Title of the Invention: Methods for the production of sub-critical water extracts of certain plants with healthcare applications

Abstract Title: Subcritical water extraction of Hawthorn Crataegus monogyna, Pueraria lobata and Centella asiatica

A method of preparing an extract from Hawthorn (Crataegus species, especially Crataegus monogyna), Pueraria lobata (also known as "Kudzu") and Centella asiatica using the technique of Sub-critical Water Extraction (SWE) preferably at 150-230°C and 15-100 bar pressure. Extracts with a composition comparable to those obtained by extraction with either aqueous alcohol or methanol, and hence expected to exhibit the established pharmacological activity of these extracts, are obtained without the use of a hazardous organic solvent. The preparations are suitable for oral and topical formulation.
FIGURE 2: TLC Profiles of Sub-critical Water Extracts of Hawthorn (Crataegus monogyna).
FIGURE 3: TLC Profiles of Sub-critical Water Extracts of *Pueraria lobata* (Kudzu) Root.
FIGURE 4: TLC Profiles of Sub-critical Water Extracts of *Centella asiatica*.
KEY TO FIGURES 2, 3 & 4.

Figure 2:

Samples L→R:
Rutin Std
50% aqueous ethanol extract of hawthorn
EXP180 SWE (150°C) extract of hawthorn
EXP181 SWE (190°C) extract of hawthorn
EXP182 SWE (230°C) extract of hawthorn
Methanol extract of hawthorn

Chromatographic Conditions:
Mobile Phase: 6:2:1 ethyl acetate/formic acid/water
Stationary Phase: Silica gel G F254
Visualisation: Dip in Anisaldehyde Reagent and heat until maximum colour develops.

Figure 3:

Samples L→R:
Rutin Std
50% aqueous ethanol extract of Pueraria lobata (Kudzu) root
EXP184 SWE (150°C) extract of Pueraria lobata (Kudzu) root
EXP185 SWE (190°C) extract of Pueraria lobata (Kudzu) root
EXP186 SWE (230°C) extract of Pueraria lobata (Kudzu) root
Methanol extract of Pueraria lobata (Kudzu) root

Chromatographic Conditions:
Mobile Phase: 6:2:1 ethyl acetate/formic acid/water
Stationary Phase: Silica gel G F254
Visualisation: Dip in Anisaldehyde Reagent and heat until maximum colour develops.

Figure 4:

Samples L→R:
Rutin Std
50% aqueous ethanol extract of Centella asiatica BRM
EXP192 SWE (150°C) extract of Centella asiatica BRM
EXP193 SWE (190°C) extract of Centella asiatica BRM
EXP194 SWE (230°C) extract of Centella asiatica BRM
Methanol extract of Centella asiatica BRM

Chromatographic Conditions:
Mobile Phase: 6:2:1 ethyl acetate/formic acid/water
Stationary Phase: Silica gel G F254
Visualisation: Dip in Anisaldehyde Reagent and heat until maximum colour develops.
Methods for the Production of Sub-critical Water Extracts of Certain Plants with Healthcare Applications.

FIELD OF THE INVENTION

The present invention relates to a method of producing an extract from certain medicinal plants without the use of an organic solvent. In particular the present invention relates to a novel method of producing extracts comparable in composition to those produced using a lower alcohol or water alcohol mixtures by using extraction with sub-critical water.

BACKGROUND TO THE INVENTION.

Well defined, standardised extracts of medicinal plants are still widely used in both pharmaceutical and also personal care applications including cosmetics. A common reason for this continued usage is that the active compounds found in the plant are complex structures which cannot be produced economically by a synthetic route. Further, plant extracts frequently contain more than one active compound with each contributing a distinct pharmacological action, and hence purification to give a single active compound is impracticable.

Conventional aqueous extraction of the botanical raw material, although cheap and simple, almost invariably results in a complex extract in which the target compound is diluted by a range of other unwanted phytochemicals derived from the plant, including proteins, sugars and other carbohydrates.

Extraction with a lower alcohol, typically ethanol or methanol, is traditionally a very popular means of obtaining a pharmacologically active preparation from a medicinal plant. The use of an alcohol has the advantage over an aqueous extraction that the highly polar compounds such as proteins, carbohydrates et cetera, which generally are inactive and serve only to add bulk and dilute the concentration of the active compounds, are not extracted.

However the obvious disadvantages of the use of an alcohol in extraction are that it is flammable, necessitating expensive precautions during processing, and also the recovery of the organic solvent for either disposal or for re-use after further purification is required. Methanol in addition is highly toxic, and residual levels must be rigorously limited in a pharmaceutical preparation. Indeed due to the high toxicity of methanol, it is frequently substituted by a mixture of the more polar water and the less polar ethanol to produce an extraction solvent of comparable polarity.

A more recent approach to the production of extracts from botanical raw material is high pressure superheated water at \( >150^\circ\text{C} \), termed "sub-critical water". Sub-critical water has the unique property that the polarity of the solvent decreases in a predictable manner as temperature is increased; thus at \( 150^\circ\text{C} \) the polarity of sub-critical water is equivalent to that of a 50:50 mixture of ethanol and water, and the polarity further decreases as the temperature is increased to \( 230^\circ\text{C} \) to a value equivalent to that of pure methanol. A further advantage of sub-critical water extraction is that the high temperature and pressure produce high diffusion rates which promote very efficient extraction of the raw material.

The application of SWE is not universally successful. The most important limitations of the technique relate to the limited range of compounds which can be solubilised, typified by the relatively polar flavonoids and other polyphenolic compounds, and the potential for degradation of thermally labile compounds even in the absence of oxygen due to the necessarily elevated temperature. However when applied to appropriate botanical raw material it provides an excellent alternative to conventional solvent extraction. This has recently been exemplified in a range of patent application filed by Advanced Extraction Technology Ltd.

The traditionally used medicinal plants for which extraction with sub-critical water is described in this invention are all reported in the scientific literature to produce a methanol or aqueous alcohol extract exhibiting important pharmacological activities.
The aqueous alcohol or methanol extracts of the aerial parts of hawthorn (Crataegus species, in particular Crataegus mongyna) contain a complex mixture of proanthocyanidins and flavonoids as the most important active components [ROHR et al, 1999]. Traditionally such extracts have formed the basis of medicines widely prescribed in continental Europe for the treatment of decreased cardiac performance [CHANG et al, 2002] and this usage has been substantiated by convincing results from well conducted modern clinical trials.

Proanthocyanidins as a class are known to exhibit anti-inflammatory activity [LI et al, 2002] and this combined with the well established anti-inflammatory activity of many flavonoids [MIDDLETON et al, 2000] largely explains the observed anti-inflammatory action of hawthorn extracts in both in-vitro and in-vivo models [KAO et al, 2005; CAI et al, 2006]. In addition proanthocyanidins generally are known to possess a wide range of useful anti-cancer activities, in particular against human colo-rectal cancer cell lines [KIM et al, 2005], and these have recently been reviewed [NANDAKUMAR et al, 2008] with the effect of raising awareness amongst the scientific community of their potential as prospective new therapies.

Related to this is the finding that hawthorn extracts are found to exert a protective effect against cellular damage resulting from exposure to ionising radiation [HOSSEINMEHR et al, 2007], which was found to correlate with polyphenol content and hence presumably the well established anti-oxidant activity of such extracts [BERNATONIENE et al, 2008].

The root of Pueraria lobata (termed “Kudzu” in traditional Asian herbal medicine) is a rich source of isoflavones such as those found in both red clover and soya. These isoflavones are quite potent phytoestrogens [OSEN et al, 2008] and consequently extracts of these plant have been used as nutritional supplements, particularly in North America [RADER et al, 2007]. However the isoflavones found in extracts of Pueraria also exhibit a range of other important pharmacological properties. Isoflavones, including several found in Pueraria have been found to inhibit proliferation and promote apoptosis of human gastro-intestinal cancer cell lines in-vitro [YANAGAHIRA et al, 1993]. This was further confirmed when the major flavonoid, puercarin, was later found to induce apoptosis of human colon cancer cells [YU & LI, 2006]. The isoflavones found in Pueraria extracts are known to exhibit an anti-inflammatory action [LEE et al, 1994] and which has subsequently been found to be related to suppression of both arachidonic acid metabolism and release of the nitric oxide pro-inflammatory mediator [JUN et al, 2005]. This will also further contribute to the anti-cancer activities of such extracts as inflammation processes are known to be important in the progression of cancer.

The aqueous alcohol or methanol extracts of the aerial parts of Centella asiatica (known as “Gotu kola” in traditional Asian herbal medicine) is traditionally used to treat minor skin conditions, with the main active compounds present in such extracts being considered the pentacyclic triterpenes, especially the glycosides asiaticoside and madecassoside [INAMADAR et al, 1996; ZHENG & QIN, 2007]. Recent pharmacological research has substantiated this usage by demonstrating both anti-inflammatory activity [WON et al, 2009; YUN et al, 2008] and the promotion of wound healing [SHUKLA et al, 1999; SUGUNA et al 1996].

In addition Centella extract has been demonstrated to suppress the excessive proliferation of keratinocytes implicated in the development of psoriasis, and this activity was found to be largely associated with the triterpenes [SAMPSON et al, 2001].

The methanol extract of Centella was found to exhibit significant anti-proliferative effects in-vitro against a range of human cancer cell types [YOSHIDA et al, 2005] and several of the individual purified triterpenes have been found to inhibit proliferation and induce apoptosis of cancer cells [TANG et al, 2009; PARK et al, 2005; BUNPO et al, 2005].

Extracts of Centella are also widely used in cosmetic applications, with the observation that collagen synthesis is stimulated and hyaluronic acid production increased [MAQUART et al, 1999] tending to give support to this usage.
Thus as the subcritical water extracts of the herbs as described in this invention are demonstrated to exhibit a composition essentially similar to that of the corresponding methanolic or aqueous alcohol extract, and in most cases also to contain the actual known active compounds, then it can be expected that these extracts will exhibit comparable pharmacological activities to these and to the compounds contained therein.

A problem limiting the use of flavonoids and related polyphenolic compounds in therapeutic situations in which systemic treatment rather than local application is required is poor water solubility. This is largely responsible for the low circulating plasma levels after a single oral dose and results in the majority of the dose being excreted without absorption. Hence this invention also describes self emulsifying formulations which increase the water solubility of these compounds, thus increasing their oral bioavailability and therapeutic efficacy.

A further enteric release formulation is described which permits the generation of active compounds locally in the intestine and which show promise as an adjunctive treatment in multi-drug resistant colon cancer.

Topical formulations of the extracts are also described which permit their use for the relief of the symptoms of inflammatory skin diseases. Such topical formulations also exhibit promise as adjunctive treatments for skin cancer, combined with conventional chemotherapy and surgery, as a consequence of the anti-cancer actions of the polyphenolic compounds which they contain. This is particularly true of the topical formulation of the Centella extract in which the additional wound healing properties would be beneficial after excision of the cancerous tissue.

LITERATURE CITED:

J.Bernatoniene et al, Medicina (Kaunas), 44 (9), 706-12 (2008).


SUMMARY OF THE INVENTION.

The present invention was made in view of the prior art described above, and the object of the present invention is to provide a means of efficiently obtaining extracts with useful pharmacological properties from certain medicinal plants without the use of an organic solvent. To solve this problem the present invention provides a method for the extraction using sub-critical water of selected medicinal plants to produce extracts of comparable composition to those obtained with methanol or aqueous alcohol mixtures. Further, examples of both oral and topical formulations to improve the bioavailability and hence efficacy of the therapeutic components of the extracts are included in the invention.

The scope of the invention is illustrated by the following series of examples, although not in any limitative sense.

Example 1:

The extraction apparatus consists of:

1. Two suitable stainless steel vessels capable of resisting high temperature and pressure connected by stainless steel tubing, the first to act as a reservoir in which sub-critical water is produced prior to introduction to the second (extraction) vessel. These are contained in a thermostatted oven.
2. The first vessel is connected via an inlet valve to a high pressure pump outside the thermostatted oven.
3. The extraction vessel is connected via an outlet valve to a stainless steel receiver vessel outside the thermostatted oven, but which is maintained at approximately 90°C.
4. A valve from the receiver vessel allows the solution that accumulates to be transferred to a suitable storage vessel.

A schematic representation of a suitable arrangement of the extraction apparatus is provided as Figure 1.

Coarsely ground hawthorn (Crataegus monogyna or a related species) botanical raw material is packed into the stainless steel extraction vessel. The system is then filled with deionised water and the temperature and pressure of the extraction vessel raised gradually to a fixed temperature in the range 150-230°C, most preferably 150-200°C, and a pressure sufficient to maintain the water in the liquid phase (85 bar) respectively. The extraction system is then held at these conditions for up to 15 minutes before the resulting solution is forced from the extraction vessel into the receiver by passing a quantity of sub-critical water into the system to continue the extraction. Typically a mass of sub-critical water equivalent to twenty times the mass of botanical raw material is passed through this over the course of up to two hours. Alternatively the same extraction may be achieved in a dynamic mode by passing the same quantity of sub-critical water continuously through the botanical raw material over the course of up to two hours.

The extract is isolated from the solution resulting from the extraction by removing the water by evaporation, preferably carried out under reduced pressure to reduce the temperature required. Alternatively the extract may be isolated from the solution by removing the water by the process of either freeze drying or spray drying.

A TLC fingerprint representative of the extract (also termed a botanical drug substance) produced in this example is given in Figure 2.
Example 2.

A method as described in example 1 wherein the botanical raw material is ground *Pueraria lobata* root. A TLC fingerprint representative of the extract produced in this example is given in Figure 3.

Example 3.

A method as described in example 1 wherein the botanical raw material is ground *Centella asiatica*. A TLC fingerprint representative of the extract produced in this example is given in Figure 4.

Example 4.

A Self Emulsifying Drug Delivery System (SEDDS) formulation for oral administration of the extracts containing the poorly water soluble active compounds can be prepared as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (selected from the extracts produced in Examples 1-3)</td>
<td>15% w/w</td>
</tr>
<tr>
<td>Lauroyl macrogolyglycerides EP (e.g. Gelucire 44/14)</td>
<td>65-85% w/w</td>
</tr>
<tr>
<td>Surfactant (e.g. Cremophor RH40 or Labrafac are suitable)</td>
<td>0-20% w/w</td>
</tr>
</tbody>
</table>

The extract is dispersed with stirring in the molten laurol macrogolglycerides at 70-80°C. The surfactant is then added and stirring continued for a further 5 minutes. Using suitable automatic or manual equipment the molten mixture is then dispensed into two piece hard shell gel capsules which are then sealed.

Example 5.

A cream formulation for topical application of the extracts can be prepared as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (selected from the extracts produced in Examples 1-3)</td>
<td>2% w/w</td>
</tr>
<tr>
<td>Cetostearyl alcohol EP</td>
<td>7% w/w</td>
</tr>
<tr>
<td>Macrogol cetoatearyl ether (e.g. Cremophor A6 or A25)</td>
<td>3% w/w</td>
</tr>
<tr>
<td>Liquid paraffin EP</td>
<td>12% w/w</td>
</tr>
<tr>
<td>Parabens (e.g. Nipastat)</td>
<td>0.2% w/w</td>
</tr>
<tr>
<td>Deionised water</td>
<td>67.8% w/w</td>
</tr>
<tr>
<td>Propylene glycol EP</td>
<td>8% w/w</td>
</tr>
</tbody>
</table>

The extract is dispersed in the propylene glycol at 70-80°C with stirring. All other ingredients except the water are mixed at 80°C and then added with stirring to the water that was heated separately to 80°C. The dispersion of extract in propylene glycol is then added to this mixture maintained at 70-80°C with stirring. The formulation is then filled into tubes.

Example 6.

A hydroalcoholic gel formulation for topical application of the extracts can be prepared as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract (selected from the extracts produced in Examples 1-3)</td>
<td>2% w/w</td>
</tr>
<tr>
<td>Ethanol EP</td>
<td>44% w/w</td>
</tr>
<tr>
<td>Carbomer (e.g. carbopol 980 NF)</td>
<td>3% w/w</td>
</tr>
<tr>
<td>De-ionised water</td>
<td>51% w/w</td>
</tr>
<tr>
<td>Sodium hydroxide (aq)</td>
<td>qs to neutralise</td>
</tr>
</tbody>
</table>
The extract is dispersed in the ethanol at 50-60°C with stirring. The carboxomer is then added slowly to the water with rapid stirring. The extract dispersion is then added to the aqueous carboxomer whilst stirring. The resulting mixture is neutralised by slowly adding aqueous sodium hydroxide to produce a smooth semi-solid. The formulation is then filled into tubes.

Example 7.

An enteric coated capsule suitable for the oral administration to give a high local concentration in the colon of the botanical drug substance can be prepared as follows:

Extract (selected from the extracts produced in Examples 1-3) 20% w/w
StarCap 1500™ (Colorcon) 80% w/w

The extract and excipient are thoroughly mixed and then using suitable automatic or manual equipment is dispensed into two piece hard shell gel capsules that are then sealed. For a size 0 capsule a target fill weight of 400mg gives a dose of 80mg of botanical drug substance. Smaller capsules may be used to produce a lower unit dose. The capsules are then enteric coated by applying an approximately 8% w/w solution of a methacrylic acid based pharmaceutical coating, Opadry Enteric 95 (Colorcon) is suitable, in an organic solvent (typically 60% propan-2-ol + 40% dichloromethane). Standard techniques (e.g. spray coating combined with coating pan) are used to give an approximate weight gain of 10% w/w for the capsules.

If desired the proprietary StarCap 1500™ excipient may be replaced by a suitable mixture of maize starch, pre-gelatinised starch and other excipients as will be recognised by those skilled in the art.

While the preferred embodiments of the invention have been described above, it will be recognised and understood by those skilled in the art that various modifications may be made therein, and the appended claims are intended to cover all such modifications which may fall within the spirit and scope of the invention.
CLAIMS

1. A method of preparing an extract containing pharmacologically active constituents from certain traditionally used medicinal plants, comprising reduction of the particle size of the raw material as appropriate, extracting the botanical raw material with sub-critical water, and then removing the water from the resulting solution to produce a pharmaceutically acceptable extract.

2. The method according to claims 1-4 wherein the extraction is carried out at a temperature in the range 150-230°C, and most preferably in the range in the range 150-200°C.

3. The method according to claim 1-4 wherein the extraction is carried out at a pressure in the range 15-100 bar sufficient to maintain the water in the liquid phase, and most preferably at 70-85 bar.

4. The method according to claims 1-4 wherein the medicinal plant is selected from the group consisting of the traditionally used parts of hawthorn (*Crataegus monogyna*), *Pueraria lobata* and *Centella asiatica*, and also including species closely related to any of the foregoing.

5. The method according to claims 1-4 wherein the water is removed to yield the extract by evaporation.

6. The method according to claims 1-4 wherein the water is removed to yield the extract by freeze drying or spray drying.

7. The method according to any preceding claim wherein the sub-critical water extraction of hawthorn (*Crataegus monogyna* or a closely related species) botanical raw material or a closely related species produces an extract with a TLC fingerprint substantially as illustrated in Figure 2, which is essentially similar to the corresponding 50% aqueous ethanol extract.

8. The method according to any preceding claim wherein the sub-critical water extraction of *Pueraria lobata* (Kudzu) or a closely related species produces an extract with a TLC fingerprint substantially as illustrated in Figure 3, which is essentially similar to the corresponding 50% aqueous ethanol extract.

9. The method according to any preceding claim wherein the sub-critical water extraction of *Centella asiatica* or a closely related species produces an extract with a TLC fingerprint substantially as illustrated in Figure 4, which is essentially similar to the corresponding 50% aqueous ethanol extract.

10. The use of a botanical drug substance as claimed in any of the preceding claims that consist essentially of botanical drug substances.

11. The use of a botanical drug as claimed in claim 10 further comprising excipients.

12. The use of a botanical drug as claimed in claim 10 wherein the botanical drug substances comprise total extracts derived from the botanical raw materials.

13. The use of a botanical drug as claimed in claim 10 wherein the botanical drug substances comprise more refined fractions derived from the total extracts of the botanical raw materials.

14. The use of a botanical drug as claimed in claim 10 wherein the botanical drug substances are standardised extracts.
15. The method according to any preceding claim wherein the pharmacologically active extract is formulated in a self emulsifying drug delivery system to improve the oral bioavailability of the active constituents.

16. The method according to claim 15 wherein the self emulsifying drug delivery system is based on the formulation described in Example 4.

17. The method according to claims 15-16 wherein the pharmacologically active extract as described in any preceding claim is selected from the list hawthorn (Crataegus monogyna), Pueraria lobata and Centella asiatica and also species closely related to any of the foregoing.

18. The method according to any preceding claim wherein the pharmacologically active extract is formulated in a topical vehicle formulated to increase efficacy of the active constituents.

19. The method according to claim 23 wherein the topical vehicle is based on the formulation described in Examples 5 & 6.

20. The method according to claim 18-19 wherein the pharmacologically active extract as described in any preceding claim is selected from the list hawthorn (Crataegus monogyna), Pueraria lobata and Centella asiatica and also species closely related to any of the foregoing.

21. The method according to any preceding claim wherein the pharmacologically active extract is formulated into an enteric release capsule to produce a high local concentration of the active constituents in the colon.

22. The method according to claim 21 wherein the enteric release capsule is based on the formulation described in Example 7.

23. The method according to claim 22-23 wherein the pharmacologically active extract as described in any preceding claim is selected from the list hawthorn (Crataegus monogyna), Pueraria lobata and Centella asiatica and also species closely related to any of the foregoing.
Amendments to the claims have been filed as follows

CLAIMS

1. A method of preparing an extract containing pharmacologically active constituents from certain traditionally used medicinal plants, comprising reduction of the particle size of the raw material as appropriate, extracting the botanical raw material with sub-critical water, and then removing the water from the resulting solution to produce a pharmaceutically acceptable extract.

2. The method according to claims 1-4 wherein the extraction is carried out at a temperature in the range 150-230°C, and most preferably in the range in the range 150-200°C.

3. The method according to claim 1-4 wherein the extraction is carried out at a pressure in the range 15-100 bar sufficient to maintain the water in the liquid phase, and most preferably at 70-85 bar.

4. The method according to claims 1-4 wherein the medicinal plant is selected from the group consisting of the traditionally used parts of hawthorn (*Crataegus monogyna*) or *Pueraria lobata*, and also including species closely related to any of the foregoing.

5. The method according to claims 1-4 wherein the water is removed to yield the extract by evaporation.

6. The method according to claims 1-4 wherein the water is removed to yield the extract by freeze drying or spray drying.

7. The method according to any preceding claim wherein the sub-critical water extraction of hawthorn (*Crataegus monogyna* or a closely related species) botanical raw material or a closely related species produces an extract with a TLC fingerprint substantially as illustrated in Figure 2, which is essentially similar to the corresponding 50% aqueous ethanol extract.

8. The method according to any preceding claim wherein the sub-critical water extraction of *Pueraria lobata* (Kudzu) or a closely related species produces an extract with a TLC fingerprint substantially as illustrated in Figure 3, which is essentially similar to the corresponding 50% aqueous ethanol extract.

9. The use of a botanical drug substance as claimed in any of the preceding claims that consist essentially of botanical drug substances.

10. The use of a botanical drug as claimed in claim 9 further comprising excipients.

11. The use of a botanical drug as claimed in claim 9 wherein the botanical drug substances comprise total extracts derived from the botanical raw materials.

12. The use of a botanical drug as claimed in claim 9 wherein the botanical drug substances comprise more refined fractions derived from the total extracts of the botanical raw materials.

13. The use of a botanical drug as claimed in claim 9 wherein the botanical drug substances are standardised extracts.

14. The method according to any preceding claim wherein the pharmacologically active extract is formulated in a self-emulsifying drug delivery system to improve the oral bioavailability of the active constituents.

15. The method according to claim 14 wherein the self-emulsifying drug delivery system is based on the formulation described in Example 3.
16. The method according to claims 14-15 wherein the pharmacologically active extract as described in any preceding claim is selected from the list hawthorn (*Crataegus monogyna*) or *Pueraria lobata* and also species closely related to any of the foregoing.

17. The method according to any preceding claim wherein the pharmacologically active extract is formulated in a topical vehicle formulated to increase efficacy of the active constituents.

18. The method according to claim 17 wherein the topical vehicle is based on the formulation described in Examples 4 & 5.

19. The method according to claim 17-18 wherein the pharmacologically active extract as described in any preceding claim is selected from the list hawthorn (*Crataegus monogyna*) or *Pueraria lobata* and also species closely related to any of the foregoing.

20. The method according to any preceding claim wherein the pharmacologically active extract is formulated into an enteric release capsule to produce a high local concentration of the active constituents in the colon.

21. The method according to claim 20 wherein the enteric release capsule is based on the formulation described in Example 6.

22. The method according to claim 20-21 wherein the pharmacologically active extract as described in any preceding claim is selected from the list hawthorn (*Crataegus monogyna*) or *Pueraria lobata* and also species closely related to any of the foregoing.
**Patents Act 1977: Search Report under Section 17**

**Documents considered to be relevant:**

<table>
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<th>Category</th>
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<th>Identity of document and passage or figure of particular relevance</th>
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<tr>
<td>X</td>
<td>1 to 23</td>
<td>KR 20090122049 A (KOREA INST SCIENCE TECHNOLOGY) See the WPI abstract AN 2009-R90941 [19]</td>
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<tr>
<td>X</td>
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<td>Journal of Supercritical Fluids 48 (2009) 'Extraction of bioactive components from Centella asiatica using subcritical water' 211 - 216 XP026004190; See the whole document</td>
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<tr>
<td>X</td>
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<td>US7208181 B1 (KING et al) The whole document</td>
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<td>X,E</td>
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<td>GB2463530 A (WHEATLEY &amp; DAVISON) The whole document</td>
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<td>WO2010/034971 A2 (WHEATLEY &amp; DAVISON) The whole document</td>
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**Categories:**

| X        | Document indicating lack of novelty or inventive step |
| Y        | Document indicating lack of inventive step if combined with one or more other documents of same category. |
| &        | Member of the same patent family |
| A        | Document indicating technological background and/or state of the art. |
| P        | Document published on or after the declared priority date but before the filing date of this invention. |
| E        | Patent document published on or after, but with priority date earlier than, the filing date of this application. |

**Field of Search:**

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC:\(^X\):

Worldwide search of patent documents classified in the following areas of the IPC:

The following online and other databases have been used in the preparation of this search report:

WPI, EPODOC, TXTE, TXTJP, TXTWOT, XPTK, BIOSIS, MEDLINE, XPESP
### International Classification:

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