ORIGINAL

ABSTRACT

PROCESS FOR PREPARATION OF GREEN 6-SHOGAOL FROM GINGER RHIZOME EXTRACT

The present invention provides a simple single pot conversion method of Gingerol to high purity green Shogaol (6-Shogaol)from the ginger rhizome SCF- CO2 extract, containing 40-45% Gingerol employing Multiple Separators of Very High Pressure Liquid Carbon dioxide by conducting basic or acidic dehydration, more preferably the conversion by 5-20% acid concentration at 80-90°C and separation of water from the crude shogaol conversion mixture by layer separation and further low pressure SCF- CO2 extraction to get crude shogaol from 20-25% & Fractionating the crude Shogaol loaded in extraction vessel into the three separators namely high pressure (H.P), medium pressure (M.P) and low pressure (L.P) separators in series by variable simulation of the pressure and temperature individually between 110 bar and 50°C, 50 bar and 40°C, 40 bar and 20°C respectively and finally High purity green Shogaol is collected from H.P Separator having purity content of 29.3-40%, lower content green Shogaol fraction is collected from M.P Separator and water and some oil are collected from L.P Separator. The green Shogaol and formulation are suitable for its application in pharmaceutical, cosmetic and food industry.

We Claim:

1. A process for preparation of 6- Shogaol from green ginger rhizome extract using single pot conversion method comprising the steps of-
   i. Conversion of Ginger rhizome extract to crude shogaol by conducting basic or acidic dehydration;
   ii. Separation of water from the crude shogaol conversion mixture by layer separation;
   iii. SCF-CO_2 fractionation of the conversion mixture at high pressure to achieve shogaol 29.3- 40% in the extract employing the green SCF- CO_2 fractionation without using any toxic chemicals.
2. The process for preparation of 6-Shogaol as claimed in claim 1 wherein the ginger rhizome extract having 40-45% gingerol is converted into shogaol by reaction with 5-20% aqueous acid and heating upto 70-90 °C under nitrogen current for 3-5 hour.
3. The process for preparation of 6-Shogaol as claimed in claim 2 wherein the acid is selected from hydrochloric, orthophosphoric, perchloric, acetic acid; most preferably hydrochloric acid.
4. The process for preparation of 6-Shogaol as claimed in claim 1 wherein the fractionization of crude shogaol is done by liquid-liquid SCF- CO_2 extraction process in an extraction vessel coupled with three separators in a series.
5. A process for preparation of 6-Shogaol as claimed in claim 4 wherein the crude shogaol is extracted from SCF- CO_2 extraction vessel maintained at a pressure ranging from 175-350 bar and temperature 50-65°C and the flow rate of liquid CO_2 pump is adjusted between 0.6-1.6 kg/min.
6. A process for preparation of 6-Shogaol as claimed in claim 5 wherein the three separator are high pressure separator (H.P.), medium pressure separator (M.P.) and low pressure separator (L.P.) and are maintained at

around optimum pressure bar and temperature 90-120 and 50-60°C, 50 Bar and 40 °C and 40-45 bar and 20 °C respectively.

7. A process for preparation of 6-Shogaol as claimed in claim 6 wherein the enriched green 6-Shogaol content upto 29.3-40% is collected from H.P. separator, low content shogaol is collected from M.P. separator and water from L.P. separator.

8. A process for preparation of 6-Shogaol as claimed in claim 6 and 7 wherein programmed logic control (PLC) system is used for maintaining the temperature and pressure of the extractor and separators.

9. A process for preparation of 6-Shogaol as claimed in claim 8 wherein Green Shogaol is heated at 40-45°C for 40-60 minutes in the presence of diluents like vegetables oil, Glyceryl mono-oleate, medium chain triglyceride and mixtures thereof are added to dilute the extract for making it 20-30% green Shogaol.

10. The process for preparation of 6-Shogaol as claimed in claim 9 wherein the excipients like aerosil, starch ester, microcrystalline cellulose, hydroxy propyl methyl cellulose, light magnesium carbonate, tribasic calcium phosphate, magnesium stearate and mixtures thereof are added in the green shogaol in a stainless steel rota-coating pan of hot water jacketed which is heated at temperature 40-50°C to prepare the powder.

Dated this 14th day of March 2012.

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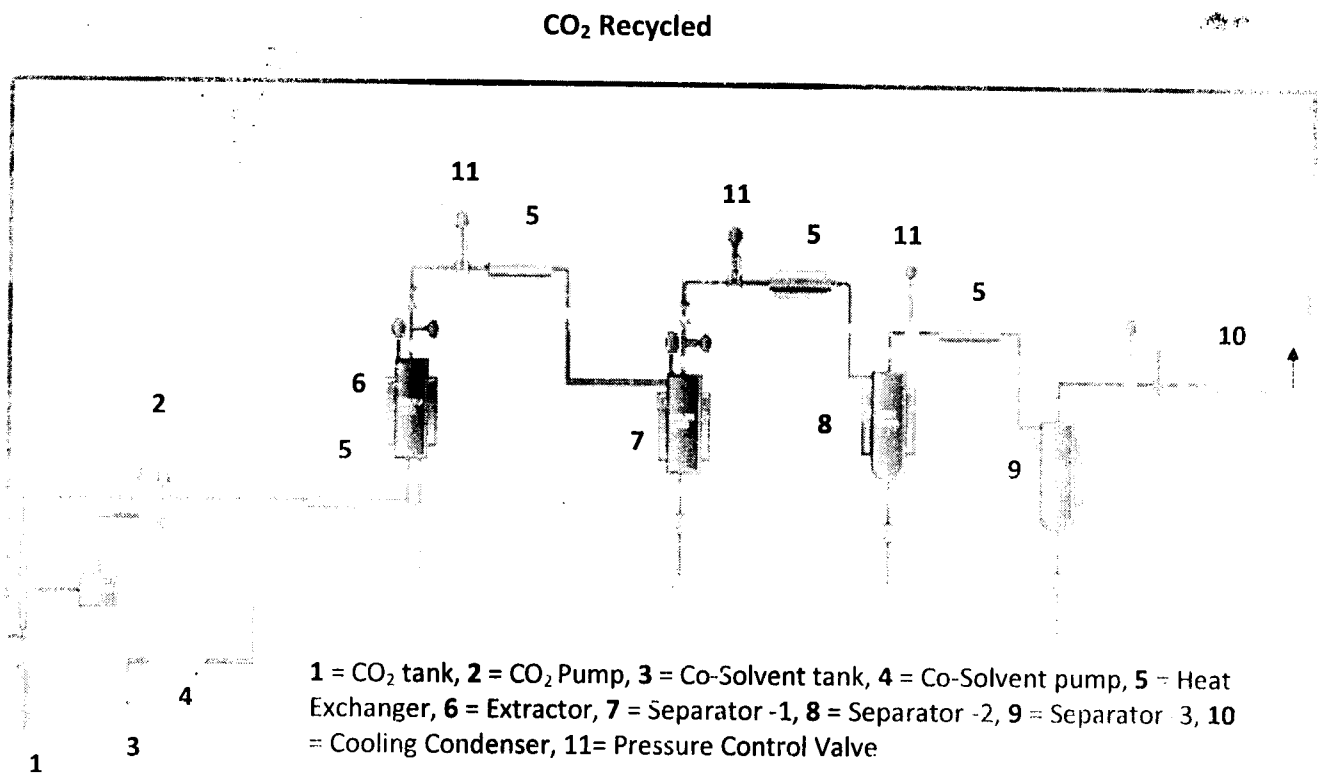

1 = CO_2 tank, **2** = CO_2 Pump, **3** = Co-Solvent tank, **4** = Co-Solvent pump, **5** = Heat Exchanger, **6** = Extractor, **7** = Separator -1, **8** = Separator -2, **9** = Separator 3, **10** = Cooling Condenser, 11= Pressure Control Valve

FIG 1: Schematic Diagram of SCF-CO_2 Extraction of Shizochytrium

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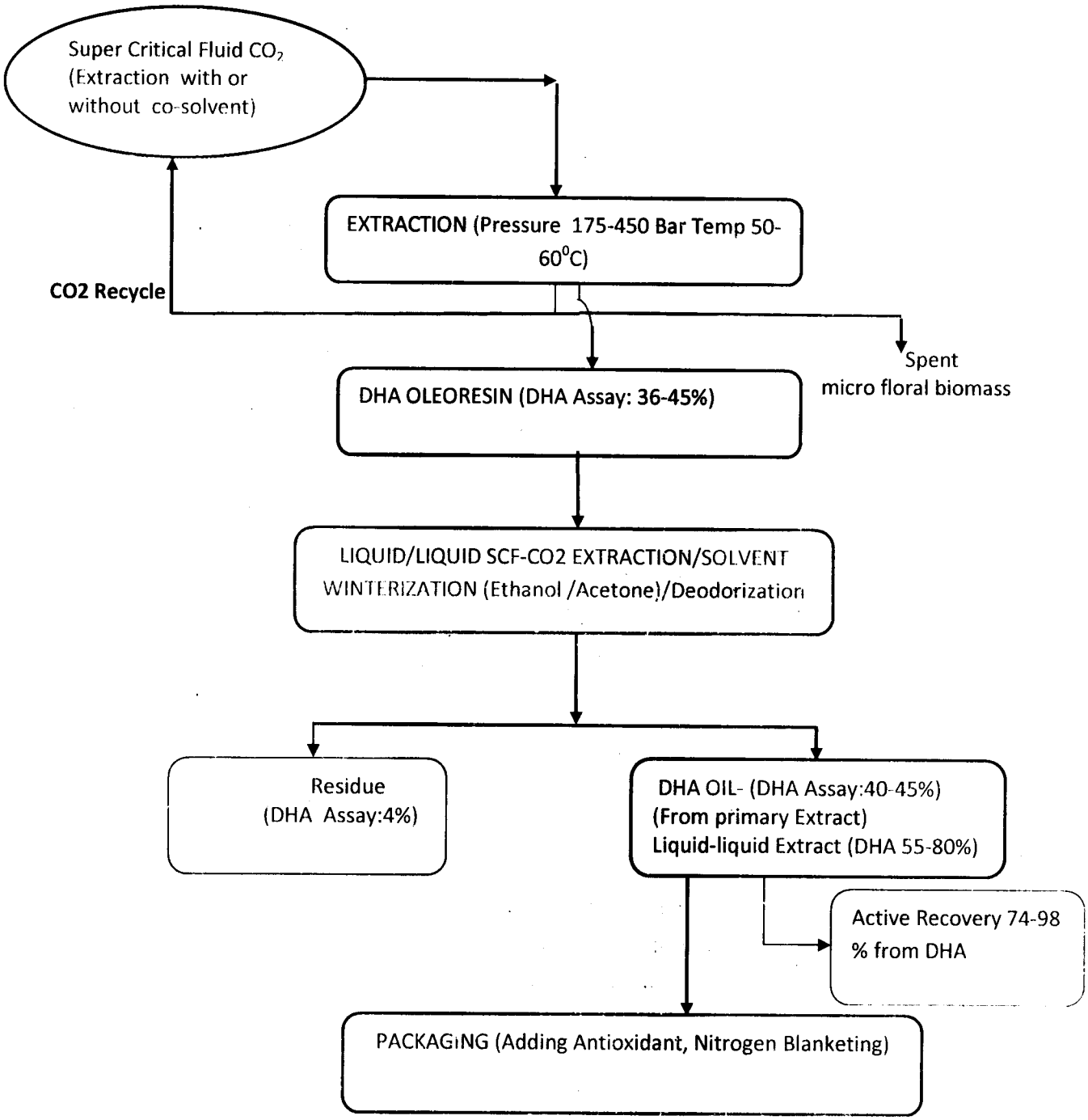

FIG-2: Flow diagram of DHA extraction and oil preparation

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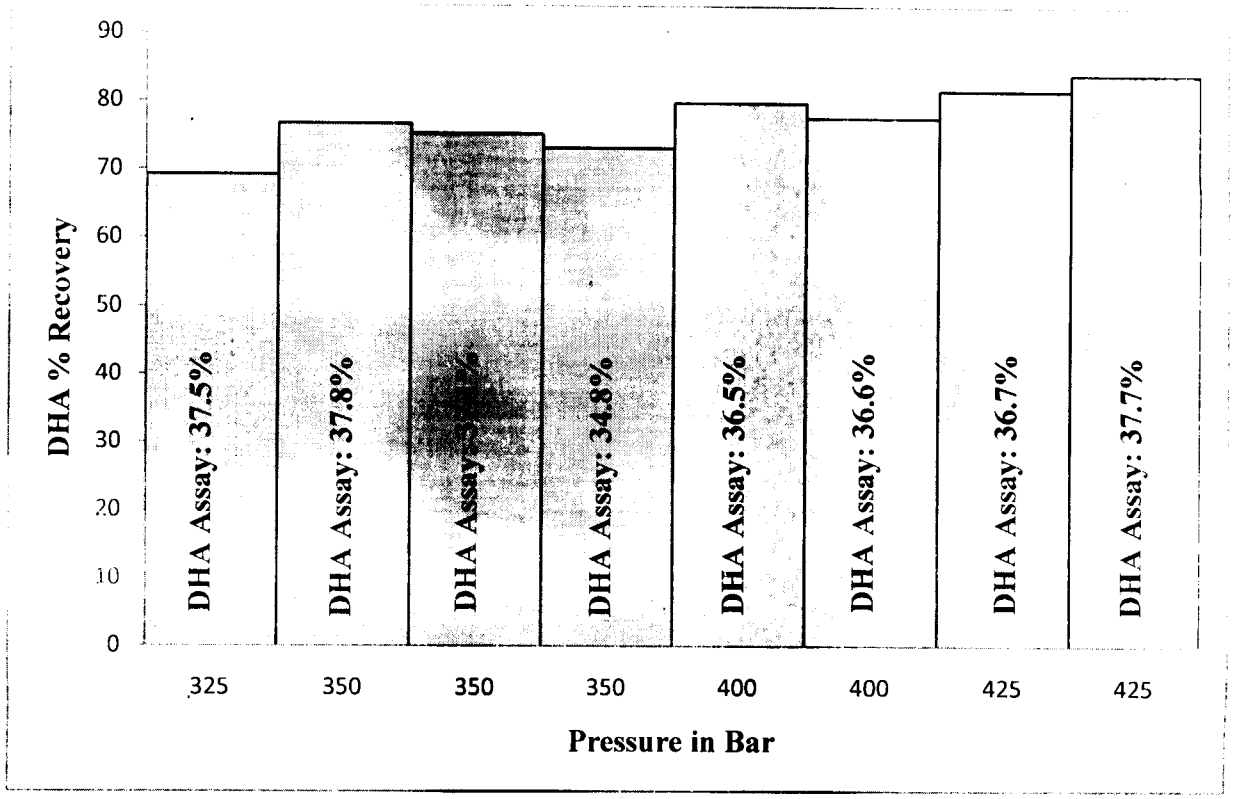

Fig.3: Bar diagram of recovery of DHA and its assay content from Schizochytrium biomass by single Stage Process of SCF-CO_2 System

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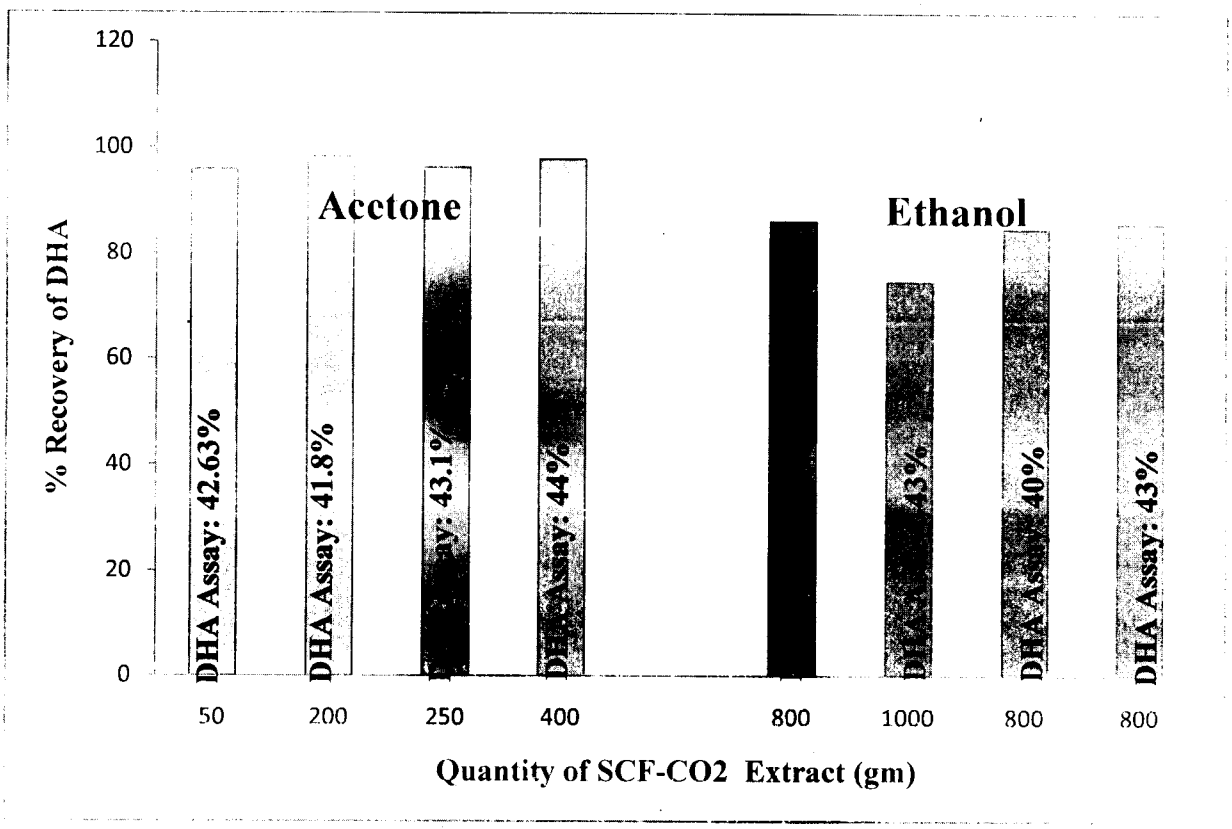

Fig. 4: Bar Diagram of recovery of DHA after winterization of SCF-CO2 Extract in Acetone and Ethanol

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PROCESS FOR PREPARATION OF GREEN 6-SHOGAOL FROM GINGER RHIZOME EXTRACT

Field of the Invention

The present invention relates to a process for preparing Green shogaol from ginger rhizome SCF-CO_2 extract employing a single step toxic-solvent free green process. In particular green shogaol is prepared using the ginger rhizome extract and carrying its reaction with an aqueous acid at 80-95°C for a duration of 3-5 hours and fractionation in 1×12L SCF- CO_2 extractor facilitated by multiple-three separator product collection system. The green shogaol so prepared also contains small quantities of 8- and 10-Shogaol and 6-gingerol. The fractionation of crude shogaol as carried out in SCF- CO_2 system yield enriched shogaol 29.3-40%.

Background of Invention

The Ginger extract isolated from ginger rhizome bears antioxidant, anti-inflammatory, and anti-tumor activities. However, it is unclear what the major active anti-tumor components in ginger are. The main principles in fresh ginger are the series of pungent oleoresin constituents known as Gingerol. Among the Gingerol, 6- Gingerol is the major constituents which have the health benefits. Whereas in dried ginger rhizome, Shogaol, the dehydration products of Gingerol during thermal processing, are predominant components. Their levels can be even higher than Gingerol in some preparations. Most researchers have considered Gingerol as the active principles and have paid little attention to Shogaol. Recent studies have shown that 6-Shogaol has greater growth inhibitory activity on lung and colon cancer cell lines than 6-Gingerol. The anti-cancerous properties has been studied in a project, 5R21CA138277-02 granted by office of Dietary supplement, National Institute of health, aimed to study the health potential of Shogaol. To study the bioavailability and biotransformation of 6-shogaol and 6-

gingerol in GEHS in comparison to that of the compound administered alone in mice. The inhibitory activities of GEHS and extract from fresh ginger, as well as 6-shogaol and 6-gingerol in a NNK-induced lung carcinogenesis model in A/J mice. NNK (4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone) is a tobacco carcinogen, and this model is highly relevant to human lung cancer formation. Owing to the wide scale health benefits of 6-shogaol, the preparation of same involving the green SCF- CO_2 extraction is investigated in the present invention.

In this direction, during the past decade many researchers have isolated, identified, and quantified Gingerol and Shogaol from ginger root extract. Qiao Q, Du Q,. 2011, has reported isolation of Gingerol and Shogaol using Flash high speed counter current chromatographic separation and isolated 6-Gingerol having 98.3% purity, 8-Gingerol having 97.8% purity and 6-Shogaol having 98.2% purity.

William J. Sanders, Jimmy L. Seidel 1992, also reported a new synthesis method of the pungent principle of ginger-zingerone and Shogaol.

DW Connell and MD Sutherland (1969) studied the pungent principles of ginger root extract, Gingerol, Shogaol and Gingerone. The active principles Shogaol and zingerone, described by Nomura et al.(2001) as ginger constituents appear to be absent but are formed by the action of alkalis or heat on Gingerol or the oleoresin of Ginger rhizome. The complex mixture of substances including a series of S-(+)-Gingerol (i.e. 1-(4- hydroxy-3-methoxyphenyl)-5-hydroxyalkan-3-ones) with 10,12, and 14 carbon atom side-chains, essential oil, palmitic and other fatty acids, and other unidentified substances.

The studies of Heba Abdel-Aziz et al. (2005) and many others indicated that the ginger extract and its constituents bear pharmacological activity. Among its active constituents, 6, 8 and 10-Gingerol as well as 6-Shogaol were shown in different in vivo studies to be at least partly responsible for the drug's anti-emetic properties. All compounds also show weak anti-cholinergic and anti-neurokininergic activities in the guinea-pig ileum (acetylcholine and substance P is mediators of the 5-HT_3 receptor effect). The vanilloid receptor did not seem to be derived from

experiments using capsazepine. These results indicate the strong potential of Gingerols and Shogaol in various applications.

Steven A. Fleming et al.1999 has studied the one step synthesis of gingerol which is a staring material for shogaol. They found that racemic 6-gingerol can be obtained in a one-pot reaction by hexanal addition to the dianion of zingerone at low temperature. Similarly, addition of octanal or decanal to the dianion provides 8-gingerol or 10-gingerol, respectively. The Gingerol on further catalytic reaction converted to Shogaol.

A study measuring the anti-inflammatory properties of red ginger by Shimoda H. et al. showed that it has very positive anti-inflammatory action. The 40% ethanolic extract from dried red ginger which contains Shogaol and Gingerol suppressed production of inflammatory mediators. The study concluded that red ginger extract has a potent suppressive effect on acute and chronic inflammation, and inhibition of macrophage activation seems to be involved in this anti-inflammatory effect.

The Gingerol and Shogaol prepared by these reactions are very stable. The stability of these was reported by Sushila Bhattarai, et al. (2001) who investigated the stability of 6-gingerol [5-hydroxy-1-(4-hydroxy-3-methoxyphenyl) decan-3-one] at temperatures ranging from 37 to 100°C in aqueous solutions, at pH 1, 4, and 7. Quantitative measurements of 6-gingerol and its major degradation product 6-Shogaol [1-(4-hydroxy-3-methoxyphenyl) decan-4-ene-3-one] were performed by HPLC. Kinetics of 6-Gingerol degradation was characterized by least square fitting of a rate equation. It was found that Gingerol exhibited novel reversible kinetics, in which it undergoes dehydration-hydration transformations with Shogaol, the major degradation product. Degradation rates were found to be pH dependent with greatest stability observed at pH 4. The reversible degradation of 6-gingerol at 100°C and pH 1 was relatively fast and reached equilibrium within 2 hrs. Activation energies for the forward and reverse reactions for 6-gingerol were

calculated from the Arrhenius equation using reaction rates obtained at temperatures ranging from 37 to 100°C

The pungent principles particularly Gingerol and shogaol have been studied in a solar dried rhizome of zinger officinalis by D.A. Balladin et al (1997). They extracted the solar dried rhizome in acetone and ethanol separately as extraction solvent.

Ginger shows promising anticancer properties. To support this not much research has been conducted to examine the pharmacokinetics of the ginger constituents 6-gingerol, 8-Gingerol, 10-Gingerol and 6-Shogaol in humans. Recently Zick, Suzanna M. 2008, Shengmin Sang et al.(2009) has conducted the clinical trial with 6-gingerol,8-Gingerol,10-Gingerol and 6-Shogaol, for examining the pharmacokinetics and tolerability. In this study, they have purified and identified eight major components, including three major Gingerol and corresponding Shogaol, from ginger extract and compared their anti carcinogenic and anti-inflammatory activities. The results showed that Shogaol 6-, 8, and 10- had much stronger growth inhibitory effects than 6, 8, and 10 Gingerol on H-1299 human lung cancer cells and HCT-116 human colon cancer cells, especially when comparing 6-Shogaol with 6-Gingerol (IC_{50} of ~8 versus ~150 μM). In addition, the 6-Shogaol had shown much stronger inhibitory effects on arachidonic acid release and nitric oxide (NO) synthesis than 6-Gingerol.

Isa Yasuka (2008) studied -6-Shogaol, 6-Gingerol and the pungent principle of ginger and found that these inhibit TNF-α mediated down regulation of adiponectin expression via different mechanisms in 3T3-L1 adipocytes. As the Adipocyte dysfunction plays an important role in the development of metabolic syndrome. Adipocyte synthesizes and secretes biologically active molecules called adipocytokines.

The 6-Shogaol and Dehydroshogaol has been studied by Wu, Hou; et al. 2010 and Imm, Jeeyoung (2010) and found that these strongly suppressed lipopolysaccharide-induced over expression of inducible nitric oxide synthase

(iNOS) and cyclooxygenase-2 (COX-2) in murine macrophages. In this study, we further compared curcumin, 6-Gingerol, and 6-Shogaol's molecular mechanism of action and their anti-tumor properties.

The Ginger extract containing Gingerol and Shogaol have been studied for their efficacy in treating colorectal cancer by Jeong CH et. al. 2009. The study carried out on specially bred mice which were given an injection of Gingerol three times a week before and after injecting human colorectal cancer cells into them. Tumors first appeared 15 days after mice were injected, but only 4 tumors were discovered in the Gingerol group, as opposed to 13 in the control mice. The results shows that Gingerol have anticancer efficacy for the prevention of colorectal cancer.

The patented process discussed above mainly focuses on the extraction of ginger rhizome to prepare the ginger extract. The extraction is mainly carried out using different solvents. The ginger extract so obtained mainly contained 6-Gingerol and small quantity of 8- and 10-Gingerol. The Shogaol is present in small quantity. A few patents describe preparation of Shogaol by using synthetic route. Besides, the application of Ginger root extract particularly Gingerol have been studied in the patent documents cited above. A few patents discuss the potential of Shogaol for controlling colorectal cancer and other diseases. The other limitation of the patents of prior art is that the Ginger extract contains only 2-3.5% Shogaol. The Flash chromatographic isolation though provides high purity gingerol and shogaol but suffer the disadvantage of involving cumbersome process and non-viability for commercial production. Owing to the health beneficial effect of the Shogaol a new process is needed to prepare Shogaol in higher concentration involving simple environmentally sustainable process. With this aim, the present investigation is aimed to overcome the limitation of all prior art and to prepare Shogaol from ginger root extract in maximum possible concentration involving green chemistry technique by the application of supercritical fluid extraction and use of low concentration of acid or alkali.

The necessity of taking present investigation and limitation of all prior art are discussed in the present invention and stress is given to prepare 6-shogaol which does not use toxic chemicals. The ginger extract as prepared in the prior art is extracted by different solvent. During the extraction with different solvents, the Gingerol are partially hydrolyzed. In addition, the solvent extracted ginger rhizomes contain higher residual solvent quantities which have adverse impact on the human health. In addition, the huge quantity of solvent required for extracting ginger extract from the rhizome will have vast burden on the petroleum resources which are depleting very fast. The process of present invention replaces the solvent with the supercritical carbon dioxide, which is a green extraction solvent and could be easily replenished. The maximum extraction of active constituents of ginger could be easily carried out by adjusting the pressure and temperature. Thus the use of supercritical carbon dioxide for the isolation of Shogaol prepared from Gingerol overcomes the disadvantages of prior art. The conversion of Gingerol to Shogaol is conducted in the CO_2 extractor or in an additional vessel which is then partitioned to obtain the extract having different Shogaol content. Thus the entire operation is possible in single equipment adding a small vessel and the total time is also reduced.

The current investigation also overcomes the limitation of prior patent 200910232593, which uses supercritical CO_2 extract for preparation of Shogaol by reacting the Ginger rhizome extract with acidic alcohol. This process also uses a number of solvent like ethyl acetate for downstream processing. In comparison the present investigation uses a vessel for conducting the conversion of 6-Gingerol to 6-Shogaol from which water is removed by layer separation. This crude Shogaol extract is again used for purification of green Shogaol using the SCF- CO_2 extraction equipment. The present investigation uses supercritical fluid CO_2 extraction having multi separator which have three separators named as high, medium and low separator. The extract having highest content of Shogaol is collected in separator-1 (high), the medium separator contain extract having lower content of Shogaol while the lower separator mainly collects the water. The

Shogaol content in the present investigation is enriched up to 40% as compared to the 20% Shogaol achieved by the patent description 200910232593.

Objective of the invention:

The objective of the present invention is to prepare green shogaol 20% and higher concentration from ginger rhizome SCF- CO_2 extract by the application of acid or base catalyst.

Another objective of the present invention is to prepare green shogaol in a single step from SCF- CO_2 extract of ginger rhizome in the SCF- CO_2 extraction unit itself by slightly amending and incorporating a vessel for the conversion process.

Further objective of the present invention is to prepare the shogaol entirely by green process therefore avoiding the toxic chemicals for the manufacturing of shogaol.

A further objective of the present invention is to enrich the shogaol to 29.3-40% purity by liquid-liquid fractionation at a pressure 175-350 bar.

Summary of the Invention:

The present invention discloses a process for preparation of 6- Shogaol and more particularly the method for preparation of 6- shogaol from green ginger rhizome extract using single pot conversion. The present method comprises of following main steps:

a) Conversion of Ginger rhizome extract by conducting basic or acidic dehydration, more preferably the conversion in 5-20% acid concentration at 80-95°C.

b) Separation of water from the crude shogaol conversion mixture by layer separation to get crude shogaol from 20-29%.

c) SCF-CO_2 fractionation of the conversion mixture at a pressure 175-350 bar to achieve shogaol 29.3- 40% in the extract employing the green SCF-CO_2 fractionation without using any toxic chemicals.

The present invention is the first ever art, and is a straight forward, one stage medium to very high pressure supercritical extraction process method to get unusually very high strength green Shogaol. Among the Shogaol, 6-Shogaol is the main constituent while 8- and 10-shogaol are present in minor quantity. The 8 and 10- shogaol which are present in minor quantities are not quantified in the present invention as the aim is to prepare and enrich 6-Shogaol from the Ginger rhizome SCF- CO_2 extract.

The Ginger rhizome extract used in the present invention is extracted by green SCF- CO_2 extraction process. The main ingredient of the ginger rhizome extract is Gingerol. The ginger rhizome extract having gingerol is treated with acid or base for conversion of gingerol to shogaol. The conversion of Ginger extract to Shogaol is itself carried out in the SCF- CO_2 extraction system by using one additional vessel for mixing of ginger extract with mild acidic water. In this first part crude shogaol is prepared which is further enriched by fractionization with liquid-liquid SCF- CO_2 system. The crude shogaol is kept in the SCF- CO_2 system extractor at pressure 175-350 bar and temperature 50-65°C. Super critical Carbon dioxide pump is stated and CO_2 gas is flown in the extractor. The extractor is coupled with three separator system which is well designed for present invention and is one of first ever key feature. The separators are named as High Pressure Separator (H.P.), Medium Pressure Separators (M.P.) and Low Pressure Separator (L.P.). The pressure and temperature of the separators is dynamically balanced and controlled by PLC and enriched 6-shogaol is collected from high pressure separator (H.P.). The balancing of the process parameters between product collection separators and enrichment of product green Ginger (Shogaol) is also a key feature of the present invention.

Thus the preparation of green Shogaol from the Gingerol in the present invention is a straight forward single stage method without adopting any two stage SCF-CO_2 process liquid-liquid extraction has lead to produce high purity Shogaol which could never be possible unless an SCF-CO_2 system used have multiple-three separator collection system than those of conventionally designed dual collection separators.

The 6-Shogoal of high strength up to 29.3-40% was obtained for the first time by using single stage SCF- CO_2 extraction. The entire process uses green solvent i.e. Supercritical carbon dioxide and water in addition to the use of food grade acid for the conversion. Shogaol so prepared by the process of present invention is formulated to 5-20% oleoresin, soft gel capsules, powder and granular formulation using vegetable oil, medium chain triglyceride antioxidant vitamin E and food grade excipients.

The high strength supercritical fluid extracted green Shogaol extract of 29.3-40% purity, toxic solvent free, having better purity are very well used in preparing different variant dosage forms by blending with diluents or excipients to get soft gel capsules, 5-30% as oil soluble extract, powders and granules as best alternative preparations to the synthetic route of Shogaol to be used for pharmaceutical, food, beverage and dietary supplement applications. Also obtaining the higher percentage extract by single stage supercritical extraction avoids the use of complex methods like the time consuming column separation and synthetic method which are using a huge quantity of solvent and the yield are low. In addition the present invention results in the green Shogaol devoid of residual solvent which are utmost requirement owing to the health implication of the residual solvent present in the different extract processed using solvent.

Brief Description of drawings:

Fig, 1 Supercritical fluid carbon dioxide extraction (SCF- CO_2) system with multi-separators

Fig. 2 Flow diagram of preparation of green shogaol from ginger rhizome extract

Fig3. Percentage recovery of 6-Shogaol under alkaline and acidic conditions

Fig4. SCF- Extraction of 6-Shogaol at different pressure conditions

Detailed Description of Invention:

The process for preparing green Shogaol from ginger rhizome SCF- CO_2 extract consist of conversion of the Gingerol to Shogaol by the addition of 5-20% aqueous acid and heating upto 60-90°C, preferably, 75-90 °C and more preferably 80-90 °C for 3-5 hours. The conversion is conducted in the SCF- CO_2 extractor itself by slightly amending it. A separate vessel for conversion of Ginger extract Gingerol to green Shogaol is used by amending the design or the conversion was conducted in a glass flask attached with the CO_2 extraction unit. The Green ginger extract is loaded in the extractor or the flask and 5-20% aqueous acid is added and the same is heated to 75-90 °C. The water is separated from the flask and the crude green Shogaol is loaded to SCF- CO_2 extractor and extracted at low pressure ranging from 175 to 350 bars. The process parameters are adjusted to get high content of green Shogaol in separator-1. Extraction pressure is increased step by step to get initially the lower content Shogaol which on further increasing the extraction pressure yield green Shogaol extract containing higher content of Shogaol. The fine tuning of pressure and temperature yields green Shogaol extract containing 29.3 to 40% high purity 6-Shogaol. The % purity of shogaol content could be easily further increased by step by step liquid-liquid extraction/ partitioning of crude shogaol extract. The process conducted may further increase the shogaol content from 40 to 50%. However, in the present investigation the Shogaol content 29.3 to 40% was aimed and the extraction /partitioning process is adopted to get this quality green shogaol extract. The process of present investigation yield high percentage of natural green shogaol as compared to the shogaol prepared by the process of all prior art by comparable methods. Though the preparation of shogaol by synthetic route is well known art and a number of studies have already been taken for

shogaol preparation by the synthesis. The preparation of green shogaol adopting the green route 40% shogaol content has never been established. The preparation of green ginger extracted from ginger rhizome yield 2.5-3.0% shogaol and further attempt to enrich the same by second stage liquid-liquid extraction does not give the desired results. This is due to the fact that the green ginger extract prepared by supercritical fluid - CO_2 extraction of Ginger rhizome contains 2-3% shogaol which on further enrichment increases the shogaol content to 2.5-3.0%. In the previous art very high percentage of shogaol has been separated by Flash high pressure separation which has its limitation for commercial application. The prevalent processes for preparing green ginger extract and green shogaol suffer from the limitation that the high content contains high residual solvent which have very adverse impact on the health of user. To overcome these shortcomings, efforts have been made in the past to prepare green shogaol by fractionation of Ginger rhizome extract but suffer from the disadvantage that the content of shogaol in the extract is very low. In view of the limitation of all patented methods and the chromatographic isolation of Shogaol an alternative processes for preparing green shogaol is needed. This has become more pertinent as the restriction has been put on the use of chemical solvent due to their health impact and the upcoming health consciousness. To achieve the desired aim, for preparing green shogaol from the green ginger extract the use of supercritical fluid extraction could be alternative process. As currently, the use of inert liquefied gases like CO_2, CH_2CH_2, CH_3CH_3, N_2O, N_2 or mixtures thereof in supercritical condition have gained popularity in applications like extraction of non-polar and moderately polar compounds. The polarity of supercritical fluids could be slightly modified by proper adjustment of pressure and temperature. Since liquid CO_2 and nitrogen are readily available as inexpensive source for extraction/isolation of various botanicals like green ginger extract from ginger rhizome and other plant extracts. Owing to these characteristic of Liquid supercritical CO_2, nitrogen and mixture thereof were preferred for conducting the present investigation after converting the green ginger extract to green shogaol crude with aqueous acid. The crude shogaol is then partitioned to prepare high

concentration of 6-shogaol using advanced technology of multiple-three separator supercritical fluid carbon dioxide (SCF-CO_2) system of very high extraction pressure. The process that is investigated describes a single pot preparation method for green shogaol using very high pressure SCF-CO_2 extraction facility up to 350 bar is never disclosed in any of referred prior arts. Further the process that is investigated describes a single pot preparation method for green shogaol, using very high pressure SCF-CO_2 extraction facility with new design of multiple-three separators is never disclosed in any of referred prior arts. This includes respectively high, medium and low pressure separators which are connected in series. The high pressure multi separator system is attached to the 1x12L extractor.

For carrying out the conversion of green ginger extract to green shogaol, 1-2 kg green ginger extract is taken in the modified vessel or in separate vessel and 5-20% aqueous acid is added. The acid used for this purpose is selected from hydrochloric, orthophosphoric, perchloric, acetic acid more preferably hydrochloric acid. The mixture is heated to 80-90 °C under nitrogen current for 3-5 hour duration. The progress of conversion is monitored by HPLC during the optimization studies. The conversion is conducted in the extraction vessel or in a modified vessel connected the extraction unit so that the product transfer from one to another vessel becomes easy. After the maximum conversion of 6-gingerol to 6-shogaol, the water is removed as it separates from the organic phase. The further conversion took 3-4 hours at the condition optimized and the analysis was conducted only after liquid-liquid extraction. The organic phase is given water wash to make it neutral.

The crude Shogaol so obtained is loaded in extraction vessel 1x12L and water is removed at very low pressure and collected in separator-3. After separation of water content the crude shogaol is extracted at a pressure ranging from 175-350 bars and temperature 50-65°C. The CO_2 pump flow rate is set through PLC and monitored physically through the flow meter. After starting the pump the CO_2 flow is adjusted between 0.6-1.6 kg/min. The flow is increased slowly-slowly. The

temperature of extractor is adjusted through heat exchangers. The pressure parameter of extractor and separators are regulated to maintain the set pressure conditions by high pressure auto control valves in concordance of temperatures regulation through heat exchangers. The pressure and temperature of separator-1 is maintained between 90-120 bar/50-55°C, separator-2 at 50-60 bar/40°C and separator-3 at 40± 5 /20 °C respectively. In principle the liquid-liquid extraction of crude shogaol prepared from ginger rhizome extract is carried by supercritical fluid carbon dioxide that is acting as dense fluid or solvent that takes away the green shogaol away from the crude material through diffusion and the green shogaol in dissolved condition in supercritical fluid is pushed to the H.P separator first. An investigation is conducted wherein the multiple separators are very much crucial in enhancing the Green shogaol content up to 29.3-40% as single stage process which is achieved by varying the pressures bar and temperatures °C of extractor 175-350 bar and 50-60°C and respective H.P, M.P and L.P. separators individually between 90-120 and 50, 40 and 40 and 22. The multi (three) separator SCF-CO_2 system allows to change the pressures and temperatures to a broad margin so that allowing for, simultaneously at single stretch, the removal and push of unwanted waxes, essential oil fraction and other impurities from the crude Shogaol. In a dual separator systems which are conventionally designed will not allow for broad variations in pressure and temperature combination selection between two separators for the removal of unwanted waxes, essential oil and unwanted impurities having low molecular weight. The current investigation uses very low quantity of acid and does not use any solvent for isolation of Shogaol from the conversion mixture. This is accomplished by the three separator SCF-CO_2 system, simultaneous extraction, refining and enhancement of Shogaol after preparation of crude Shogaol to H.P. Separator and the content of Shogaol in the Green Shogaol extract is 18-25%. The separator-3 L.P. separator collects the lighter oils and water. The process of present investigation thus avoids the second stage partitioning to enrich the Shogaol content and the high percentage green Shogaol is obtained in one step only. A pressure required in M. P. separator to collect and retain the unwanted waxes and oils that are removed from crude

extract of first separator to be at least between 40-70 bar at temperatures of 40-50^{0}C. Otherwise conditions that are commonly designed for final recovery and recycling of CO_2 in separator-2 of dual separator SCF-CO_2 system will carry the waxes and oils to the receiver and re-carry the material to the extractor vessel thus making the extraction process always incomplete. Thus second and third separators as in case of three separator system are must to enrich the extract without carry of any liquid-liquid extraction at one stretch with high 6-shogaol content in green Shogaol extract.

In the three separator system controlling the unwanted waxes, essential oil fraction in the green Shogaol become possible maintaining the process parameters dynamically between well designed separator systems. Wherein the three separator SCF-CO_2 system the pressure and temperature of extractor are kept 175-350 bar and 50-60°C for achieving 29.3-40% shogaol. The oleoresin containing high shogaol content is collected in H.P. separator. The lighter oils and waxes are carried to separator-3. The high strength supercritical fluid extracted green Shogaol extract of 29.3-40% purity, toxic solvent free, having better purity are very well used in preparing different variant dosage forms by blending with diluents or excipients to get soft gel capsules, 5-30% as oil soluble extract, powders and granules as best alternative preparations to the synthetic route of Shogaol to be used for pharmaceutical, food, beverage and dietary supplement applications. Also obtaining the higher percentage extract by single stage supercritical extraction avoids the use of complex methods like the time consuming column separation and synthetic method which are using a huge quantity of solvent and the yield are low. In addition the present invention results in the green Shogaol devoid of residual solvent which are utmost requirement owing to the health implication of the residual solvent present in the different extract processed using solvent.

Examples:

1. The multiple-three Separator supercritical CO_2 extraction system (Fig.1) with an additional vessel for conversion of ginger rhizome extract to crude Shogaol was used in the present invention process. 3-4 kg of ginger rhizome extract having total 40-45% gingerol and 30-36% 6-Gingerol was used for the conversion to green Shogaol. The extract 3-4kg, more preferably (1.5-2.0 kg) is charged in a vessel and 5-20% sodium hydroxide or potassium hydroxide is added. The reaction is carried for 3-5 hrs at 80-90 °C. The maximum Shogaol content prepared by base catalysis was 19% Shogaol. The higher concentration alkali above 10% was not investigated as the recovery was low in two experiment studied which were having alkali concentration more than 10%. To increase the assay of Shogaol in crude Shogaol, further conversion of the SCF- CO_2 extract of Ginger rhizome is carried out with 5-20% aqueous acid preferably hydrochloric acid, sulfuric acid or orthophosphoric acid; most preferably hydrochloric acid is used for the conversion of Ginger rhizome SCF- CO_2 extract by heating to 75-90°C under the current of nitrogen. This heating is continued for 3-5 hours and water is separated and removed as it forms separate layer. The crude mixture after removal of water is loaded to 1x12L extraction vessel. The results of both acid and base catalyzed reaction undertaken for conversion of SCF- CO_2 ginger rhizome extract to crude Shogaol are presented in Table1.

2. The crude Shogaol prepared by above method is loaded in the extraction vessel for carrying out the liquid-liquid extraction. In the experiment, very much broad changes are made in pressure bar and temperature (°C) of respective multiple H.P, M.P and L.P. separators between 90-120 and 60 °C 50-55 and 40 °C and 40-45 bar and 20 °C respectively. At the end the optimized extraction parameters are set on programmed logic control (PLC) system for extractors (bar/°C) 175-300/60°C, high pressure separator 110/55°C, medium pressure separator 50/40°C and low pressure separator

40-45/20°C and also carried the experiments at various conditions tabulated in Table-1 with process flow diagram Fig.2

The flow rate of CO_2 on the pumps is set for 0.6-1.6 kg/min. Start CO_2 pump with 0.6 kg/min and the flow rate is gradually increased up to 1.6 kg/min by observing that the pump is running in healthy condition. Collect water from low pressure separator every 15 minute. Collect green Shogaol extract from H.P separator and the green Shogaol containing lower content from M.P. separator and oil and water from L.P. separator.

Yield:

The extraction yield from crude Shogaol with respect to the Shogaol content of the conversion and the Gingerol content of extract as obtained in different separators is presented below. The yield presented here is for the experiment conducted for ginger extract containing 40-45% Gingerol and 2-3.5% Shogaol.

	Extraction Yield (From crude Shogaol)	**% Green Shogaol content**
1) High pressure separator	55-66%	Assay 29.3-40.0%
2) Medium pressure separator	14-20%	Assay 18.0-25.0%
3) Low pressure separator	water	ND

3. Green Shogaol (containing approximately 29.3-40% pure 6-Shogaol) is taken in a hot water/steam jacketed stainless steel vessel and heated at 40-45°C for 40-60 minutes.

The diluents like vegetables oil, Glyceryl mono-oleate, medium chain triglyceride and mixtures thereof are added to dilute the extract for making it 10-30% green Shogaol.

4. Green Shogaol (29.3-40%) is taken in stainless steel rota-coating pan of hot water jacketed and then heated at 40-50°C. The excipients like aerosil, starch ester, microcrystalline cellulose, hydroxy propyl methyl cellulose, light magnesium carbonate, tribasic calcium phosphate, magnesium stearate and mixtures thereof are added and the powder so prepared is sieved to form uniform size particles/granules

5. Green Shogaol (assay 29.3-40%) is used for preparing soft gel capsules by adding pharmaceutical grade excipients.

Table1: Conversion of Ginger Rhizome Extract (Gingerols) to Shogoal

Exp. No.	Ginger oleoresin (Gingerols)				Shogoals				Process parameters
	Quantity (g)	Gingerols Assay (%)	6-Gingerol Assay (%)	Active 6-Gingerol (g)	Quantity (g)	Assay (%)	Active 6-shogaol (g)	Active Recovery (%)	
001	750	45	32	240	550	10.60	58.0	24.2	5% NaOH , 750ml water, 80 °C, 3hr
002	750	45	35	87.5	220	13.40	29.4	33.6	10% NaOH, 2.5L water, 80-85 °C 4hr
003	1000	45	32	320	980	10.76	105	32.8	5%KOH, 6L water, 60°C, 3 hr
004	1000	45	32	320	890	19.15	170	53.1	10%KOH, 100ml water, 80°C, 3 hr
005	1000	45	32	320	900	20.90	188	58.8	10%KOH, 160ml water, 85°, 6 hr
006	1000	45	32	320	923	28.00	258.4	80.8	1L 10% HCl, 85-90°C, 4hr
007	2000	45	30	600	1980	24.60	487	81.6	1L, 15% HCl,85-90°C, 3hr
008	2000	45	30	600	1950	26.00	507	84.5	1L 20% HCl, 85-90°C, 3hr
009	2000	45	30	600	1920	27.00	513	86.4	1L 20% HCl, 85-90°C, 3hr
010	2000	45	32	640	1900	29.80	566	88.4	2L 20% HCl, 80-90 °C, 4hr

Table 2: Recovery of Green Shogaol (6-Shogaol) from conversion of Ginger rhizome extract

Exp. No.	RM Source	Crude Shogaol: Loaded Qty. of RM (g)	Crude Shogaol: Shogaol content in crude %	Crude Shogaol: Shogaol Content in RM (g)	Extraction Condition#				6-Shogaol Extract Quantity, g				6-Shogaol Assay			Green Shogaol Active, (g)	
					Extractor (bar/°C)	Seperator-1 (bar/°C)	Seperator-2 (bar/°C)	Seperator-3 (bar/°C)	Seperator-1	Seperator-2	Seperator-3	Seperator-1 + Seperator-2 (Extract g)	Seperator-1	Seperator-2	Seperator-3	Seperator-1	Seperator-2
001	EBGN0L07	1000	24.6	246	175/60	110/50	50/40	45/ 22	216	74	T	290	33.0	18.4	NA	71.3	13.6
					200/60	110/50	50/40	45/ 20	217	23	T	240	37.0		NA	80.3	4.9
					250/60	110/50	50/40	45/ 20	150	17	T	207	22.0		NA	33.0	3.1
					300/60	110/60	50/40	40/ 20	60	10	T	70	12.8		NA	7.7	0.9
		RECOVERY OF GREEN SHOGAOL=87.0%															
002		2000	25.0	500	175/60	110/50	50/40	45/ 22	350	100	T	450	35.0	17.0	NA	122.5	17.0
					200/60	110/50	50/40	45/ 20	500	60	T	540	39.0	17.8	NA	195.0	10.7
					250/60	110/50	50/40	45/ 20	300	40	T	340	26.0	17.0	NA	78.0	6.8
					300/60	110/60	50/40	40/20	150	10	T	160	10.0	9.0	NA	15.0	0.90
		RECOVERY OF GREEN SHOGAOL=89.2%															
003		4500	27.0	1215	175/60	110/50	50/40	45/ 22	820	244	T	1064	34.0	18.0	NA	278.8	43.9
					200/60	110/50	50/40	45/ 20	1094	86	T	1180	39.0	18.5	NA	426.7	15.9
					250/60	110/50	50/40	45/ 20	930	45	T	975	25.0	14.8	NA	232.5	5.3
					300/60	110/60	50/40	40/ 20	456	50	T	506	8.9	7.0	NA	40.6	3.5
		RECOVERY OF GREEN SHOGAOL=86.2%															

Pressure & temperature varies with ± 5% of set parameters on PLC (**Programmable logic controller**); RM= Raw Material crude shogaol, T= Traces, NA= Not applicable