and *Boswellia carterii* volatile oil fraction (BcVOIL) obtained from gum resin of the *Boswellia carterii* species.
4. 'Boswellia low polar gum resin extract fraction' or 'Boswellia low polar gum resin extract' or 'BLPRE' used herein refers to non-acidic Boswellia gum resin extract oil fraction comprising sesquiterpenes, diterpenes, triterpenes and other oily phytochemicals obtained after removing the volatile components from Boswellia oil obtained from gum resin of any of the Boswellia species by any of the processes described.
5. '*Boswellia serrata*low polar gum resin extract fraction' or '*Boswellia serrata* low polar gum resin extract' or 'BsLPRE' used herein refers to non-acidic *Boswellia serrata* gum resin extract oil fraction comprising sesquiterpenes, diterpenes, triterpenes and other oily phytochemicals obtained after removing the volatile components from Boswellia oil obtained from gum resin of *Boswellia serrata* species by any of the processes described.

6. '*Boswellia carterii* low polar gum resin extract fraction' or '*Boswellia carterii* low polar gum resin extract' or 'BcLPRE' used herein refers to non-acidic *Boswellia carterii* gum resin extract oil fraction comprising sesquiterpenes, diterpenes, triterpenes and other oily phytochemicals obtained after removing the volatile components from *Boswellia carterii* oil obtained from gum resin of *Boswellia carterii* species by any of the processes described.
7. 'Boswellia volatile oil fraction' or 'Boswellia volatile oil' or 'volatile oil' or 'volatile fraction' or 'BVOIL' used herein refers to the volatile fraction/extract comprising monoterpenes, sesquiterpenes, volatile oils and other oily phytochemicals obtained from gum resin of any of the Boswellia species by any of the processes described.
8. '*Boswellia serrata* volatile oil fraction' or '*Boswellia serrata* volatile oil' or 'serrata volatile oil' or 'serrata volatile fraction' or 'BsVOIL' used herein refers to the volatile fraction/extract comprising monoterpenes, sesquiterpenes, volatile oils and other oily phytochemicals obtained from gum resin of the *Boswellia serrata* species by any of the processes described.
9. '*Boswellia carterii* volatile oil fraction' or '*Boswellia carterii* volatile oil' or 'carterii volatile oil' or 'carterii volatile fraction' or 'BcVOIL' used herein refers to the volatile fraction/extract comprising monoterpenes, sesquiterpenes, diterpenes, volatile oils and other oily phytochemicals obtained from gum resin of the *Boswellia carterii* species by any of the processes described.
10. 'Gum' or 'Gum resin' or 'resin' used herein refers to an exudate of Boswellia plant species.
11. 'Phytochemical' refers to a pure or semi-pure compound or compounds isolated from plants.
12. Cognition refers to acquisition, processing and retention of information.
13. Cognition enhancer(s) refers to substance(s) that enhances concentration and memory.
14. Nootropic agent(s) refers to smart drugs, memory enhancers and cognitive enhancers, dietary supplements, nutraceuticals, functional ingredients and functional foods that are purported to improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration.

15. Biological agent(s) refer to one or more agents selected from biologically active ingredient(s), anti-oxidant(s), dietary supplements, herbal ingredients, nutraceuticals, functional ingredients, functional foods and nootropic agents andoil(s) their mixtures obtained from plant(s)/animal(s)/microorganism(s)/ synthesis or semi synthesis.
16. 'Biologically active ingredient(s)' refers to any pharmaceutically or dietetically acceptable active ingredient(s); compound(s), extract(s), fraction(s), phytochemical(s), synthetic drug(s) or their salts or mixtures thereof derived from plants, animals or microorganisms or obtained by chemical synthesis/semi-synthesis.
17. 'functional ingredient(s)' refers to any herbal ingredients, dietary supplements, antioxidants, vitamins, minerals, amino acids, fatty acids, essential oils, fish oils, enzymes, glucosamine, Chondroitin and probiotics or their salts or mixtures thereof derived from plants or animals or microorganisms or chemical synthesis or semi-synthesis.

The gum resin of Boswellia has been very widely used since ancient times. The gum resin of various species of Boswellia such as *Boswellia serrata, Boswellia carterii* or *Boswellia papyrifera* is a complex mixture comprising Boswellia oil fraction (BOIL) containing essential oil/Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia low polar gum resin extract fraction (BLPRE); boswellic acids, sugars and polysaccharide fraction. The *Boswellia serrata /Boswellia carterii/Boswellia papyrifera* extracts widely available in the international markets are acidic fractions separated from the gum resin which are standardized to contain 65% or 85% total Boswellic acids by titrimetric method of analysis. During the execution of commercial process for regular Boswellia extracts derived from *Boswellia serrata/Boswellia carterii/Boswellia papyrifera* (85% total Boswellic acids), the acidic fraction, which contains predominantly triterpene acids including Boswellic acids is separated from the rest of gum resin components. The sugars and other polymeric materials get separated out into the aqueous phase during the enrichment process for total Boswellic acids. The remaining water immiscible low polar compounds are separated as Boswellia oil fraction/extract. These low polar compounds are either absent or present at very low concentration in both, commercial Boswellia

extracts standardized to boswellic acids and Boswellia extracts selectively enriched in 3-O-acetyl-11-keto-β-Boswellic acid (AKBA).

Process for obtaining non-acidic Boswellia oil (BOIL) fraction:

A representative process for obtaining Boswellia oil comprises:

a) procuring the gum resin of one or more of the plant(s) selected from but not limited to *Boswellia serrata* or *Boswellia carterii* or *Boswellia papyrifera* or mixtures thereof,

b) extraction of the gum resin with a water immiscible organic solvent,

c) filtering the extract carefully to remove the insoluble resin material,

d) washing the organic solvent extract repeatedly with an aqueous alkali solution such as aqueous potassium hydroxide,

e) washing the organic layer with water and brine,

f) evaporating the organic layer under vacuum and high temperature to obtain the oily residue (BOIL).

Processes for obtaining Boswellia volatile oil (BVOIL) fraction:

The process for obtaining Boswellia volatile oil (BVOIL) is through steam distillation or using high vacuum from Boswellia gum resin.

A representative process for obtaining Boswellia volatile oil comprises:

a) procuring the gum resin of Boswellia and

b) separating the Volatile oil component by either steam distillation or distillation under high vacuum, low temperature from the said gum resin to obtain BVOIL.

In an alternative process,

a) BOIL is prepared according to the process described above,

b) BOIL is then subjected to steam distillation or vacuum distillation to collect Boswellia volatile oil (BVOIL)

Processes for obtaining Boswellia low polar gum resin extract (BLPRE) fraction:

A representative procedure for obtaining Boswellia low polar gum resin extract (BLPRE) comprises:

a) extraction of the gum resin of Boswellia species with a water immiscible organic solvent and filtering the extract carefully to remove the insoluble resin material,

b) washing the organic solvent extract repeatedly with an aqueous alkali solution such as aqueous potassium hydroxide,

c) washing the organic layer obtained after the alkali wash, with water and brine,

d) evaporating the said organic layer under vacuum and high temperature to obtain the oily residue,

e) removing the volatile compounds from the said oily residue under high vacuum and very high temperature to obtain BLPRE.

Another representative procedure for obtaining Boswellia low polar gum resin extract (BLPRE) comprises:

a) preparing the alcohol or hydroalcohol extract of Boswellia gum resin,

b)partitioning the alcohol extract between an aqueous alkali solution and a water immiscible organic solvent,

c) separation of the organic solvent layer, followed by evaporation of the solvent to obtain oily residue,

d) removal of volatile compounds from the said oily residue under high temperature and high vacuum to obtain BLPRE.

Yet another representative procedure for obtaining Boswellia low polar gum resin extract (BLPRE) comprises:

a) extracting the gum resin of Boswellia species with alcohol or hydro alcohol,

b) evaporating the organic solvent to an optimum level of total solids and then

c) adjusting the pH to the alkaline side, preferably pH 9 – 12,

d) repeatedly extracting the solution with an organic solvent,

e) evaporating the organic solvent under vacuum and high temperature to obtain the oily residue,

f) evaporating the volatiles from the said oily residue under high vacuum and high temperature to obtain BLPRE.

A representative procedure for obtaining *Boswellia serrata* volatile oil (BsVOIL) comprises:

a) procuring the gum resin of *Boswellia serrata.*

b) separating the Volatile oil component by either steam distillation or distillation under high vacuum, low temperature from the said gum resin to obtain BsVOIL.

Yet another representative procedure for obtaining *Boswellia carterii* volatile oil (BcVOIL) comprises:

a) procuring the gum resin of *Boswellia carterii.*

b) separating the Volatile oil component by either steam distillation or distillation under high vacuum, low temperature from the said gum resin to obtain BcVOIL.

The representative processes for obtaining Boswellia volatile oil (BVOIL) from *Boswellia serrata, Boswellia carterii* are described above. However, a similar process or processes can be applied to any of the gum resin obtained from Boswellia species for producing Boswellia volatile oil (BVOIL).

A representative procedure for obtaining *Boswellia serrata*low polar gum resin extract (BsLPRE) comprises:

a) Procuring the gum resin of *Boswellia serrata.*

b) extraction with an water immiscible organic solvent and the insoluble gum materials were separated by filtration and discarded,

c) washing the organic solvent extract repeatedly with dilute aqueous alkali solution to remove the acidic compounds,

d) washing the organic layer successively with water and brine,

e) evaporating the organic layer under vacuum at 60 - 70°C to obtain an oily residue,

f) the volatile components are then removed from the said oily residue under high vacuum and very high temperature to obtain a viscous oil, which is referred herein after as *Boswellia serrata* low polar gum resin extract (BsLPRE).

Alternatively, the BsLPRE can also be prepared by a process comprising:

a) preparing the alcohol or hydroalcohol extract of *Boswellia serrata* gum resin,

b) partitioning the alcohol extract between an aqueous alkali solution and a water immiscible organic solvent,

c) separation of the organic solvent layer , followed by evaporating the organic layer under vacuum at 60 - 70°C to obtain an oily residue,

d) the volatile components are then removed from the said oily residue under high vacuum and high temperature to obtain a viscous oil , which is referred herein after as *Boswellia serrata* low polar gum resin extract (BsLPRE) .

A representative procedure for obtaining *Boswellia carterii* low polar gum resin extract (BcLPRE) comprises:

a) procuring the gum resin of *Boswellia carterii*,

b) extracting the gum resin with an water immiscible organic solvent and the insoluble gum materials were separated by filtration and discarded,

c) washing the organic solvent extract repeatedly with dilute aqueous alkali solution to remove the acidic compounds,

d) washing the organic layer successively with water and brine,

e) evaporating the organic layer under vacuum at 60 - 70°C to obtain an oily residue.

f) the volatile components are then removed from the said oily residue under high vacuum and high temperature to obtain a viscous oil, which is referred herein after as *Boswellia carterii* low polar gum resin extract (BcLPRE).

Alternatively, the BcLPRE can also be prepared by process comprising:

a) preparing the alcohol or hydroalcohol extract of *Boswellia carterii* gum resin,

b) partitioning the alcohol extract between an aqueous alkali solution and a water immiscible organic solvent,

c) separation of the organic solvent layer, followed by evaporating the organic layer under vacuum at 60 - 70°C to obtain an oily residue,

d) the volatile components are then removed from the said oily residue under high vacuum and high temperature to obtain a viscous oil, which is referred herein after as *Boswellia carterii* low polar gum resin extract (BcLPRE).

The representative processes for obtaining Boswellia low polar gum resin extract (BLPRE) from *Boswellia serrata* and *Boswellia carterii* are described above. However, a similar process or processes can be applied to any of the gum resin obtained from Boswellia species for producing the low polar gum resin extract.

The said intact Boswellia oil (BOIL) or Boswellia volatile oil (BVOIL) or Boswellia low polar gum resin extract (BLPRE) constitute significant components in Boswellia gum resin. However, it has very limited commercial utility and it is mostly discarded as a waste material. Potential utilization of these fractions have been long overdue. The inventors found very unexpectedly that *Boswellia serrata* low polar gum resin extract (BsLPRE), a fraction obtained after removing the volatile compounds from the *Boswellia serrata* oil, has several beneficial properties.

In our earlier Indian patent application 2229/CHE/2008 filed 15th September, 2008 and PCT application # PCT/IN2009/000505 filed 14th September, 2009 we disclosed synergistic compositions comprising AKBA enriched fraction and *Boswellia serrata* non-acidic extract (BNRE). BNRE composition and method of identification are also disclosed.

In our recent Indian patent application 394/CHE/2010 filed 15th February, 2010 we disclosed non Boswellic acid fraction and its synergistic compositions.

As a part of developing new agents for improving brian/mental function and alleviating disease conditions related to cognition and memory deficits, the inventors have screened large number of plant extracts for their inhibitory property on Acetylcholinesterase enzyme activity. The assay was performed in vitro by the method of Ellman *et al.*, with minor modifications, using acetylthiocholine iodide as a substrate (Lee J. H., et. al. *Arch Pharm Res* 2004, 27(1): 53-56). It was found very unexpectedly that the non-acidic extract, *Boswellia serrata* oil (BsOIL),*Boswellia serrata* low polar gum resin extract (BsLPRE) fraction and *Boswellia serrata* volatile oil (BsVOIL) fractions, were potent inhibitors of acetylcholinesterase in vitro. BsLPRE for example potently inhibited

acetylcholinesterase enzyme activity in vitro as shown in **Table 2.** Its in vitro efficacy against acetylcholinesterase enzyme is comparable to commercial drug Neostigmin. BsLPRE exhibited an IC50 value of 37.01 ng/mL compared to 43.29 ng/mL shown by neostigmin. Its acetylcholinesterase inhibitory activity was also evaluated by a cell based in vitro assay in Rat pheochromocytoma PC 12 cells. The inhibitory property of BsLPRE on the enzyme activity was assessed in β-amyloid peptide induced-rat pheochromocytoma PC 12 cells. Rat pheochromocytoma PC 12 cells were equally distributed with phenol red free Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10% fetal bovine serum (FBS) in 24-well plate. Cells were pretreated separately with BLPRE and positive control Neostigmin for 1h. Thereafter, cells were induced with 1 μg/mL of β-amyloid peptide (Calbiochem, USA) for 24h at 37 ^{o}C. After 24h, cells were collected and washed twice with 1x PBS by centrifugation at 1200 rpm for 5min at 4 ^{o}C. The cell extracts were prepared in solubilization buffer and the cell lysates were analysed for acetylcholine esterase (AChE) activity. The BsLPRE showed 25.3% inhibition at 100ng/mL concentration, where as Neostigmin showed 49.1% inhibition at 20 ng/mL as summarized in **Table 4.**

In order to understand the chemical composition of BsLPRE, the inventors have carried out extensive separation of BsLPRE using repeated column chromatography and high performance liquid chromatography (HPLC), and isolated several diterpenoid and triterpenoid compounds. The structures of the compounds were rigorously characterized using ^{1}H NMR, ^{13}C NMR, DEPT, HSQC and HMBC, Mass spectral data. The compounds so obtained and identified are guiol (**1**), nephthenol (**2**), serratol (**3**), diterpene X (**4**), lupeol (**5**), olean-12-ene-3β-ol (**6**), olean-12-ene-3α-ol (**7**), lanosta-8, 24-diene-3α-ol (**8**) and urs-12-ene-3α-ol (**9**) as depicted in **Figure I.** The fraction, *Boswellia serrata* low polar gum resin extract (BsLPRE) was then standardized to three or more of the phytochemical marker compounds selected from **1** to **9**. The typical results obtained are summarized in the **Table 1** and a typical chromatogram depicting the profile of BsLPRE is presented in **Figure II.** However, the inventors have also found that this composition of BsLPRE or any other Boswellia low polar gum resin extract composition (BLPRE) obtained from any other species may vary based on several factors such as Boswellia

species used, age of the plant, season of collection of gum resin, geographic location and manufacturing process employed.

The foregoing results manifest that BsLPRE is a novel composition comprising unique combination of sesquiterpenoids, diterpenoids and triterpenoids and other phytochemical(s). A compound tentatively identified as diterpene X (**4**) and compounds guiol (**1**), nepthenol (**2**) and Lanosta-8, 24-diene-3α-ol (**8**) are not known to be metabolites of *Boswellia serrata* gum resin.

The low polar gum resin extract of these as well as other Boswellia species comprise a composition having some similarity to that of *Boswellia serrata.* However, the low polar gum resin extract of *Boswellia carterii* (BcLPRE) has shown biological activity and synergistic effect very similar to that exhibited by BsLPRE as summarized in the following in vitro and in vivo studies. The experimental studies are discussed in the examples.

The acetylcholinesterase inhibitory of different boswellic acids was also evaluated in both enzyme based assay and cell based assay and the inhibitory activities are summarized in **Tables 3 and 5.**

Oxidative stress induced increased ROS is critical for neuronal damage, which is a serious complication with regard to brain health. Interestingly, the low polar gum resin extract of Boswellia serrata (BsLPRE) showed potent inhibition of reactive oxygen species (ROS) generation in RAW 264.7 mouse macrophages (**Table 6**). In addition, BsLPRE also showed protection from oxidative stress induced cytotoxic damage of human neuroblastoma cells. In a cell based assay, oxidative stress induced by H_2O_2 showed potent cytotoxic effect on the proliferation of IMR32 human neuroblastoma cells. However, the treatment with BsLPRE significantly attenuates the proliferation index of IMR32 human neuroblastoma cells back to the normal level (**Table 7**). Hence the observations confirm that the low polar gum resin extract (BLPRE) offers protection from neuronal damage and support improving brain health.

The in vivo efficacy of BsLPRE on learning and memory improvement was proven in rats using elevated radial arm maze (RAM) method. Oral administration of BsLPRE (250 mg/kg) significantly ($P< 0.01$) decreased the number of days required to make the rats learned as per set criteria andsignificantly ($P< 0.05$) decreased the time taken to find the food by the learned rats in the elevated RAM model. The positive control Piracetin (150 mg/kg) also showed significant improvement in spatial learning like reduction in latency and Number of wrong entries, when compared with the control group and the results are as stated below (Figure IIIA to IIIC). The test product BsLPRE also significantly improves cognition and memory retention (Figure IVA and IVB). These results confirm the efficacy shown by BsLPRE in vitro and suggest that the use of BsLPRE improves spatial learning and memory retention. According to these findings, BsLPRE is a promising candidate for facilitation of learning and memory.

Synergistic compositions comprising Boswellia extracts:

The inventors have conducted several cell based and enzyme based in vitro anti-acetylcholinesterase studies on broad array of Boswellia extracts standardized to boswellic acids and *Boswellia serrata* low polar gum resin extract (BsLPRE), in addition to other herbal extracts. The individual extracts and different combination of these extracts were tested for their efficacy to inhibit acetylcholinesterase enzyme. It was found very surprisingly that a composition (composition-1) comprising *Boswellia serrata* extract standardized to 85% total boswellic acids (BSE85%) and *Boswellia serrata* low polar gum resin extract (BsLPRE) showed potent inhibition of acetylcholinesterase (AChE).

Hence, the foregoing shows that BOIL, BVOIL and BLPRE alone as well as in combination with Boswellic acid(s)/ Boswellia extract(s) or fractions(s) containing boswellic acid(s)/ extracts standardized to boswellic acids / one or more Nootropic agents are potent inhibitors of acetylcholinesterase and as such can be used for the prevention, control and treatment of cognitive disorders and improving memory and alleviating disease conditions related to cognition and memory deficits.

Pure boswellic acids and commercially available *Boswellia serrata* extract standardized to 85% boswellic acids have been used to demonstrate the present invention. However,

any *Boswellia serrata* standardized to 40% - 100% total boswellic acids by titrimetric method of analysis or standardized to 30% - 100% total boswellic acids by HPLC method of analysis can also be used.

Similarly, a composition (composition-34) containing low polar gum resin extract (BLPRE) in combination with α-mangostin offers better protection from neuronal damage (**Table 6**) and hence can improve brain health. In addition, composition-34 also showed better protection from oxidative stress induced cytotoxic damage of human neuroblastoma cells in a cell based assay (**Table 7**). This result further confirms the potential role of the composition containing BsLPRE in the improvement of brain health.

Different embodiments of the present invention are as outlined below:

In the primary aspect, the invention provides non-acidic Boswellia low polar gum resin extract (BLPRE) fraction, Boswellia volatile oil (BVOIL) fraction and Boswellia oil fraction (BOIL) comprising BLPRE and BVOIL for improving mental condition/brain health, treating impaired memory and alleviating memory and cognition related disorders and other associated diseases in warm blooded animals.

In the other primary aspect the invention provides compositions comprising atleast one fraction selected from Boswellia oil (BOIL), Boswellia volatile oil (BVOIL) and Boswellia low polar gum resin extract (BLPRE) in combination with one or more biological agents or Nootropic agents for improving mental condition/brain health, treating impaired memory and alleviating memory and cognition related disorders and other associated diseases in warm blooded animal.

In another embodiment the invention provides, composition comprising atleast one Boswellia derived non-acidic extract/fraction selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction (BOIL) in combination with at least one component selected from biological agents, phytochemicals, vitamins, amino acids, minerals; pharmaceutically or dietetically acceptable excipients, vehicles, carriers and diluents or mixtures thereof for

improving mental condition/brain health;enhancing brain functions such as cognition, memory, learning, communication; for treating impaired memory, and for preventing, control or treating memory and cognition related disorders/diseases.
In another embodiment the invention provides methods for improving brain health and brain functions such as cognition, memory, learning, communication or treating impaired memory in a subject or warm blooded animal in need thereof, wherein the method comprises supplementing the said subject or warm blooded animal with an effective dose of Boswellia derived non-acidic extract/fraction or their composition(s).

In another embodiment the invention provides methods for preventing, control or treating memory and cognition related disorders/diseases in a subject or warm blooded animal in need thereof, wherein the method comprises supplementing the said subject or warm blooded animal with an effective dose of Boswellia derived non-acidic extract/fraction or their composition(s).

In another embodiment the invention provides methodsof preventing, control or treating memory and cognition related disorders/diseases, wherein memory and cognition related disorders/diseases include but not limited to senile dementia, multi-infarct dementia, dyslexia, aphasia, organic brain syndrome, myasthenia gravis, vascular dementia, mild cognitive impairment (MCI), Attention-deficient Hyperactivity Disorder (ADHD), Lewy body dementia, Wemicke-Korsakoff-syndrome, Alzheimer's, Parkinson's disease, hypoxia, anoxia, cerebrovascular insufficiency, epilepsy, myoclonus and hypocholinergic dysfunctions, memory impairment disorders and neurodegenerative disorders.

In another aspect, the invention provides Boswellia low polar gum resin extract (BLPRE) fraction, Boswellia volatile oil (BVOIL) fraction and Boswellia oil (BOIL) fraction comprising BLPRE and BVOIL individually or their composition(s) comprising useful for the prevention, control and treatment of brain related diseases comprising Attention-deficit Hyperactivity Disorder (ADHD) and memory deficits or to enhance brain functions such as cognition, memory, learning and communication. Importantly, the said fractions and compositions of the present invention help in making the brain healthy.

In another aspect, the invention provides compositions comprising atleast one component selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction (BOIL) in combination with at least one pharmaceutically/dietetically acceptable excipients/diluents, further optionally comprising one or more agents selected from natural antioxidants, anti-inflammatory agents and immune modulators.

In another embodiment the invention provides the composition comprising atleast one Boswellia derived non-acidic extract/fraction in combination with at least one pharmaceutically/dietetically acceptable excipients/diluents, wherein said pharmaceutically or dietetically acceptable excipients, carriers, vehicles and diluents include but not limited to glucose, fructose, sucrose, maltose, lactose, yellow dextrin, white dextrin, silicon dioxide, microcrystalline cellulose powder, calcium stearate, magnesium stearate, sorbitol, stevioside, corn syrup, citric acid, tartaric acid, malic acid, succinic acid, lactic acid, L-ascorbic acid, dl-alpha-tocopherol, glycerin, propylene glycol, glycerin fatty ester, poly glycerin fatty ester, sucrose fatty ester, sorbitan fatty ester, propylene glycol fatty ester, acacia, carrageenan, casein, gelatin, pectin, agar, nicotinamide, calcium pantothenate, calcium salts, pigments, flavors, preservatives, distilled water, saline, aqueous glucose solution, alcohol, propylene glycol and polyethylene glycol, various animal and vegetable oils, white soft paraffin, paraffin and wax.

In another aspect, the invention provides Bowellia non acidic extracts/fractions selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction (BOIL) and their compositions to prevent, control and treat brain related diseases/disorders which include but not limited to senile dementia, multi-infarct dementia, dyslexia, aphasia, organic brain syndrome, myasthenia gravis, vascular dementia, mild cognitive impairment (MCI), Lewy body dementia, Wemicke-Korsakoff-syndrome, Alzheimer's, Parkinson's disease, Attention-deficit Hyperactivity Disorder (ADHD), hypoxia, anoxia, cerebrovascular insufficiency, epilepsy, myoclonus and hypocholinergic dysfunctions, memory impairment disorders and neurodegenerative disorders

In another aspect, the invention provides Boswellia derived non-acidic extract/fraction or their composition(s) for improving mental condition/brain health by slowing down memory deterioration, functional loss, by inhibiting beta-amyloid plaque deposition, by controlling blood pressure and blood circulation in the brain.

In another aspect, the Nootropic agent(s) used for making the composition comprise one or more agent(s) selected from smart drugs, memory enhancers and cognitive enhancers; dietary supplements, herbal ingredients, nutraceuticals, functional ingredients and functional foods that improve mental functions such as cognition, memory, intelligence, motivation, attention and concentration.

In another aspect, the Nootropic agents can be selected from one or more components selected from the extract(s)/ fraction(s)/ phytochemicals derived from herbs including but not limited to Bacopa species, Curcuma species or Rosmarinus species.

In another aspect, the herbal ingredients that can be used for preparing compositions are selected from including but not limited to *Boswellia serrata, Boswellia carterii, Bacopa monniera, Curcuma longa, Withania somnifera, Rosmarinus officinalis, Garcinia mangostana,* α-mangostin, *Annona squamosa* and *Sphaeranthus indicus.*

In another aspect of the invention, the Nootropic agents can be selected from extract(s)/fraction(s)/phytochemicals, extracts/fractions enriched in one or more phytochemicals selected from including but not limited to Bacopa monnieri, Withania somnifera, Emblica officinalis, Centella asiatica; extract or fraction enriched in one or more phytochemicals selected from including but not limited to Bacoside A3, Bacopaside II, Jujubogenin isomer of bacopasaponin C, Bacopasaponin C, Bacopaside I, Bacosine, Apigenin, Luteolin and Sitosterol-D-glucoside, curcumin, demethoxycurcumin, bisdemethoxycurcumin, monodemethylcurcumin, bisdemethylcurcumin, tetrahydrocurcumin, tetrahydrodemethoxycurcumin, tetrahydrobisdemethoxycurcumin and ar-turmerone, carnosic acid, rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol, rosmanol and their salts thereof. .

In yet another aspect, the invention provides compositions comprising therapeutically effective combination of Boswellia oil (BOIL/ Boswellia volatile oil BVOIL)/ Boswellia low polar gum resin extract (BLPRE) in combination with at least one Boswellia derived component selected from the extract(s), fraction(s) enriched with one or more boswellic acids/ pure boswellic acid compounds or Nootropic agents for improving memory, impaired memory and alleviating memory and cognition related disorders and other associated diseases.

In another aspect, the invention provides compositions for the cognition enhancement achieved through one or more biological actions comprising inhibition of Acetylcholinesterase, increase in Butyrylcholinesterase and inhibition of β-amyloid aggregation.

In yet another aspect, the invention further provides compositions comprising Boswellia oil (BOIL)/ Boswellia volatile oil (BVOIL)/ Boswellia low polar gum resin extract (BLPRE) and atleast one component, derived from gum resin of Boswellia species, which include but not limited to α-boswellic acid, β-boswellic acid, 3-O-acetyl-α-boswellic acid, 3-O-acetyl-β-boswellic acid, 3-O-acetyl-11-keto-α-boswellic acid and 3-O-acetyl-11-keto-β-boswellic acid or mixtures thereof for improving memory, impaired memory and alleviating memory and cognition related disorders and other associated diseases.

In yet another important aspect, the invention further provides compositions comprising *Boswellia serrata* low polar gum resin extract (BsLPRE) or BsVOIL or BsOIL and at least one Boswellia derived component selected from the extracts or fractions enriched in or standardized to one or more compounds derived from the gum resin of Boswellia which include but not limited to α-boswellic acid, β-boswellic acid, 3-acetyl-α-boswellic acid, 3-acetyl-β-boswellic acid, 3-acetyl-11-keto-α-boswellic acid and 3-acetyl-11-keto-β-boswellic acid or mixtures thereof for improving memory, improving impaired memory and alleviating memory and cognition related disorders and other associated diseases.

In another aspect, the invention further provides compositions comprising *Boswellia serrata* low polar gum resin extract (BsLPRE) or BsVOIL or BsOIL and a *Boswellia serrata* extract standardized to 30 – 100 % total boswellic acids by titrimetric method of analysis or 20 - 100% total boswellic acids by HPLC method of analysis.

In preferred aspect, the invention further provides compositions comprising *Boswellia serrata* low polar gum resin extract (BsLPRE) or BsVOIL or BsOIL and *Boswellia serrata* extract standardized to 85% total boswellic acids by titrimetric method of analysis or 65% total boswellic acids by titrimetric method of analysis.

In another preferred aspect, the invention provides compositions comprising *Boswellia serrata* low polar gum resin extract (BsLPRE) or BsVOIL or BsOIL and *Boswellia serrata* extract selectively enriched in AKBA concentration varying from 3 - 99% by HPLC method of analysis.

In other preferred embodiment, the invention further provides a process for producing the Boswellia low polar gum resin extract (BLPRE), which include extraction of the gum resin of Boswellia species with a water immiscible organic solvent followed by washing the organic solvent extract with an aqueous alkali solution such as aqueous potassium hydroxide, followed by water and brine, and then finally evaporating the organic layer under vacuum to obtain an oil, followed by removing the volatile compounds under high vacuum and temperature to obtain BLPRE. The water immiscible organic solvent can be selected from hexane, chloroform, dichloromethane, ethyl acetate, methyl isobutyl ketone or any other water immiscible solvent or mixtures thereof.

The process for producing the *Boswellia serrata* low polar gum resin extract (BsLPRE) is variable and the alternative process for example comprise, extracting the gum resin with alcohol or hydroalcohol, and then evaporating the organic solvent to optimum concentration of total solids and then adjusting the solution to pH to 9 – 11, followed by repeatedly extracting the solution with a low polar organic solvent and then evaporating the organic solvent followed by removing the volatiles under vacuum at high temperature to obtain BsLPRE.

In a further embodiment, the *Boswellia serrata* intact oil can also be used in place of BsLPRE for improving memory, impaired memory and alleviating memory and cognition related disorders and for making the compositions of the present invention.

The water immiscible organic solvent in the above process can be selected from the solvents but not limited hexane, chloroform, dichloromethane, ethyl acetate, methylisobutylketone, tert-butanol or any other water immiscible solvent.

The *Boswellia serrate* extract standardized to 30 – 100 % total boswellic acids by a titrimetric method of analysis or 20 – 100% total boswellic acids by HPLC method of analysis can be prepared from the gum resin using a known procedure or obtained from a group of commercially available *Boswellia serrata*extracts standardized to boswellic acids.

In another aspect of the invention, the non acidic extracts Boswellia oil (BOIL), Boswellia volatile oil (BVOIL),Boswellia low polar gum resin extract (BLPRE) used for the demonstration of the invention can be obtained from the Boswellia species selected from *Boswellia serrata, Boswellia carterii, Boswellia papyrifera, Boswellia ameero, Boswellia bullata, Boswellia dalzielii, Boswellia dioscorides, Boswellia elongata, Boswellia frereana, Boswellia nana, Boswellia neglecta, Boswellia ogadensis, Boswellia pirottae, Boswellia popoviana, Boswellia rivae, Boswellia sacra* and *Boswellia socotrana.*

In another aspect of the invention one or more of the Curcuma species that can be used for making the compositions of the present invention can be selected from *Curcuma longa, Curcuma aromatica, Curcuma domestica, Curcuma aeruginosa, Curcuma albicoma, Curcuma albiflora, Curcuma alismatifolia, Curcuma angustifolia, Curcuma elata, Curcuma ferruginea, Curcuma flaviflora, Curcuma yunnanensis* and *Curcuma zedoaria.*

In yet another aspect of the present invention, BOIL, BVOIL or BLPRE alone or in combination with one or more Boswellia derived extracts selectively enriched in boswellic acids/ commercially available boswellic extract(s) standardized to 50 – 100 % total boswellic acids/ *Boswellia serrata* extracts wherein AKBA concentration varies from 3 -99% HPLC method of analysis and optionally contains one or more of pharmaceutically/ nutraceutically/ dietically acceptable excipient(s), diluents, salt(s), additive(s), natural antioxidants or natural anti-inflammatory agents.

In another aspect, the invention provides the usage of BOIL, BVOIL or BLPRE alone or their compositions as it is or in comminuted form and/or in unmodified form as granules or powder or paste or the active ingredients are formulated into a solid, semi-solid or liquid dosage form by adding a conventional biologically or pharmaceutically acceptable salt(s) or additive(s) or excipient(s).

In yet another aspect, the invention provides use of therapeutically effective amount of BOIL, BVOIL or BLPRE alone or their compositions with one or more biological agents or Nootropic agents for administration in a specific dosage form such as orally, topically, transdermally, parenterally or in the form of a kit to a subject or patient in need thereof. Specific dosage form for formulation of the compositions of the present invention include but not limited to oral agents such as tablets, soft capsule, hard capsule, soft gel capsules, pills, granules, powders, emulsions, suspensions, syrups, pellets, food, beverages, concentrated shots, drops and the like; parenteral agents such as injections, intravenous drip and the like; suppositories; transdermal agents such as patches, topical creams and gel; ophthalmic agents and nasal agents.

In another aspect, the present invention provides compositions containing at least one extract/fraction selected from BOIL, BVOIL or BLPRE in combination with one or more functional ingredient(s) comprising herbal ingredients, dietary supplements, antioxidants, vitamins, minerals, amino acids, fatty acids, essential oils, fish oils, enzymes, glucosamine, Chondroitin and probiotics or their salts or mixtures thereof derived from plants or animals or microorganisms or chemical synthesis or semi-synthesis.

In a further aspect, the present invention provides BOIL, BVOIL or BLPRE alone or their compositions further optionally combined with effective amounts of one or more pharmaceutical/ nutraceutical/ dietically acceptable agents including but not limited to antioxidant(s), adaptogen(s), anti-acetylcholinesterase agent(s), anti-inflammatory agent(s), anti-diabetic agent(s), antiobese agent(s), antiatherosclerotic agent(s), bio-protectants and/or bio-availability enhancer(s) and trace metals.

The examples of the biologically/pharmaceutically acceptable carriers employed in the present invention include, but are not limited to, surfactants, excipients, binders, diluents, disintegrators, lubricants, preservatives, stabilizers, buffers and suspensions.

In alternative aspect of the invention, the BOIL, BVOIL or BLPRE alone or their compositions can be optionally delivered in the form of controlled release dosage forms;and by using techniques including nanotechnology, microencapsulation, colloidal carrier systems and other drug delivery systems. The said formulation can be designed for once a daily administration or multiple administrations per day.

In accordance to the present invention, the BOIL, BVOIL or BLPRE alone or their compositions can also be formulated into or added to existing or new food and beverage form(s) and animal feeds as a healthy food or beverage or feed.

In accordance to the present invention, the BOIL, BVOIL or BLPRE alone or their compositions can also be formulated into or added to existing or new food and beverage form(s) and animal feeds as a healthy food or beverage or feed for prevention, control and treatment of brain related diseases/disorders.

In yet another embodiment, the composition can comprise 10%-99% by the weight of *Boswellia serrata* derived component selected from the extract(s) and fraction(s) enriched with one or more boswellic acids, pure boswellic acid compounds and mixtures thereof and 90%-10% by weight of *Boswellia serrata* low polar gum resin extract BsLPRE or BsVOIL or BsOIL.

The following examples, which include preferred embodiments, will serve to illustrate the practice of this invention, and it being understood that the particulars shown are by way of example and for purpose of illustrative discussion of preferred embodiments of the invention and they are not to limit the scope of the invention.

Example 1:

A process for preparation of the non-acidic Boswellia extract (BOIL) comprises:

g) Procuring the gum resin of one or more of the plant(s) selected from but not limited to *Boswellia serrata* or *Boswellia carterii* or *Boswellia papyrifera* or mixtures thereof,

h) Extraction of the gum resin with a water immiscible organic solvent,

i) Filtering the extract carefully to remove the insoluble resin material,

j) Washing the organic solvent extract repeatedly with an aqueous alkali solution such as aqueous potassium hydroxide,

k) Washing the organic layer with water and brine,

l) Evaporating the organic layer under vacuum and high temperature to obtain the oily residue (BOIL).

Example 2:

A process for preparation of the non-acidic Boswellia volatile oil fraction (BVOIL) comprises:

a) Procuring the gum resin of one or more of the plant(s) selected from but not limited to *Boswellia serrata* or *Boswellia carterii* or *Boswellia papyrifera* or mixtures thereof,

b) Separating the Volatile oil component by either steam distillation or distillation under high vacuum andtemperature from the said gum resin to obtain Boswellia volatile oil fraction (BVOIL).

Example 3:

A process for preparation of the non-acidic Boswellia low polar gum resin extract fraction (BLPRE) comprises:

a) Procuring the gum resin of one or more of the plant(s) selected from but not limited to *Boswellia serrata* or *Boswellia carterii* or *Boswellia papyrifera* or mixtures thereof,

b) Extraction of the gum resin with a water immiscible organic solvent,

c) Filtering the extract carefully to remove the insoluble resin material,

d) Washing the organic solvent extract repeatedly with an aqueous alkali solution such as aqueous potassium hydroxide,

e) Washing the organic layer with water and brine,

f) Evaporating the organic layer under vacuum and high temperature to obtain the oily residue (Boswellia oil).

g) Taking the said oily residue and removing the volatiles under high vacuum and high temperature to obtain Boswellia low polar gum resin extract fraction (BLPRE).

Example 4:

Representative procedure for the preparation of *Boswellia serrata* low polar gum resin extract fraction (BsLPRE): The *Boswellia serrata* gum resin (100 g) was dispersed in 600 mL of methyl isobutyl ketone (MIBK) solvent and stirred at room temperature for 60 min. The insoluble gum materials were separated by filtration. The MIBK solution was extracted repeatedly with 2% KOH solution (3 x 200 mL) to remove the acidic compounds. The MIBK layer was then washed successively with water (400 mL) and brine (200 mL). The MIBK layer was evaporated under reduced pressure at 60 – 70°C and the volatile components are removed from the oily residue under high vacuum at 75 – 110°C to obtain BsLPRE as a viscous oil (12 g).

Alternatively, the gum resin (250 g) collected from *Boswellia serrata* was extracted with methanol (300 mLx3) and the combined methanol extract was concentrated. The residue (50 g) was dissolved in ethyl acetate (400 mL) and extracted thrice with 1N KOH (3 x 100 mL). The organic layer was washed with water (2 x 200 mL) and brine (200 mL) and evaporated to obtain Boswellia oil. The volatile compounds were evaporated from the oil under vacuum at high temperature (75-110°C) to obtain 22 g of BsLPRE.

The BsLPRE was subjected to column chromatography over normal silica gel using solvents of increasing polarity starting from hexane to hexane/ethyl acetate mixtures to ethyl acetate. The identical fractions were combined based on TLC and combined fractions were subjected individually to repeated column over silica gel using mixtures of hexane/ethyl acetate or hexane/acetone as eluants to obtain pure compounds. Some of the impure fractions were further subjected to preparative HPLC using a reversed phase C18 silica column to obtain pure compounds. The structures were established by analyzing the ^{1}H NMR, ^{13}C NMR, DEPT, HSQC and HMBC and mass spectral data and then comparing the data with that of known compounds. Nine of the prominent compounds are identified as guiol (**1**), nephthenol (**2**), serratol (**3**), diterpene X (**4**), lupeol (**5**), olean-12-ene-3β-ol (**6**), olean-12-ene-3α-ol (**7**), lanosta-8,24-diene-3α-ol (**8**) and urs-12-ene-3α-ol (**9**) as depicted in **Figure I**. The pure compounds were then utilized to standardize the *Boswellia serrata* low polar gum resin extract (BsLPRE) using HPLC method. The novel composition of BsLPRE evaluated based on analytical HPLC method along with the retention times (R_t) is summarized in **Table 1**. The HPLC chromatogram for BsLPRE is depicted in **Figure II**.

Table 1

Composition of *Boswellia serrata* low polar gum resin extract fraction (BsLPRE)

S. No	Test substance	R_t in min	Percentage
1	Guiol (**1**)	4.5	0.96
2	Nephthenol (**2**)	7.087	2.01
3	Serratol (**3**)	8.027	13.32
4	Diterpene X (**4**)	15.777	0.12
5	Lupeol (**5**)	26.901	0.06
6	Olean-12-ene-3β-ol(**6**)	31.460	1.29

7	Olean-12-ene-3α-ol(**7**)	33.718	5.36
8	Lanosta-8,24-diene-3α-ol (**8**)	35.371	1.34
9	Urs-12-ene-3α-ol(**9**)	37.207	4.55

Example 5

Boswellia serrata extract standardized to 50 - 100% total boswellic acids (titrimetric method): *Boswellia serrata* extracts standardized to 85% or 65% total boswellic acids are commercially available. These extracts are standardized using titrimetric method of analysis. These extracts can be prepared using a known procedure. For example, by extracting the gum resin of *Boswellia serrata* using a water immiscible solvent and then selectively extracting the acidic compounds from the organic solvent extract using aqueous alkali solution through phase separation. Finally acidification of the alkali solution to precipitate the boswellic acids followed by vacuum drying to yield *Boswellia serrata* extract enriched to 85% boswellic acids (BE85%). *Boswellia serrata* extracts standardized to a selected concentration of total boswellic acids in the range of 40 – 100% by titrimetric method of analysis or 30 – 100% by HPLC method of analysis can be obtained by purification of the gum resin or the extracts or by dilution of higher grade material.

Example 6:

Determination of acetylcholinesterase inhibitory activity of BsOIL, BsLPRE, BcLPRE, BsVOIL and different boswellic acid compound in an in vitro enzymatic assay:

Acetylcholinesterase activity is measured using the substrate acetylthiocholine iodide, which is converted to thiocholine. The reaction of thiocholine with the chromogenic substrate Dithionitrobenzoic acid (DTNB) leads to the formation of a yellow anion, Nitrobenzoic acid, which absorbs strongly at 412nm. Incubation was done for 10min.

The AChE assay was performed by the method of Ellman *et al.,* with minor modifications, using acetylthiocholine iodide as a substrate (Lee J. H., et. al. *Arch Pharm Res* 2004, 27(1): 53-56). Ellmans reaction mixture contains 0.5 mM acetylthiocholine

iodide and 1mM 5,5'-dithio-bis-(2-nitrobenzoic acid) in a 50 mM sodium phosphate buffer (pH 8.0). The assay mixture contained 50µl of 50 mM phosphate buffer at pH – 8.0, 30 µl of test substance (BsOIL or BsLPRE or BcLPRE or BsVOIL, different boswellic acids and positive control Neostigmin) at various concentrations and 20 µl of (100 mU/mL) enzyme. For blanks enzyme was replaced with phosphate buffer. The reaction mixture was mixed thoroughly, 100 µl of Ellman's reagent was added and incubated at room temperature for 10 min. The absorbance was measured at 412nm using microplate reader. The percentage inhibition of enzyme activity was calculated by comparing OD's of tests wells with that of control wells using the following formula. Calculations: % inhibition = [(control- sample)/control] x 100. The results of Acetylcholinesterase inhibitory activity of BsOIL, BsLPRE, BcLPRE, BsVOIL and boswellic acids are summarized in **Table 2** and **3.**

Table 2

Acetylcholinesterase inhibitory activity of BsOIL, BsLPRE, BcLPRE, BsVOIL

Name of the compound	**% Inhibition at concentrations of**			**IC50 ng/mL**
	10 ng	**25 ng**	**50 ng**	
BsOIL	10.65	17.09	32.69	> 50
BsLPRE	31.5	43.7	57.87	37.01
BcLPRE	15.6	23.18	42.56	> 50
BsVOIL	16.5	25.49	33.01	>50
BSE-85	-	4.22	6.27	
Composition-1				42.7
Neostigmin	25.17	37.19	54.69	43.29

Table 3

Acetylcholinesterase inhibitory activity of Pure Boswellic acids

Name of the Product	% Inhibition at concentrations of				IC50 ng/mL
	10 ng	25 ng	50 ng	100 ng	
11-keto-β-boswellic acid	-	11.82	16.36	24.55	>100
3-O-acetyl-11-keto-β-boswellic acid	-	9.64	16.07	27.5	>100
α-boswellic acid	-	13.85	21.92	39.23	>100
β-boswellic acid	-	16.75	21.23	31.37	>100
3-O-acetyl-α-boswellic acid	-	14.86	21.28	33.11	>100
3-O-acetyl-β-boswellic acid	-	36.29	40.32	52.02	91.77
Neostigmin	25.17	37.19	54.69	-	43.29

Example 7:

Acetylcholine esterase inhibitory activity of BsOIL, BsLPRE, BcLPRE, BsVOIL and boswellic acids in PC 12 cells: The inhibitory property on the enzyme activity was assessed in β-amyloid peptide induced-rat pheochromocytoma PC 12 cells. Rat pheochromocytoma PC 12 cells were equally distributed with phenol red free Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10% fetal bovine serum (FBS) in 24-well plate. Cells were pretreated with test agents (BsOIL,

BsLPRE, BcLPRE, BsVOIL and different boswellic acids and positive control Neostigmin) for 1h. Thereafter, cells were induced with 1 μg/mL of β-amyloid peptide (Calbiochem, USA) for 24h at 37°C. After 24h, cells were collected and washed twice with 1x PBS by centrifugation at 1200 rpm for 5min at 4°C. Cell extracts were prepared in solubilization buffer (10mM Tris, pH 7.2; 100mM NaCl, 50 mM MgCl2, 1% Triton X-100). The cell extracts were used as samples for measuring the acetylcholine esterase (AChE) activity.

Cell extract samples (100 μl) were dispensed into each well of 96-well microtitre plate. Fifty micro litres of DTNB (Dithiobisnitro benzoate) solution was added to each well and incubated for 5 min at room temperature. After incubation, 50 μl of acetyl choline iodide solution was added to each well and absorbance was read immediately at 405 nm for 12 min at 2 min intervals. A standard curve was constructed by using serial concentrations of acetyl cholinesterase (0-100 mU). Total protein present in 100 μl aliquot of cell extract was calculated by Bradford method and the enzyme activity was normalized and expressed as unit activity per milligram of protein. Efficacy of test samples was expressed in terms of percent inhibition of AChE activity and compared with a standard drug, Neostigmin as the positive control.

Results: Table 4 and **Table 5** are summary of the acetyl cholinesterase inhibitory activities exhibited by various non acidic extracts from *Boswellia serrata* and *Boswellia carterii* (BsOIL, BsLPRE, BcLPRE and BsVOIL) and different boswellic acids. A standard drug, Neostigmin was used as the positive control for comparing the AChE inhibitory efficacies of the boswellia products.

Table 4

Acetyl cholinesterase inhibitory property of BsOIL, BsLPRE, BcLPRE and BsVOIL

Test samples	Treatment Conc.	% inhibition in AChE activity
BsOIL	100 ng/ml	19.92
BsLPRE	100 ng/mL	25.31
BcLPRE	100 ng/mL	18.67
BsVOIL	100 ng/mL	16.46
Neostigmin	20 ng/ml	49.09

Table 5

Comparative efficacy of different boswellic acids in inhibiting Acetyl cholinesterase activity

Test samples	Treatment Conc.	% inhibition in AChE activity
11-keto-β-boswellic acid	100 ng/ml	31.39
3-O-acetyl-keto-β-boswellic acid	100 ng/ml	41.67
α-boswellic acid	100 ng/ml	38.42
β-boswellic acid	100 ng/ml	46.54
3-O-acetyl-α-boswellic acid	100 ng/ml	29.22
3-O-acetyl-β-boswellic acid	100 ng/ml	37.34
Neostigmin	20 ng/ml	54.65

Example 8

Preparation of composition-1: Composition-1 was prepared by mixing unit doses of the following components; four parts of *Boswellia serrata* low polar gum resin extract (BsLPRE) (4g) and one part of *Boswellia serrata* extract standardized to 85% total Boswellic acids (BSE 85%) (1 g).

Example 9

Composition-2: Composition-2 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* volatile oil (BsVOIL) (1 g) and four parts of *Boswellia serrata* extract standardized to 85% total Boswellic acids (BSE 85%) (4 g).

Example 10

Composition-3: Composition-3 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* non acidic oil (BsOIL) (1 g) and four parts of *Boswellia serrata* extract standardized to 85% total Boswellic acids (BSE 85%) (4 g).

Example 11

Composition-4: Composition-4 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g) and four parts of *Boswellia serrata* extract standardized to 85% total Boswellic acids (BSE 85%) (4 g).

Example 12

Composition-5: Composition-5 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii*volatile oil (BcVOIL) (1 g) and three parts of *Boswellia carterii* extract standardized to 85% total Boswellic acids (BCE 85%) (3 g).

Example 13

Composition-6: Composition-6 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g),

four parts of *Boswellia serrata* extract enriched with 20% of 3-O-acetyl-11-keto-β-Boswellic acid (AKBA) (4 g).

Example 14

Composition-7: Composition-7 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g), four parts of *Boswellia carterii* extract enriched with 20% of 3-O-acetyl-11-keto-β-Boswellic acid (AKBA) (4 g).

Example 15

Composition-8: Composition-8 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BcLPRE) (1 g), four parts of *Boswellia serrata* extract enriched with 40% of 3-O-acetyl-11-keto-β-Boswellic acid (AKBA) (4 g).

Example 16

Composition-9: Composition-9 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g), three parts of *Boswellia carterii* extract enriched with 40% of 3-O-acetyl-11-keto-β-Boswellic acid (AKBA) (3 g).

Example 17

Composition-10: Composition-10 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and three parts of *Bacopa monniera* standardized extract (3 g).

Example 18

Composition-11: Composition-10 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* volatile oil fraction (BsVOIL) (1 g) and three parts of *Bacopa monniera* standardized extract (3 g).

Example 19

Composition-12: Composition-12 was prepared by mixing unit doses of the following components; one part of non-acidic *Boswellia serrata* oil fraction (BsOIL) (1 g) and three parts of *Bacopa monniera* standardized extract (3 g).

Example 20

Composition-13: Composition-13 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of Bacopa monniera water extract (4 g).

Example 21

Composition-14: Composition-14 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of *Bacopa monniera* 90% methanol extract (4 g).

Example 22

Composition-15: Composition-15 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and three parts of *Bacopa monniera* standardized extract (3 g).

Example 23

Composition-16: Composition-16 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of *Bacopa monniera* extract standardized to 25% bacopasaponins (4 g).

Example 24

Composition-17: Composition-17 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g) and four parts of *Bacopa monniera* extract standardized to 25% bacopasaponins (4 g).

Example 25

Composition-18: Composition-18 was prepared by mixing unit doses of the following components; one part of *Boswellia papyrifera* low polar gum resin extract (BpLPRE) (1 g) and four parts of *Bacopa monniera* extract standardized to 25% bacopasaponins (4 g).

Example 26

Composition-19: Composition-19 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of *Curcuma longa* extract standardized to 95% total Curcuminoids (CLE 95%) (1 g).

Example 27

Composition-20: Composition-20 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* volatile oil fraction (BsVOIL) (1 g) and four parts of *Curcuma longa* extract standardized to 95% total Curcuminoids (CLE 95%) (4 g).

Example 28

Composition-21: Composition-21 was prepared by mixing unit doses of the following components; one part of non-acidic *Boswellia serrata* oil fraction (BsOIL) (1 g) and four parts of *Curcuma longa* extract standardized to 95% total Curcuminoids (CLE 95%) (4g).

Example 29

Composition-22: Composition-22 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and three parts of curcumin (3 g).

Example 30

Composition-23: Composition-23 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and three parts of bisdemethylcurcumin (BDMC) (3 g).

Example 31

Composition-24: Composition-24 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of *Withania somnifera* methanol extract (4 g).

Example 32

Composition-25: Composition-25 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g) and four parts of standardized *Withania somnifera* extract (4 g).

Example 33

Composition-26: Composition-26 was prepared by mixing unit doses of the following components; one part of Boswellia low polar gum resin extract (BLPRE) (1 g) and four parts of standardized *Rosmarinus officinalis* extract (4 g).

Example 34

Composition-27: Composition-27 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of standardized *Rosmarinus officinalis* extract (4 g).

Example 35

Composition-28: Composition-28 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g) and four parts of standardized *Rosmarinus officinalis* extract (4 g).

Example 36

Composition-29: Composition-29 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and four parts of *Rosmarinus officinalis* extract standardized to 30% Rosmarinic acid (RA 30%) (4 g).

Example 37

Composition-30: Composition-30 was prepared by mixing unit doses of the following components; one part of *Boswellia carterii* low polar gum resin extract (BcLPRE) (1 g) and three parts of *Rosmarinus officinalis* extract standardized to 30% Rosmarinic acid (RA 30%) (3 g).

Example 38

Composition-31: Composition-31 was prepared by mixing unit doses of the following components; one part of Boswellia non acidic oil (BOIL) (1 g) and three parts of *Garcinia mangostana* methanol extract (3 g).

Example 39

Composition-32: Composition-32 was prepared by mixing unit doses of the following components; one part of non-acidic *Boswellia serrata* oil (BsOIL) (1 g) and three parts of *Garcinia mangostana* methanol extract (3 g).

Example 40

Composition-33: Composition-33 was prepared by mixing unit doses of the following components; one part of *Boswellia serrata* low polar gum resin extract (BsLPRE) (1 g) and three parts of *Garcinia mangostana* methanol extract (3 g).

Example 41

Composition-34: Composition-34 was prepared by mixing unit doses of the following components; two parts of *Boswellia serrata* low polar gum resin extract (BsLPRE) (2 g) and one part of α-mangostin (1 g).

Example 42

Composition-35: Composition-35 was prepared by mixing unit doses of the following components; four parts of *Boswellia serrata* low polar gum resin extract (BsLPRE) (4 g) and one part of α-mangostin (1 g).

Example 43

Composition-36: Composition-40 was prepared by mixing unit doses of the following components; one part of Boswellia low polar gum resin extract fraction (BLPRE) (1 g) and three parts of *Sphaeranthus indicus* ethyl acetate extract (3 g).

Example 44

Composition-37: Composition-40 was prepared by mixing unit doses of the following components; one part of non acidic Boswellia oil fraction (BOIL) (1 g) and four parts of *Sphaeranthus indicus* ethyl acetate extract (4 g).

Example 45

Composition-38: Composition-40 was prepared by mixing unit doses of the following components; one part of Boswellia volatile oil fraction (BVOIL) (1 g) and four parts of *Sphaeranthus indicus* ethyl acetate extract (4 g).

Example 46

Assay for measuring reactive oxygen species (ROS)

Formation of ROS was measured using of the fluorescent probe DCFH-DA. The method is based on the incubation of the RAW 264.7 mouse macrophages with DCFH-DA, which diffuses passively through the cellular membrane. Intracellular esterase activity results in the formation of DCFH, which emits fluorescence when oxidized to 20, 70-dichlorofluorescein (DCF). Briefly, the cells (final concentration 2 x 10^6/ ml suspension) were incubated with DCFH-DA (5 mM) in HEPES-buffered (20 mM) HBSS ($CaCl_2$ 1.26 mM, KCL 5.37 mM, KH_2PO_4 0.44 mM; $MgCl_2$ 0.49 mM, MgSO4 0.41 mM, NaCl 140 mM, $NaHCO_3$ 4.17 mM, Na_2HPO_4 0.34 mM) with glucose (5 mM) at 37°C for 15 min. Following centrifugation, the extracellular buffer with DCFH-DA was exchanged with fresh buffer and the suspension was mixed gently. The cells ($2x10^6$/ml, 125 ml) were transferred to 250 ml wells (microtiter plate reader, 96 wells) containing 125 ml buffer with the different concentrations of test samples (α-mangostin, BsLPRE, Composition-34 and Composition-35) in presence or absence of100 mM H_2O_2. Fluorescence was recorded using excitation wavelength 485 nm, emission wavelength 530 nm in a Modulus luminescence spectrometer (Turner

Biosystems, USA) for 120 min. Results are calculated as area under the curve (AUC) and the percentage of inhibition of intracellular ROS generation was calculated from the cultures treated with H_2O_2. The results are summarized in Table 6.

Table-6

Anti-oxidant properties of herbal products and their combinations

Serial no	Treatments	Inhibition of Reactive Oxygen Species (ROS) generation (IC50)
1	α-mangostin	49.817 ug/ml
2	BsLPRE	53.879 ug/ml
3	Composition-34	33.342 ug/ml
4	Composition-35	50.776 ug/ml

Example 47

Cell proliferation assay

Effect of BsLPRE or α-mangostin or their combination (Composition-34) on cell growth was tested in oxidative stress induced IMR32 human neuroblastoma cells SW 982 human synovial cells by using MTT based cell proliferation assay. Briefly, IMR32 human neuroblastoma cells were cultivated in Dulbecco's modified Eagle's red medium (DMEM) (Sigma Life Science, USA) containing 10% fetal bovine serum (FBS). Equal number of IMR32 cells was seeded in to each well of 96 well microplate and incubated at 37°C with 5% CO_2. The cells were treated with 250 uM H2O2 in presence or absence of different concentrations of BsLPRE or α-mangostin or their combination (Composition-34) for 72 h. Control wells were supplemented with 0.05% DMSO. After 72 h of treatment, equal volume of MTT reagent (R&D Systems, USA) was added to each well and incubated for 4 h. Thereafter, 50 µl of solublization buffer (R&D Systems, USA) was added to each well to solubilize the colored formazan crystals produced by the reduction of MTT. After 24 h, the optical density was measured at 550 nm using microplate reader (Bio-Rad, USA). In each assay, the vehicle control and the treatments were done in

quadruplicates. The average OD obtained in the vehicle control wells is considered as the cell proliferation index of 100. The results are summarized in Table-7 below.

Table 7

Protection from oxidative stress induced cytotoxic damage of human neuroblastoma cells

S. No	Treatments	Treatment conc.	Cell proliferation Index
1	Vehicle Control		100.00
2	H2O2	250 uM	78.71
3	α-mangostin	10ng/ml	80.32
		25ng/ml	86.78
		50ng/ml	87.21
		100ng/ml	91.78
4	BsLPRE	10ng/ml	87.81
		25ng/ml	92.24
		50ng/ml	95.19
		100ng/ml	97.30
5	Composition-34	10ng/ml	92.81
		25ng/ml	95.82
		50ng/ml	99.05
		100ng/ml	101.33

Example 48

Inhibition of acetylcholinesterase activity by herbal products and their combinations in Beta Amyloid protein induced PC12 cells: The acetylcholinesterase inhibitory activity of α-mangostin, BsLPRE and Composition-34 was measured using the procedure described in example 7.

Serial No.	Treatments	Treatment conc.	% inhibition in Acetylcholinesterase (AChE) activity
1	α-mangostin	100ng/ml	26.83
		250ng/ml	29.48
2	BsLPRE	100ng/ml	30.99
		250ng/ml	35.15
3	Composition-34	100ng/ml	37.97
		250ng/ml	40.57

Example 49

Determination of spatial learning and memory improvement efficacy of BsLPRE in rats using elevated radial arm maze method: The animal study protocol was approved by institutional animal ethics committee. Sprague Dawley rats were acclimatized for one week and healthy rats were selected for the study. The selected rats were pre-trained in elevated radial arm maze (RAM) adapted rats were allocated to various treatment groups each containing eight rats. After completion of pre-training, the oral treatment was initiated to the animals and continued daily up to two weeks. During this treatment phase the rats were placed on the RAM for 10 min each to recognize the food pellets present in the three different colored arms. During training the spatial learning was estimated by measuring various parameters like number of days required to learn the task, latency in finding food and number of wrong entries/attempts. After this treatment and training the animals were given rest without treatment or training for one week (3^{rd} week). On 4^{th} week, the animals were treated with allocated doses of test products and memory retention test was assessed using the same animals by measuring latency and number of wrong entries. The data was analyzed using ANOVA followed by a suitable post-hoc test.

Result (Spatial learning):

Oral administration of BsLPRE (250 mg/kg) significantly ($P< 0.01$) decreased the number of days required to make the rats learned as per set criteria andsignificantly ($P< 0.05$)

decreased the time taken to find the food by the learned rats in the elevated RAM model. Piracetam showed significant improvement in spatial learning represented by reduction in latency and Number of wrong entries, when compared with the control group and the results are as stated below (Figure IIIA to IIIC). The test product BsLPRE also significantly improves cognition and memory retention (Figure IVA and IVB). These results suggest that the use of BsPREimproves spatial learning and memory retention. According to these findings, BsLPREis a promising candidate for facilitation of learning and memory.

It will be appreciated to those of ordinary skill in the art that changes could be made to the embodiments described above without departing from the broad inventive concept thereof. It is understood, therefore, that this invention is not limited to the particular embodiments or examples disclosed, but is intended to cover modifications within the objectives and scope of the present invention.

We claim,

1. A non-acidic extract/fraction selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and Boswellia oil fraction (BOIL) derived from the gum resin of Boswellia species for improving mental condition/brain health;enhancing brain functions such as cognition, memory, learning, communication; for treating impaired memory, and for preventing, control or treating memory and cognition related disorders/diseases.
2. A composition comprising atleast one Boswellia derived non-acidic extract/fraction selected from Boswellia low polar gum resin extract fraction (BLPRE), Boswellia volatile oil fraction (BVOIL) and non-acidic Boswellia oil fraction (BOIL) in combination with at least one component selected from biological agents, phytochemicals, vitamins, amino acids, minerals; pharmaceutically or dietetically acceptable excipients, vehicles, carriers and diluents or mixtures thereof for improving mental condition/brain health; enhancing brain functions such as cognition, memory, learning, communication; for treating impaired memory, and for preventing, control or treating memory and cognition related disorders/diseases.
3. The Boswellia derived non-acidic extract/fractions as claimed in claims 1 and 2, wherein the Boswellia species can be selected from *Boswellia serrata, Boswellia carterii, Boswellia papyrifera, Boswellia ameero, Boswellia bullata, Boswellia dalzielii, Boswellia dioscorides, Boswellia elongata, Boswellia frereana, Boswellia nana, Boswellia neglecta, Boswellia ogadensis, Boswellia pirottae, Boswellia popoviana, Boswellia rivae, Boswellia sacra* and *Boswellia socotrana.*
4. The Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2, to prevent, control and treat memory and cognition related disorders/diseases, which include but not limited to senile dementia, multi-infarct dementia, dyslexia, aphasia, organic brain syndrome, myasthenia gravis, vascular dementia, mild cognitive impairment (MCI), Attention-deficient Hyperactivity Disorder (ADHD), Lewy body dementia, Wemicke-Korsakoff-syndrome, Alzheimer's, Parkinson's disease, hypoxia, anoxia, cerebrovascular

insufficiency, epilepsy, myoclonus and hypocholinergic dysfunctions, memory impairment disorders and neurodegenerative disorders.

5. The Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2, for improving mental condition/brain health by slowing down memory deterioration, functional loss, by inhibiting beta-amyloid plaque deposition, by controlling blood pressure and blood circulation in the brain.
6. The composition according to claim 2, wherein the biological agent(s) used for making the composition comprise one or more agent(s) selected from dietary supplements, herbal ingredients, nutraceuticals, functional ingredients, functional foods and nootropic agents.
7. The compositions according to claim 2, wherein the biologicalagent(s) is selected from natural antioxidants, neuroprotecting agents, anti-inflammatory agents and immune modulators.
8. The biological agent(s) used for making the compositions as claimed in claim 6, where in the herbal ingredients are selected from extracts, fractions, standardized extracts/fractions or pure phytochemicals derived from plants or mixtures there-of.
9. The biological agent used for making the compositions as claimed in claim 2 is a *Boswellia serrata* derived component selected from the extract(s) or fraction(s) containing boswellic acids, extracts or fractions selectively enriched with one or more boswellic acids, pure boswellic acid compounds or mixtures thereof.
10. The biological agent used for making the compositions as claimed in claim 2 is a *Boswellia serrata* extract standardized to 30 – 100 % total boswellic acids by titrimetric method of analysis or 20 – 100% total boswellic acids by HPLC method of analysis.
11. The biologically active ingredientused for making the compositions as claimed in claim 2 is a Curcuma longa derived component selected from the extract(s), fraction(s) extracts or fractions selectively enriched with one or more curcuminoids, pure curcuminoid compounds or mixtures thereof.
12. The Nootropic agents according to claim 6, selected from extract(s), fraction(s) or phytochemicals derived from Bacopa monnieri, Withania somnifera, Emblica officinalis, Centella asiatica; extract or fraction enriched in one or more

phytochemicals selected from including but not limited to Bacoside A3, Bacopaside II, Jujubogenin isomer of bacopasaponin C, Bacopasaponin C, Bacopaside I, Bacosine, Apigenin, Luteolin and Sitosterol-D-glucoside, curcumin, demethoxycurcumin, bisdemethoxycurcumin, monodemethylcurcumin, bisdemethylcurcumin, tetrahydrocurcumin, tetrahydrodemethoxycurcumin, tetrahydrobisdemethoxycurcumin and ar-turmerone, carnosic acid, rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol, rosmanol and their salts thereof.

13. The composition comprising atleast one Boswellia derived non-acidic extract/fraction as claimed in claim 2, wherein said pharmaceutically or dietetically acceptable excipients, vehicles, diluents and carriers comprises surfactants, binders, disintegrators, lubricants, preservatives, stabilizers, buffers and suspensions.
14. The Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2,wherein said extract/fraction or their composition(s) is formulated as oral agents such as tablets, soft capsule, hard capsule, soft gel capsules, pills, granules, powders, emulsions, suspensions, syrups, pellets, food, beverages, concentrated shots, drops and the like; parenteral agents such as injections, intravenous drip and the like; suppositories; transdermal agents such as patches, topical creams and gel; ophthalmic agents and nasal agents.
15. The Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2, wherein said extract/fraction or their composition(s) is delivered in the form of controlled release dosage forms by using techniques including nanotechnology, microencapsulation, colloidal carrier systems and other drug delivery systems.
16. The Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2,wherein said extract/fraction or their composition(s) is formulated into or added to existing or new food and beverage form(s) as a healthy food for subject or warm blooded animal.
17. A method of improving brain health and brain functions such as cognition, memory, learning, communication or treating impaired memory in a subject or warm blooded animal in need thereof, wherein the method comprises

supplementing the said subject or warm blooded animal with an effective dose of Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2.

18. A method of preventing, control or treating memory and cognition related disorders/diseases in a subject or warm blooded animal in need thereof, wherein the method comprises supplementing the said subject or warm blooded animal with an effective dose of Boswellia derived non-acidic extract/fraction or their composition(s) according to claims 1 and 2.

19. The method of preventing, control or treating memory and cognition related disorders/diseases as claimed in claim 18, wherein memory and cognition related disorders/diseases include but not limited to senile dementia, multi-infarct dementia, dyslexia, aphasia, organic brain syndrome, myasthenia gravis, vascular dementia, mild cognitive impairment (MCI), Attention-deficient Hyperactivity Disorder (ADHD), Lewy body dementia, Wemicke-Korsakoff-syndrome, Alzheimer's, Parkinson's disease, hypoxia, anoxia, cerebrovascular insufficiency, epilepsy, myoclonus and hypocholinergic dysfunctions, memory impairment disorders and neurodegenerative disorders.