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(54) Title: THE USE OF WINTER SAVORY (*SATUREJA MONTANA*) OR THE EXTRACTS THEREOF FOR THE PREPARATION OF MEDICAMENTS FOR THE TREATMENT OF THE PREMATURE EJACULATION

(57) Abstract: The use of Winter savory or its purified extracts rich in rosmarinic acid, the use of rosmarinic acid and the extracts containing it for the preparation of medicaments for the treatment of premature ejaculation.

THE USE OF WINTER SAVORY (*SATUREJA MONTANA*) OR THE EXTRACTS THEREOF FOR THE PREPARATION OF MEDICAMENTS FOR THE TREATMENT OF THE PREMATURE EJACULATION

The present invention relates to the use of Winter savory or purified extracts thereof rich in rosmarinic acid, and the use of rosmarinic acid or extracts containing it for the preparation of medicaments for the treatment of premature ejaculation.

TECHNOLOGICAL BACKGROUND

Premature ejaculation, which is ejaculation occurring before the individual wishes, is a dysfunction affecting more than 30% of male population, mainly adolescent, but that may also persist in the adult. Three main causes are apparently involved:

1) *organic factors*, such as anatomical or physiological alterations of the reproductive system;

2) *psychological factors*, deriving from wrong sexual education or deep-seated problems related to improper sexual development during growth;

3) *external factors*, such as use of drugs (amphetamines, hallucinogens), excessive consumption of alcoholics, food and medications.

Premature ejaculation can ultimately be ascribed to alterations of the neuroconduction and neuromodulation processes which modulate the sexual behaviour both at the central and peripheral levels. Therefore, pharmacological treatment usually consists in the administration of benzodiazepines (alprazolam 0.25 mg), antidepressants acting on the serotoninergic system (paroxetine 20 mg), or in the local application of anaesthetics (prilocaine 25 mg + lidocaine 25 mg).

As this type of therapy does not always prove effective and also involves side effects such as sedation or cardio circulatory problems, there is the need for novel active principles.

It has now been found that Winter savory (*Satureja montana*) or the extracts thereof, as well as rosmarinic acid or the extracts containing it, can be used for the preparation of medicaments for the treatment of premature ejaculation.

DETAILED DISCLOSURE OF THE INVENTION

Winter savory (*Satureja montana*) is a plant belonging to the Lamiaceae family, already known in traditional medicine as a remedy against asthenia or as aphrodisiac.

The object of the invention is the use of Winter savory or the extracts thereof, and of rosmarinic acid or extracts containing rosmarinic acid, for the preparation of medicaments for the treatment of premature ejaculation.

The invention further relates to a Winter savory total extract useful for the preparation of medicaments for the treatment of premature ejaculation.

A further object of the invention is the use of rosmarinic acid or extracts containing it for the preparation of medicaments for treatment of premature ejaculation.

According to the invention, the process for the preparation of the Winter savory purified extract comprises the following steps:

a) milling Winter savory dried aerial parts;

b) extracting them one or more times with water and recovering the extraction solvent;

c) filtering the extraction solvent or the combined extraction solvents and subsequent hot concentration under reduced pressure to obtain a concentrated solution;

d) eluting the concentrated solution from step c) on a resin column;

e) washing the resin with water;

f) eluting with ethanol;

g) hot concentrating the organic phase under reduced pressure;

h) drying the resulting syrup under vacuum at 60°C for 24 hours;

i) milling the resulting solid.

Alternatively to steps d)-f), the solution from step c) is extracted once or repeatedly with butanol; the organic phase (or combined organic phases) is then subjected to steps g), h) and i).

The resulting extract was solubilised in Tween 80 (10%) and water and administered to test animals through a gastric probe, in a volume of 5 ml/kg, at different doses, 45 minutes before the *in vivo* test.

After acute administration per os (25, 50 and 100 mg/kg), a statistically significant increase in ejaculation latency has been observed.

HPLC analysis of Winter savory purified extract evidenced the presence of rosmarinic acid that, when tested under the same experimental conditions at doses of 10 and 20 mg/kg, showed equivalent activity to that of the extract.

Qualitatively similar, although quantitatively different, results were obtained replacing the purified extract with the total aqueous extract of Example I and with the alcoholic extract of Example IV.

For the treatment in humans, the compounds of the invention can be incorporated in pharmaceutical formulations suitable for the oral, intramuscular, transdermal administrations, with conventional excipients and methods. Doses can range from 100 mg to 2000 mg daily, preferably from 200 to 400 mg daily.

The invention is described in more detail by the following examples.

EXAMPLE I: PREPARATION OF TOTAL AQUEOUS EXTRACT

One kg of finely ground *Satureja montana* dried leaves is extracted by infusion at 70°C with 4 volumes of water for 4 hours. The procedure is repeated for 5 times. The hot extracts are filtered, combined and concentrated

under vacuum to give a soft extract, which is subsequently dried under vacuum at 60°C to give 290 g of dry extract.

EXAMPLE II: PURIFICATION OF TOTAL AQUEOUS EXTRACT WITH BUTANOL

100 g of the extract of Example I is redissolved in 10 volumes of water. The solution is extracted at room temperature with 3x0.5 litres of water-saturated n-butanol. Combined butanolic extracts are concentrated to a soft extract. The butanol residue is replaced with water. The extract is dried under vacuum at 60°C to give 11 g of a dry extract with 9.74% content in rosmarinic acid.

EXAMPLE III: PURIFICATION OF TOTAL AQUEOUS EXTRACT WITH ADSORBING RESINS

50 g of the extract of Example I is redissolved in 10 volumes of water, absorbed on a column containing 1 litre of duolite XAD761, and washed with 5000 ml of water. Washings are discarded and the column is eluted with 3000 ml of 95% ethanol. The hot ethanol fraction is concentrated under reduced pressure to obtain a soft extract which is subsequently dried under vacuum at 60°C for 24 hours. 5 g of purified extract is obtained, having 10.48% content in rosmarinic acid.

EXAMPLE IV: PREPARATION OF WINTER SAVORY ALCOHOLIC EXTRACT

Dried aerial parts of Winter savory (100 g) are macerated in 500 ml of 90% (v/v) ethanol for 24 hours; the suspension is then filtered and evaporated to dryness.

EXAMPLE V: COPULATORY ACTIVITY

Sprague-Dawley rats were used, both males (weighing approx. 220 g) and females (weighing approx. 160 g), from Harlan Italy (Udine, Italy). The animals were housed under controlled temperature and humidity conditions

(22±1°C, 60% humidity), with 12 hours inverted light-darkness cycle, with lights on at 7 a.m. Females were ovariectomized and subcutaneously injected with estradiol valerate (500 μg) and progesterone (2 mg) 48 hours after, to induce estrus. Male rats with very short ejaculation latency time were chosen through starting screening consisting of 7 pre-tests [A. Ågmo, Male rat sexual behavior, *Brain Research Protocols 1*: 203-209, 1997].

The resulting extract according to Example II or rosmarinic acid were solubilised in Tween 80 (10%) and water and administered to animals, by gastric probe, in a volume of 5 ml/kg, at different doses, 45 minutes before carrying out all the *in vivo* tests.

Sexual behaviour was evaluated under quite conditions, with feeble red light, according to the standard procedure [A. Ågmo, Male rat sexual behavior, *Brain Research Protocols 1*: 203-209, 1997]. The main parameters recorded during the test were:

1) mount latency (ML);
2) intromission latency (IL);
3) ejaculation latency (EL);
4) postejaculatory interval (PEI);
5) mount frequency (MF);
6) intromission frequency (IF).

RESULTS

Table 1 shows that all of the doses of Winter savory extract administered (25, 50, and 100 mg/kg) induced slowing down of the copulatory activity, and a statistically significant prolonging of ejaculation latency. The same results were obtained with rosmarinic acid at doses of 10 and 20 mg/kg (Table 2).

***Table 1**: Effect of the administration of Winter savory extract on copulatory activity*

Treatment (mg/kg)	ML (sec)	MF (n°)	IL (sec)	IF (n°)	EL (sec)	PEI (sec)
Control	155.0±41.7	2.5±0.5	155.1±41.7	21.0±3.2	469.2±80.2	392.2±20.4
Winter savory 25	499.7±132.8*	1.2±0.2**	499.8±132.7*	23.8±3.6	1420.0±215.9*	686.7±101.7*
Control	247.5±42.1	3.1±0.6	283.1±48.2	27.8±3.8	496.0±107.4	508.6±67.2
Winter savory 50	451.1±130.0	1.2±0.2*	451.1±130.0	29.6±4.7	1346.0±161.7*	562.0±127.0
Control	37.7±13.2	8.9±0.5	73.6±26.2	12.7±0.7	470.4±10.9	301.9±6.6
Winter savory 100	160.4±88.8	6.2±1.1	189.2±86.4	9.5±0.8*	1552.2±208.9	458.2±74.2***

*Values are expressed as mean±SEM (n= 8-10). Mann-Whitney test: *p<0.05, **p<0.01, ***p<0.001 vs. the respective controls.*

***Table 2**: Effect of the administration of rosmarinic acid on copulatory activity*

Teatment (mg/kg)	ML (sec)	MF (n°)	IL (sec)	IF (n°)	EL (sec)	PEI (sec)
Control	135.2±33.4	2.4±0.3	147.1±37.5	22.3±3.9	483.3±60.6	383.1±18.3
Rosmarinic acid 10 mg/kg	382.4±93.6*	1.6±0.3	393.9±89.8*	19.8±1.2	1282.2±90.4*	548.5±45.5*
Control	184.3±45.3	2.9±0.3	199.7±37.3	24.1±2.3	470.9±71.1	444.4± 6.6
Rosmarinic acid 20 mg/kg	425.8±83.2*	1.1±0.1*	408.4±48.6*	27.3±0.7	1493.1±81.9**	602.2±102.4*

*Values are expressed as mean±SEM (n = 8-10). Mann-Whitney test: *p<0.05, **p<0.01, vs. respective controls.*

CONCLUSIONS

The results reported above prove that the Winter savory purified extract acts on copulatory activity, significantly prolonging the ejaculation time. The effect is dose-dependent after oral administration and appears after 30-45 minutes.

CLAIMS

1. The use of Winter savory or the extracts thereof, and of rosmarinic acid or extracts containing rosmarinic acid, for the preparation of medicaments for the treatment of premature ejaculation.

2. The use as claimed in claim 1 of Winter savory or extracts thereof.

3. The use as claimed in claim 1 of rosmarinic acid.

4. The use as claimed in claim 1 of extracts containing rosmarinic acid.

5. A process for the preparation of a Winter savory extract which comprises the following steps:

a) milling Winter savory dried aerial parts;

b) extracting them one or more times with water and recovering the extraction solvent;

c) filtering the extraction solvent or the combined extraction solvents and subsequent hot concentration under reduced pressure to obtain a concentrated solution;

d) eluting the concentrated solution from step c) on a resin column;

e) washing the resin with water;

f) eluting with ethanol;

g) hot concentrating the organic phase under reduced pressure;

h) drying the resulting syrup under vacuum at 60°C for 24 hours;

i) milling the resulting solid.

6. A process as claimed in claim 5 in which, alternatively to steps d)-f), the solution from step c) is extracted once or repeatedly with butanol and the organic phase (or the combined organic phases) is then subjected to steps g), h) and i).

7. The extract obtained by the process as claimed in claims 5-6.