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(54) Title: AN IMPROVED PROCESS FOR PREPARATION AND PURIFICATION OF BOSWELLIC ACIDS

(57) Abstract: The invention disclosed herein is an improved, cost effective, ecofriendly process for purification of Boswellic acid extracted from the gum resin exudates of *Boswellia serrata* using hydro alcoholic solvents, specifically ethanol, to produce standardized Boswellic acid fraction comprising 70-90% of total Boswellic acids.

"AN IMPROVED PROCESS FOR PREPARATION AND PURIFICATION OF BOSWELLIC ACIDS"

Technical field of the Invention:

The present invention relates to an improved, cost effective, ecofriendly process for preparation of standardized Boswellic acids from gum resin exudates of *Boswellia serrata* and purification thereof.

Background and Prior art of the Invention:

Boswellia gum, known in the vernacular language as Salai guggal is the dried exudate of plant *Boswellia serrata Roxb.* (Burseraceae) which grows abundantly in central hilly regions of India. It is also called as Dhup, Indian Frankincense or Indian Olibanum. The gum has long been used for the management of rheumatism, respiratory diseases and liver disorders in Ayurvedic system of medicine. Modern studies reveal that an extract of the gum exhibits anti-inflammatory, anti-arthritic, anti-ulcerogeinc, anti-tumor, anti-cancer and Immuno modulatory activities.

Boswellia gum resin is a mixture of various terpenoids, polysaccharides, and inorganic salts. The triterpenoid fraction of the gum contains certain active principles responsible for its pharmacological activities. These active principles include among others pentacyclic triterpenic acids which are commonly known as Boswellic acids.

Boswellia extracts are being marketed under various dosage forms for the management of inflammation and arthritis. These extracts contain Boswellic acids as the active principles which include beta-Boswellic acid, acetyl-beta-Boswellic acid, 11-keto-beta-Boswellic acid, acetyl- 11-keto-Boswellic acid, alpha-Boswellic acid and acetyl-alpha-Boswellic acid. The Boswellic acids content in commercially available Boswellia gum extract generally varies between 50%-90% by weight estimated as total organic acids. These extracts are being prepared using various organic solvents which have potential health risks due to its toxicity and hazardous properties.

US Patent No. 5629351 describes a purification process of extracting the gum using polar solvent such as alcohol, acetone, ethyl acetate or their mixtures and purifying using

dichloromethane, chloroform, hexane, benzene, petroleum ether or their mixtures thereof. Boswellic acids are further separated by column chromatography over silica gel or by preparative HPLC.

WIPO Publication No. 2002085921 describes a method for solvent extraction and purification of Boswellic acids by ion exchange resin using solvents selected from alcohols, aliphatic hydrocarbons, organic ethers, organic chlorine compounds, ester, ketones or organic acids.

WIPO Publication No. 2010105821 describes a process for extraction of plant resins like Boswellia gum using a solvent mixture containing glycerol, glycols, alcohol, polysorbate and water in presence of surfactant.

US Patent Publication No. 20060234990 describes a process for extracting the Boswellia resin using alcohol and purifying further using ester or ketones as solvents. US'990 also describe supercritical extraction of Boswellic acids using liquid carbon dioxide.

The column chromatographic process involves adsorption of an alcohol, ketone, ether, ester or hexane extract of Boswellia gum on silica gel and subsequent separation of Boswellic acids from other lower terpenes and non-polar impurities by eluting With suitable alcohol, ketone, ether, ester, hexane or chlorinated solvents or its mixtures.

Ion exchange process for the purification of Boswellic acid involves loading an alcohol, ketone, ether, ester or hexane extract of Boswellia gum on anionic form of an ion exchange resin, washing the resin by alcohol, ketone, ether, ester, hexanes or chlorinated solvents or its mixtures and then eluting the Boswellic acids using an organic acid preferably formic acid, acetic acid or trifluoro acetic acid.

However, the above said processes have a major disadvantage in the use of toxic and hazardous organic solvents for removal of gum to obtain a standardized Boswellic acid fraction. Prolonged and high concentration exposure of these solvents poses health risk to people and can cause occupational diseases. Further, organic solvents are non¬ biodegradable thus making significant environmental pollution. A complete recovery and

reuse of the solvent is practically impossible which leads to the generation of large volume of hazardous flammable waste.

A process for extraction of Boswellic acids by liquid carbon dioxide has been reported in the prior art. Carbon dioxide is used as a solvent to extract the lipophilic compounds. This process too has its disadvantages like poor separation of Boswellic acids from other terpenes, low levels of Boswellic acid concentration and high operational cost.

There is therefore a need in the art to provide a process for preparation of standardized fractions of Boswellic acids that ameliorates the disadvantages of the prior art processes, specifically it eliminates the use of harsh and unsafe solvents such as Hexanes, ethers, Ketones, esters, benzene, toluene and chlorinated hydrocarbons.

Summary of the Invention:

In accordance with the above, the main object of the present invention is to provide an improved, cost effective and ecofriendly process for preparation of standardized fractions of Boswellic acids using hydro alcohol, ethanol/water as solvent which obviates the use of any other organic solvent.

The other object of the invention is to provide standardized Boswellic acid fraction comprising 70%-90% total Boswellic acids by weight.

In an aspect, the present invention provides an improved, cost effective process for preparation of standardized fractions of Boswellic acid using a mixture of alcohol and water, to obtain the terpenoid fraction containing Boswellic acids, alkalizing and concentrating solvent fraction, precipitation and separation of the gum by the addition of water (DM) followed cooling, acidification of the mixture followed by filtration and repeated washings using water (DM) to obtain 70-90% of total Boswellic acids by weight.

Detailed Description of the Invention:

The invention will now be described in detail in connection with certain preferred and optional embodiments, so that various aspects thereof may be more fully understood and

appreciated. However, any skilled person will appreciate the extent to which such embodiments could be extrapolated in practice.

The present invention discloses an improved, cost effective, environment friendly process for preparation of standardized fractions of Boswellic acid from gum resin exudates of *Boswellia serrata*, using hydro alcoholic solvents.

In an embodiment, the present invention discloses an improved, cost effective process for the preparation of Boswellic acid fraction from gum resin exudates of *Boswellia serrata* and purification thereof comprising;

a) extracting *Boswellia serrata* gum resin multiple times using an alcohol-water mixture and filtering to remove insoluble compounds;
b) adjusting the pH of the extract of step (a) between 8.0-9.5 using an aqueous alkaline solution, followed by concentrating the extract to 20%-50% of its initial volume;
c) adding water to 40-60% of the concentrated volume of step (b) to precipitate lower terpenes and non-polar impurities/other gums;
d) cooling the mixture of step (c) to a temperature less than 10°C and allowing the gums to sediment over a period of 15 to 30 hours;
e) separating the solvent layer from the sediments of step (d) by filtration or decantation or siphoning;
f) concentrating the solvent layer to the volume in step (b) and adding water to 20%-30% of the concentrated volume to precipitate the remaining gums;
g) cooling the mixture of step (f) to a temperature less than 10°C and allowing the gums to sediment over a period of 15 to 30 hours;
h) separating the solvent layer from the sediments of step (g) by filtration or decantation or siphoning followed by evaporating the ethanol;
i) diluting the extract with water and precipitating the Boswellic acids with an inorganic acid at a pH between 1.0-4.0;
j) filtering the precipitate and washing with water till neutral pH followed by drying under vacuum to obtain standardized Boswellic acids fractions.

The standardized fractions of Boswellic acid contain 70-90%, preferably 75% to 85% of total Boswellic acids by weight estimated as total organic acids by titration method. The standardized Boswellic acids fraction comprises Boswellic acids such as beta-Boswellic acid, acetyl-beta-Boswellic acid, 11-keto-beta-Boswellic acid, acetyl-11-keto-Boswellic acid, alpha-Boswellic acid, acetyl-alpha-Boswellic acid.

The standardized Boswellic acids content obtained from the above process is quantified using known titrimetric methods, preferably by titrating against a standard 0.1 molar sodium methoxide solution using thymol blue indicator.

The concentration of alcohol-water mixture used in the extraction is between 70:30 to 95:15 by volume, more preferably 80:20 to 85:15 by volume.

The alcohol used in the process is selected from lower CI to C6 alcohols comprising ethanol methanol, isopropanol and the like; preferably ethanol.

The aqueous alkaline solution used in the process is selected from sodium hydroxide solution or potassium hydroxide solution at concentration between 20%-30% w/v.

The inorganic acid used in the process is selected from mineral acid such as hydrochloric acid, sulphuric acid, nitric acid or phosphoric acid. The inorganic acids used is at a concentration ranges from 10%-30% v/v.

According to the invention, in the first step Boswellia gum resin is extracted multiple times using ethanol-water (DM) mixture at a ratio between 70:30 to 95:15, more preferably 80:20 to 85:15 followed by filtering the extract through a suitable filter to remove insoluble materials to produce an extract consists mainly terpenoids and gums. The pH of the extract is adjusted between 8.0 to 9.5 more preferably between 8.40 to 8.60 using an aqueous alkaline solution and concentrating the extract to 20%-50% of its volume, more preferably to 30%-35% of its original volume using jacket steam at 60-85°C under vacuum. The so obtained extract is referred herein as the first concentrated volume.

Water (DM) is added to the first concentrated volume to an amount equivalent to 40%-60% of it to precipitate the gum containing lower terpenes and relatively non-polar impurities/other gums, cooling the mixture to a temperature in the range of 5-10°C using jacketed chilled water and maintaining the temperature and allowing the non-polar impurities/other gums to sediment over a period of 15-30 hours, preferably 20-24 hours.

The sediments are then separated from the solvent fraction by filtration, decantation or siphoning and further concentrating the solvent fraction to the first concentration volume using jacket steam at 60-85°C under vacuum.

The process steps comprising precipitating terpenes and other gums using water (DM), cooling the mixture and separating the sediments from the solvent fractions is carried out until the fractions are completely extracted in the solvent and the absence of non-polar impurities/other gums in the solvent fraction is ensured.

This is followed by evaporating ethanol completely from the solvent fraction and diluting the solvent fraction further with equivalent amount of water to obtain dilute solution. pH of the dilute solvent fraction is adjusted between 1.0-4.0, more preferably to 1.5-2.5 using an inorganic acid. The precipitate is filtered to collect the insoluble organic acid fraction containing Boswellic acids which are further washed with water (DM) till the pH of water reaches to neutral, preferably 6.0-7.0 and drying the precipitate under vacuum to obtain standardized fractions of Boswellic acid.

The Boswellic acids content in the dried product is further quantified using known titrimetric methods, preferably by titrating against a standard sodium methoxide solution using thymol blue indicator.

In an embodiment, the present invention provides a pharmaceutical composition comprising atleast one standardized fraction of Boswellic acid obtained by the instant process with atleast one pharmaceutically acceptable excipient and to the use of said pharmaceutical composition for the treatment and management of rheumatism, respiratory diseases, liver disorders, as anti-inflammatory, anti-arthritic, anti-ulcerogeinc, anti-tumor, anti-cancer and Immuno modulatory activities.

In yet another embodiment, the present invention provides a method for treating said disorders comprising administering the said pharmaceutical composition to the subject by the method known in the art.

In yet another embodiment, the process of the present invention eliminates the use of harsh and unsafe solvents such as Hexanes, ethers, Ketones, esters, benzene, toluene and chlorinated hydrocarbons

Further details of the process of the present invention will be apparent from the examples presented below. Examples presented are purely illustrative and are not limited to the particular embodiments illustrated herein but include the permutations, which are obvious as set forth in the description.

Example 1: Process for preparation of standardized fractions of Boswellic acids from gum resin exudates of *Boswellia serrata* and their purification.

To 550 kg of Boswellia gum resin charged in a suitable extractor was added 1600 litres of ethanol:water (DM) at a ratio 85:35 by volume. The ethanol was heated to reflux temperature using jacketed steam followed by extracting the gum at reflux condition for 3 hours and cooling the extract to room temperature. The extract was filtered through a micron filter to remove the insoluble particles followed by repeating the extraction twice using 1600 litres of ethanol: water (85:15). All three filtered extracts was combined and the pH was adjusted to 8.4-8.6 using 25% w/v sodium hydroxide solution. The combined extract was then concentrated in a suitable reactor to approximately 1600 litres by using jacket steam at 60°-85°C under vacuum.

800 litres of water (DM) was added to the concentrated extract to precipitate out the lower terpenes and other gums. This was followed by cooling the mixture to 5-8°C using jacketed chilled water and was maintained at the same temperature for 24 hours. The clear solvent layer was separated from sediments by siphoning and filtering. The solvent layer was concentrated to approximately 1600 litres in a suitable reactor using jacket steam at 60°- 85°C under vacuum. This was followed by addition of 400 litres of water

(DM) to the concentrated extract to precipitate the terpenes and other gums. The mixture was cooled to 5-8°C using jacketed chilled water and maintained at the same temperature for another 24 hours.

The clear solvent layer was separated from sediments by siphoning and subsequent filtration. The solvent layer was then evaporated in a suitable reactor using jacket steam at 60°-85°C under vacuum to remove the ethanol. The concentrated aqueous layer was diluted with water to get a volume of 2000 litres, followed by acidification with 10% hydrochloric acid to reach a pH upto 2.0. The precipitated Boswellic acids were filtered using a suitable filter which was followed by washing with water (DM) until pH was 6.0-6.5. The precipitate was dried in an oven at a temperature below 75°C under vacuum to obtain the standardized Boswellic acid fractions.

The present invention ensures that the process for preparation of standardized fractions of Boswellic acid and purification thereof using alcohol-water mixture is ecofriendly, cost effective and technically easy to implement without use of any additives and toxic solvents such as hexanes, ethers, ketones, esters and chlorinated hydrocarbons, which the prior art processes has failed to achieve. The standardized fractions of Boswellic acid are obtained in good yield and purity by the instant process with advantageous properties for pharmaceutical use.

We Claim,

1. An improved, cost effective process for preparation of standardized fractions of Boswellic acid from gum resin exudates of *Boswellia serrata* and purification thereof comprising;
    a) extracting *Boswellia serrata* gum resin multiple times using an alcohol-water (DM) mixture and filtering to remove insoluble compounds;
    b) adjusting the pH of the extract of step (a) between 8.0-9.5 using an aqueous alkaline solution, followed by concentrating the extract to 20%-50% of its initial volume;
    c) adding water (DM) to 40-60% of the concentrated volume of step (b) to precipitate lower terpenes and non-polar impurities/other gums;
    d) cooling the mixture of step (c) to a temperature less than 10°C and allowing the gums to sediment over a period of 15 to 30 hours;
    e) separating the solvent layer from the sediments of step (d) by filtration or decantation or siphoning;
    f) concentrating the solvent layer to the volume in step (b) and adding water to 20%-30% of the concentrated volume to precipitate the remaining gums;
    g) cooling the mixture of step (f) to a temperature less than 10°C and allowing the gums to sediment over a period of 15 to 30 hours;
    h) separating the solvent layer from the sediments of step (g) by filtration or decantation or siphoning followed by evaporating the ethanol;
    i) diluting the extract with water and precipitating the Boswellic acids with an inorganic acid at a pH between 1.0-4.0; and
    j) filtering the precipitate and washing with water (DM) till neutral pH followed by drying under vacuum to obtain standardized Boswellic acids fractions.

2. The process according to claim 1, wherein the concentration of alcohol-water mixture is 70:30 to 95:15 by volume.

3. The process according to claim 1, wherein the alcohol selected from lower CI to C6 alcohols comprising ethanol, methanol or isopropanol.

4. The process according to claim 1, wherein the alcohol used is Ethanol.

5. The process according to claim 1, wherein aqueous alkaline solution selected from sodium hydroxide solution or potassium hydroxide solution.

6. The process according to claim 1, wherein inorganic acids selected from hydrochloric acid, sulphuric acid, nitric acid or phosphoric acid.

7. The process for purification of Boswellic acid according to claim 1, wherein standardized Boswellic acid fractions comprising of 70-90% of total Boswellic acids.

8. The process according to claim 1, wherein standardized Boswellic acids fraction comprises boswelic acids such as beta-Boswellic acid, acetyl-beta-Boswellic acid, 11-keto-beta-Boswellic acid, acetyl-11-keto-Boswellic acid, alpha-Boswellic acid, acetyl-alpha-Boswellic acid.