EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Orthosiphon stamineus* Benth., folium

Based on Article 10a of Directive 2001/83/EC as amended (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC as amended (traditional use)

Final

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Orthosiphon stamineus* Benth., folium
Herbal preparation(s)	Liquid extract (1:1, ethanol 25% m/m) Dry extract (5-7:1, water) Dry extract (8-12:1, ethanol 60% V/V) Dry extract (7-8:1, ethanol 70% V/V)
Pharmaceutical forms	Herbal substance or herbal preparations in solid dosage forms or as herbal tea for oral use
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1. Introduction

The aim of this report is to assess the available preclinical and clinical data on Orthosiphonis folium (Java tea) for preparing a community herbal monograph. This report is based on the documentation provided by the European Medicines Agency (EMA) completed by additional researches and information taken from monographs on Orthosiphonis folium (Commission E Monographs, 1998; ESCOP monographs, 2003).

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Java tea leaf and top of stems.

The composition of Java tea is very complex. The most characteristic compounds are minerals (potassium 3%), diterpenes (orthosiphols A-E 0.2%), triterpenes, essential oil.(0.02 -0.06%) (sesquiterpenes), lipophilic flavones like sinensetin (0.1 – 0.19%), isosinensetin and eupatorin flavonol glycosides; rosmarinic acid (0.1 – 0.5%), and other caffeic acid depsides like mono and dicafeyl tartric acid as well as lithospermic acid, pytosterols as b-sitosterol and up to 0.7% of essential oil, isositol, pimarane, isopimarane and staminane diterepnes, triterpenes and chromenes (ESCOP 2003; BRUNETON 1998, MATSUURA 1973;, BOMBARDELLI 1972;, PARIS and MOYSE 1971).

The European Pharmacopeia prescribes not less than 0.5% of sinensetin.

- Herbal preparation(s)

Powder, dry extracts, liquid extract.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

Not applicable.

1.2. Information about products on the market in the Member States

Java tea, as single herbal substance, is authorized in Belgium, France, Germany, Poland and Spain.

The active substance is present on the market as:

- Herbal substance

Dried leaves for herbal tea (Poland, 15 years; France, 1974).

- Herbal preparation

Powder (Belgium, 1997; France, 1989; Spain, 1987, 1991).

Liquid extract (solvent ethanol 25% m/m, DER 1:1) (France, 1952, 2006).

Dry extract (solvent water, DER 5-7:1) (Germany, at least 1976, 1995).

Dry extract (solvent ethanol 25% m/m, DER 4-5:1) (France, 1991).

Dry extract (solvent ethanol 30% V/V, DER 4:1) (France, 1988).

Dry extract (solvent ethanol 60% V/V, DER 8-12:1) (Germany, at least 1976).

Dry extract (solvent ethanol 70% V/V, DER 7-8:1) (Germany, at least 1976).

Regulatory status overview

Member State	**Regulatory Status**				**Comments (not mandatory field)**
Austria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only in combinations
Belgium	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Denmark	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	Only in combination
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products, may be as food supplements
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
France	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☒ Other Specify:	Only as food or food supplements
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal

Member State	Regulatory Status				Comments (not mandatory field)
					products
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Norway	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Poland	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	
Spain	☐ MA	☒ TRAD	☐ Other TRAD	☐ Other Specify:	
Sweden	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify:	No herbal medicinal products

MA: Marketing Authorisation
TRAD: Traditional Use Registration
Other TRAD: Other national Traditional systems of registration
Other: If known, it should be specified or otherwise add 'Not Known'
This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

1.3. Search and assessment methodology

This report is based on the documentation provided by the European Medicines Agency (EMA) and other national agencies completed by additional researches (Embase, Pubmed) and information taken from monographs on Orthosiphonis folium (Commission E Monographs, 1998; ESCOP monographs, 2003).

2. Historical data on medicinal use

2.1. Information on period of medicinal use in the Community

Orthosiphon stamineus Benth., syn. *O. spicatus* Bak., syn. *O. aristatus* Miq., belongs to the *Lamiaceae* family. The plant is found in an area extending from tropical Asia to tropical Australia, and is a 40 to 80 cm high herb. The medicinal parts are the leaves and stem tips collected during the flowering

season. Various herbal preparations (notably aqueous and ethanolic extracts) are used in traditional medicines.

Orthosiphonis folium (Java tea) has traditionally been used in Java for the treatment of *hypertension* and *diabetes* (Awale et al, 2003c)

It has also been used in folk medicine for bladder and kidney disorders, gout and rheumatism (Arafat et al, 2008)

European countries became interested in Java tea with the scientific work made by the Dutchman Van Itallie in 1886 (Paris and Moyse, 1971)

Java tea was mentioned in the Dutch Pharmacopoeia in 1926 and it was also listed in the French Pharmacopoeia in 1974 as an herbal that has been present in the previous pharmacopoeias.

Early studies are published since the twenties and Java tea has been used as herbal substance or herbal preparations since 1965 in France and 1976 in Germany.

2.2. Information on traditional/current indications and specified substances/preparations

Four monographs are currently available. For each monograph, the indications, the Posology and the method of administration are given:

1. The complete German Commission E Monographs (1998)

The monograph Java tea was published on March 13, 1986.

Therapeutic indication: "*Irrigation therapy for bacterial and inflammatory diseases of the lower urinary tract and renal gravel".*

Dosage: Unless otherwise prescribed daily dosage: 6-12g herb; equivalent preparation.

Method of administration: Cut herb for infusions and other galenical preparations for oral use.

2. European Scientific Cooperative on Phytoterapy (ESCOP) 2003

The monograph Java tea was published on 1996.

Therapeutic indication: "*Irrigation of the urinary tract, especially in cases of inflammation and renal gravel, and as an adjuvant in the treatment of bacterial infections of the urinary tract".*

Dosage:

Adults: An infusion of 2-3g of dried material in 150ml of water two to three times per day; equivalent preparations.

Method of administration: For oral administration

Duration of administration: No restriction.

3. French Health Authority: Cahiers de l'Agence n°3 (AFSSAPS, 1998)

The first text on orthosiphon was published on 1986.

Therapeutic indication: "*Traditionally used to facilitate urinary and digestive elimination functions".*

"*Traditionally used to promote the renal elimination of water".*

"*Traditionally used as an adjuvant to slimming regimes".*

4. British Herbal Medicine Association (BHMA) British Herbal Pharmacopoeia 1996

Therapeutic indication: *"Diuretic".*

In Belgium and Germany, Orthosiphonis folium is a well-established herbal medicinal product. The current therapeutic indications in these European countries are:

In Belgium: to enhance the renal elimination of water, after all serious pathologies have been excluded.

In Germany: as a purging in bacterial and inflammatory diseases of the urinary tract collection system and in renal gravel.

In Spain, Poland and France, Orthosiphonis folium is a traditional herbal medicinal product. The current therapeutic indications in these European countries are:

In Spain: traditionally used to increase the amount of urine.

In Poland: traditionally used as an adjuvant in the treatment of mild bacterial infections of the urinary tract and as adjuvant in renal gravel.

In France: traditionally used to promote the renal elimination of water, or as an adjuvant to slimming regimes.

2.3. Specified strength / posology / route of administration / duration of use for relevant preparations and indications

- Current posology for herbal medicines used as "well established use":

Dry aqueous extract (DER 5-7:1): 360 mg 3 to 4 times daily or 500 mg 3 times daily.

Dry extract (solvent: ethanol 60% V/V, DER 8-12:1): 200 to 400 mg 3 times daily.

Dry extract (solvent: ethanol 70% V/V, DER 7-8:1): 277.5 mg 3 times daily.

Powder: 250 to 500 mg, 3 to 4 times daily.

- Current posology for "traditional herbal medicines":

Java tea for herbal tea: 6 to 12 g daily in divided doses.

Powder: 650 mg 2 times daily.

Liquid extract (solvent ethanol 25% m/m, DER 1:1): 2 g 2 times daily.

Dry extract (solvent ethanol 25% m/m, DER 4-5:1): 200 mg 2 times daily.

Dry extract (solvent ethanol 30% V/V, DER 4:1): 150 to 300 mg 3 times daily.

3. Non-Clinical Data

Overall strategy

This literature-based nonclinical assessment report provides a critical review of data related to the experimental pharmacology, pharmacokinetics and toxicology studies performed with the herbal drug or herbal preparations. When needed, data obtained with isolated substances were also taken into consideration.

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Primary pharmacodynamics

Diuretic activity

To support the traditional use of herbal preparations obtained from *Orthosiphon stamineus*, the diuretic activity of various extracts (aqueous or hydro-ethanolic) was evaluated *in vivo* in rats. The activity of a few isolated compounds was also studied by some authors. Table 1 summarizes the overall study results for extracts and Table 3 for isolated compounds. The corresponding studies are further detailed in the paragraphs below.

Englert and Harnischfeger (1992) – see Table 1

To study the diuretic activity of an aqueous extract prepared from leaves of *Orthosiphon stamineus*, male rats were administered via oral gavage doses of 0 (water), 125, 750 and 1000 mg/kg. The loop diuretic furosemide (100 mg/kg) was used as a reference compound.

Compared to controls, the urine volume measured in rats treated with either the extract or furosemide was not increased. According to the authors, the extract enhanced ion excretion (Na^+, K^+, Cl^-) to a level comparable to that obtained with furosemide and optimum activity was reached at the dose of 750 mg/kg. In addition, the hypothesis that increased ion excretion is due to large amounts of potassium (1-3%) in the extract was not confirmed based on results obtained in K^+-aspartate fed rats.

The ratios of active doses in rats vs. therapeutic / traditionally used doses in humans amounted to 80-180 for furosemide and 80 for Orthosiphonis folium. Therefore, it was suggested that rat is rather a poor model for the known diuretic activity of furosemide in humans. Consequently, it was recommended to further test the diuretic activity of Orthosiphon extract in a more appropriate model such as the dog.

Assessor's comment

In animals treated at 750 mg/kg, the urinary excretion of sodium and potassium ions was twice that measured in controls and the urinary excretion of chloride ions increased almost 3-fold. This effect did not further increase with dose for both potassium and chloride ions, whereas sodium ions excretion further increased. The authors conclude that ion excretion obtained in animals treated with Orthosiphon is comparable to that obtained with furosemide. This is not fully endorsed because the effect of furosemide on sodium and chloride ions excretion appeared much more intense in furosemide-treated rats than in the group treated with the extract at 750 mg/kg (please refer to Table 1).

The aqueous extract of Orthosiphon stamineus and furosemide did not induce an increase in urine volume. This result is questionable at least for furosemide, which usually increases diuresis. The authors indicate that rat is rather a poor model for furosemide, but it is also noted that furosemide (30 mg/kg) was shown to increase diuresis in rats in the study performed by Olah et al, 2003.

Overall, this study did not demonstrate that the extract tested has diuretic activity in male rats, but it was shown to increase urinary excretion of sodium, chloride and potassium ions at doses of 750 mg/kg and above, without a dose-effect relationship. The figures obtained were not tested for statistical significance.

Kavimani et al (1997) – see Table 1

The diuretic activity of an aqueous extract of Orthosiphon thymiflorus (whole plant) was evaluated in male rats. The study design was comparable to that used by Englert and Harnischfeger (1992). In particular, the route of administration, doses of extract and furosemide were the same.

According to the authors, optimum activity of the extract was noted at 750 mg/kg. No increase in urine volume was observed. Sodium and chloride ions excretions increased 2.7-fold compared to controls. In furosemide-treated rats, urinary excretion of sodium and chloride ions increased 6-fold and 4-fold, respectively. It is concluded that the extract did not show any aquaretic activity but enhanced considerably ion excretion almost to an extent similar to that produced by furosemide.

Assessor's comment

The results obtained by Kavimani et al (1997) are comparable to those obtained by Englert and Harnischfeger (1992). Again, the results obtained do not clearly allow to state that the effect of O. thymiflorus extract on ion excretion is comparable to the effect obtained with furosemide, which is more pronounced. Similarly to what was observed previously, the effect of the extract is not related to the dose regarding potassium and chloride ions excretion.

An increase in urine volume was not reported, so that it cannot be concluded that the extract or the positive control furosemide demonstrated diuretic activity in this study. No statistical test was performed.

Olah et al (2003) – see Table 1

Extracts of *Orthosiphon stamineus* (leaves) were obtained either with ethanol 50% (v/v) or ethanol 70% (v/v). The diuretic activity was then tested in male rats after oral administration of water (control), or 700 mg/kg of each extract. Furosemide (30 mg/kg, oral route) was used as a reference compound.

Whereas urine volume was 2.5-fold higher in furosemide treated rats than in controls, it was only slightly increased in rats treated with the 50% ethanolic extract (1.3-fold), and not increased in animals receiving the 70% ethanolic extract. Sodium excretion was enhanced in all treated animals compared to controls, and the natriuretic effect of the 50% ethanolic extract was above that of furosemide. Potassium excretion was also increased, but remained below that obtained with furosemide. Uric acid elimination was also improved.

Assessor's comment

Compared to both studies presented before, furosemide administration induced a diuretic effect. This seems surprising considering that the dose administered was 3-fold lower than that administered by Englert and Harnischfeger (1992) and Kavimani et al (1997).

Otherwise, this study showed that the 50% ethanolic extract induced an increase in urine volume compared to controls when administered orally at 700 mg/kg to rats. In terms of intensity, the effect was half that observed in furosemide-treated animals. No effect on urine volume was reported in rats treated with the 70% ethanolic extract, thus showing the importance of well-characterizing the mode of preparation of herbal preparations. In addition to the effect on urine volume, the excretion of sodium and potassium ions increased with both extracts. No statistical analysis was performed to test the significance of the effects on urine volume or ion excretion.

Table 1: studies performed to test the diuretic activity of Orthosiphon extracts upon acute administration

Reference	Extract / Substance			Species	Route	Duration of urine collection	Doses (mg/kg)	Urinary parameters (compared to controls)					
	Plant	Plant part	Type					Urine volume	Ion concentration in urine			Ion quantity in urine	
									$[Na^+]_u$	$[K^+]_u$	$[Cl^-]_u$	Na^+_u	K^+_u	Cl^-_u
Adam et al, 2009	O.stam	Leaves	Aq	Rat, males	Oral	4 hours	5 10 Furosemide (10) HCTZ (10)	x7.3 **x15.5** **x23.4** **x21.6**				x2.1 x1.8 x14.8 x19.6	x5.4 x10 x2 x2.6	x4 x3.3 x10.1 x11.9
Arafat et al, 2008	O.stam	Leaves	MeOH MeOH-water	Rat, male	Oral	24 hours	See text							
Beaux et al, 1999	O.stam	?	HA	Rat, males	IP	8 hours	50 HCTZ (10)	↑ ↑	– ↑	↑ ↑				
						24 hours	50 HCTZ (10)	↑ –	– –	– –				
Casadebeig-Lafon et al, 1989	O.stam	Leaves	Aq	Rat, males	Oral	6 hours	18 180	**x1.6** **x1.4**	**x2.4** **x2.2**	x1.0 x1.1	x1.4 **x1.6**			
			HA (70%)	Rat, males	Oral	6 hours	13.5 135	**x1.6** **x1.4**	x1.2 x1.8	x0.7 x0.9	x0.9 **x1.3**			
Englert and Harnischfeger, 1992	O.stam	Leaves	Aq	Rat, males	Oral	Not known	125 750 1000 Furosemide (100)	x1.1 x1.2 x1.0 x0.9	x1.3 x2.0 x5.9 x5.8	x1.1 x2.0 x1.7 x1.3	x2.0 x2.8 x1.7 x4.2			
Kavimani et al, 1997	O.thym	Whole plant	Aq	Rat, males	Oral	5 hours	125 750 1000 Furosemide (100)	x0.8 x0.9 x0.9 x1.3	x1.3 x2.7 x4.6 x5.8	x1.1 x1.3 x1.3 x1.3	x2.2 x2.7 x2.1 x4.0			

Reference	Extract / Substance			Species	Route	Duration of urine collection	Doses (mg/kg)	Urinary parameters (compared to controls)					
								Urine volume	Ion concentration in urine			Ion quantity in urine	
	Plant	Plant part	Type						$[Na^+]_u$	$[K^+]_u$	$[Cl^-]_u$	Na^+_u	K^+_u	Cl^-_u
Adam et al, 2009	O.stam	Leaves	Aq	Rat, males	Oral	4 hours	5 10 Furosemide (10) HCTZ (10)	x7.3 **x15.5** **x23.4** **x21.6**				x2.1 x1.8 x14.8 x19.6	x5.4 x10 x2 x2.6	x4 x3.3 x10.1 x11.9
Arafat et al, 2008	O.stam	Leaves	MeOH MeOH-water	Rat, male	Oral	24 hours	See text							
Olah et al, 2003	O.stam	Leaves	HA (50%)	Rat, males	Oral	24 hours	700 Furosemide (30)	x1.3 x2.5				x1.6 x1.3	x2.1 x4.6	
			HA (70%)	Rat, males	Oral	24 hours	700 Furosemide (30)	x0.9 x2.5				x1.3 x1.3	x1.5 x4.6	
Chow et al, 1979	O.stam	? (herba)	HA (50%)	Dog	IV	?	18.8 mg/kg/min	**x1.3**[a]				**x1.3**[a]	**x1.6**[a]	**x1.3**[a]

O.stam: *Orthosiphon stamineus*; O.thym: *Orthosiphon thymiflorus*; HA: hydro-alcoholic; Aq: aqueous; MeOH: methanol; HCTZ: hydrochlorothiazide

[a] no control group included, urinary parameters were compared to values obtained in the same animals before treatment

in bold: statistically significant

Casadebaig-Lafon et al (1989) – see Table 1

Two types of extract produced from *Orthosiphon stamineus* (leaves) were tested for diuretic activity: an aqueous extract, or a hydro-alcoholic (70%) extract. The oral doses administered to male rats amounted to 18 and 180 mg/kg, or to 13.5 and 135 mg/kg, respectively. Urines were collected for 6 hours after administration of the test article. No positive control was used.

The increase in urine volume noted in all treated groups (compared to water-treated controls) was statistically significant. The authors note that the aqueous extract is particularly interesting because the increased diuresis occurred simultaneously with increased excretion of sodium at both dose levels. At the highest dose level, the excretion of chloride ions was also significant. The same effect on chloride ions excretion is observed in rats treated at the highest dose of alcoholic extract, without any concomitant effect on sodium excretion. In all treated groups, the urinary excretion of potassium was not enhanced.

Assessor's comment

Casadebaig-Lafon et al reported in rats a diuretic effect for 2 extracts (aqueous and 70% ethanolic) of Orthosiphon stamineus leaves, as shown by statistically increased urine volumes in treated animals vs. controls. It can also be mentioned that this effect was not dose-dependent. Statistical increases in sodium and/or chloride urinary excretion were also noted, notably in animals treated with the aqueous extract.

The inclusion of a group treated with a reference compound would have allowed to better assess the intensity of the effects observed.

Beaux et al, 1999 – see Table 1

The diuretic activity of a commercial hydro-alcoholic extract of *Orthosiphon stamineus* was tested by intraperitoneal route in male rats. The dose administered to animals amounted to 50 mg/kg, and hydrochlorothiazide (10 mg/kg) was used as positive control. Urines were collected for 8 and 24 hours post-administration.

The urine volume collected was significantly increased (compared to controls) from 2 to 24 hours and from 2 to 8 hours post-dose in animals treated with the extract and with hydrochlorothiazide, respectively. In extract-treated animals, no effect was observed on sodium or chloride ion excretion, while potassium excretion increased at 8 hours post-dose. In hydrochlorothiazide-treated animals, sodium and potassium excretion were enhanced at 8 hours post-dose but not thereafter.

According to the authors, this experiment justifies the use of *Orthosiphon stamineus* (aerial parts) as a diuretic agent in traditional medicine.

Assessor's comment

A significant diuretic effect was obtained with the extract, but potassium excretion was enhanced in the first 8 hours following extract administration. No effect on sodium or chloride ions excretion was observed.

The therapeutic relevance of this experiment is questioned as the route of administration is not what is used clinically. In addition, some elements are missing for the extrapolation of the results such as proportion of ethanol in the extraction solvent and the part of the plant used.

Chow et al, 1979 – see Table 1

The pharmacological effect of a 50% hydro-ethanolic extract of Orthosiphonis herba was studied in pentobarbital-anaesthetized dogs under saline diuresis. The urine volume, excretion of electrolytes (Na^+, K^+, Cl^-) and fractional water excretion (V/GFR) were significantly increased by IV infusion of the

drug (18.8 mg/kg/min) in dogs. A significant decrease in re-absorption of sodium and chloride ions was also noted in renal tubules. A significant increase in the plasmatic concentration of potassium ions was also observed, whereas those of sodium and chloride ions remained unaltered. The authors also reported that the clearances of creatinine and para-aminohippuric acid (PAH), the urinary pH and blood pH were not altered.

Assessor's comment

This is the only study aiming at evaluating the diuretic activity of Orthosiphon stamineus in a non-rodent species. It showed that an ethanolic (50% v/v) extract of Orthosiphon stamineus caused significant increase of urine volume and electrolyte excretion (Na^+, K^+, Cl^-), and significant reduction of reabsorption of Na^+ and Cl^- ions in renal tubules. Plasmatic concentration of potassium increased.

It should be noted that the route of administration used is not therapeutically relevant, and that the plant part used to prepare the extract is not known.

Arafat et al, 2008

The diuretic effect of different methanol extracts of *Orthosiphon stamineus* leaves was examined by treating different groups of male Sprague–Dawley rats with either single (2000 mg/kg) or repeated (500 g/kg/day for 7 days) oral doses of methanol and methanol-water (1:1) extracts. Hydrochlorothiazide (10 mg/kg) was used as a positive control in the acute study only. Control animals were administered tap water. Cumulative urine volume and electrolytes (Na^+ and K^+) concentrations at different time intervals were measured.

In the acute study, it was shown that a single dose of methanol or methanol-water extract induced no significant increase in urinary output, contrary to hydrochlorothiazide. Increases in urinary pH, and sodium and potassium excretion were also noted with both extracts. Detailed results are reported in Table 2.

Table 2: effect of oral administration of HCTZ 10 mg/kg, MeOH and MeOH:water (1:1) extracts 2g/kg on pH, cumulative urinary volume and cumulative urinary excretion of sodium and potassium in rats (Arafat et al, 2008)

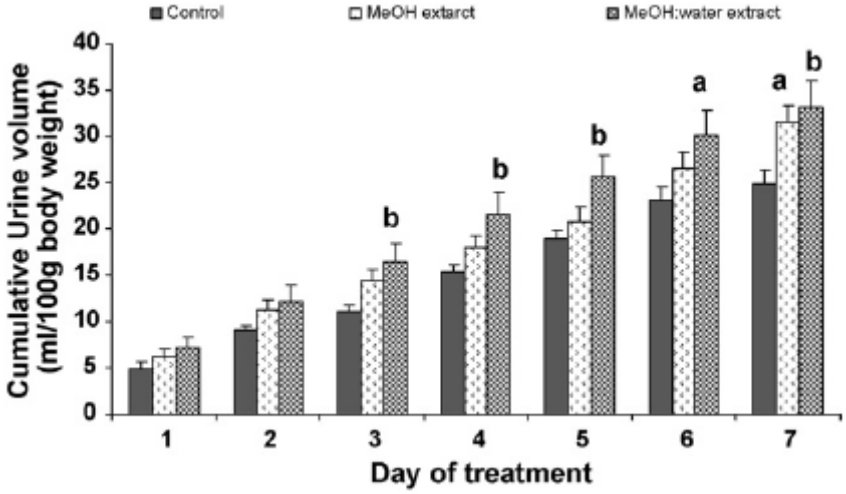
Time (h)	Control	Hydrochlorothiazide	MeOH	MeOH:water
pH				
2	8.4 ± 0.4	8.2 ± 0.1	9.0	8.7 ± 0.1
4	7.4 ± 0.2	7.8 ± 0.2	8.3 ± 0.3	7.5 ± 0.3
6	7.3 ± 0.3	7.5 ± 0.2	7.8 ± 0.3	7.8 ± 0.3
8	7.7 ± 0.3	7.5 ± 0.5	7.9 ± 0.1	8.4 ± 0.4
24	8.0 ± 0.1	8.0 ± 0.1	8.7 ± 0.3*	8.9 ± 0.1*
Cumulative urine volume (ml/100 g body weight)				
2	0.5 ± 0.1	1.9 ± 0.2*	0.8 ± 0.2	0.9 ± 0.2
4	1.0 ± 0.2	3.1 ± 0.3*	1.6 ± 0.5	1.4 ± 0.3
6	1.6 ± 0.3	4.1 ± 0.4*	1.9 ± 0.5	1.7 ± 0.3
8	1.8 ± 0.3	4.5 ± 0.4*	2.3 ± 0.5	2.3 ± 0.4
24	4.4 ± 0.4	7.2 ± 0.8	5.0 ± 1.5	4.9 ± 0.8
Na^+ excreted (mmol/100 g body weight)				
2	37.7 ± 12.8	200.6 ± 25.7*	111 ± 33.5	99.5 ± 23.2
4	96.1 ± 18.6	344.2 ± 35.9*	235.3 ± 52.2*	230.9 ± 35.4*
6	165.9 ± 23.2	476.4 ± 42.6*	313.4 ± 63.9	326.9 ± 45.6*
8	169.6 ± 21.6	485.5 ± 44.3*	364.5 ± 63.2*	380.6 ± 47.9*
24	555.3 ± 61.8	708 ± 51.5	605.4 ± 79.5	636.9 ± 48.6
K^+ excreted (mmol/100 g body weight)				
2	29.5 ± 9.8	79.1 ± 9.4*	85.9 ± 22.1*	117.9 ± 16.5*
4	71.3 ± 15.8	145 ± 10.9	167.8 ± 34.9*	185.4 ± 26.1*
6	115 ± 23.1	208.5 ± 23.9	208.3 ± 35.2	237.7 ± 40*
8	131.4 ± 21.8	213.9 ± 24.4	236.9 ± 31.4	293.2 ± 44.9*
24	501.2 ± 101.2	587.3 ± 117.3	459.9 ± 117.2	782.2 ± 128.5

Mean ± S.E.M. $n = 9$. Na^+: Sodium; K^+: potassium.
* $p < 0.05$ vs. control.

Repeated administrations of methanol:water (1:1) extract at a dose of 500 mg/kg increased the urinary output significantly from the 3rd day compared to the negative control group. In the group administrated the methanol extract, a significant increase in the cumulative urinary volume was noted on day 7 only. Results are shown in Figure 1. In addition, both extracts significantly increased urinary sodium and potassium excretion from day 4 and 2, respectively.

Figure 1: effects of MeOH and MeOH:water extracts (500 mg/kg/day for 7 days) on cumulative urine volume (mean±SEM)

a: p< 0.05 – b: p<0.01



The authors conclude that the delayed diuretic effect of the methanol extract compared to that of methanol:water extract can be explained by the presence of more polar components such as flavonoids and rosmarinic acid which may act synergistically in the methanol:water extract.

Assessor's comment

Methanol and methanol:water extracts are not reported to be used traditionally in humans, so that the clinical relevance of this study can be discussed. However, it is interesting to note that while no significant diuretic activity was reported after a single oral administration of each extract, repeated administrations of the same extracts over 7 days induced an increased urinary volume. The effect was observed earlier with the methanol:water extract, which contained more polar compounds (flavonoids, rosmarinic acid).

This is the only study dealing with diuretic activity of Orthosiphon stamineus administered repeatedly.

Adam et al, 2009 – see Table 1

Water extracts were administered orally at doses of 0, 5 and 10 mg/kg to Sprague–Dawley rats. Positive control groups were given either furosemide or hydrochlorthiazide at 10 mg/kg. Urine volume, urine pH, urine density and urine electrolytes were determined every hour for 4 h. Blood was assayed for glucose, albumin, blood urea nitrogen (BUN) and creatinine.

O. stamineus extract exhibited dose-dependent diuretic activity. However, excretion of Na+ and Cl– was not markedly elevated, but urinary excretion of K+ was significantly increased. *O. stamineus* extracts slightly increased the serum BUN, creatinine and blood glucose level. Although these levels were statistically significant when compared to control, they were still within the normal range.

The authors conclude that *O. stamineus* exhibited diuretic activity, but was less potent than furosemide and hydrochlorothiazide. Care should be taken when consuming this herb as a slight increase of kidney function enzymes was recorded.

Assessor's comment

A diuretic effect is reported for this aqueous extract of O. stamineus administrered once, but it is less potent than that of furosemide or hydrochlorothiazide administered at the same dose level. This diuretic effect seems to be dose-related. Decreased kalemia is also noted.

Significant increases in renal function markers are reported (BUN, creatinine), but it is mentioned that the values are within the normal range. The lack of an adequate repeat-dose toxicity study does not allow putting this result in perspective, but current guidelines for traditional herbal medicinal products indicate that the lack of repeat-dose toxicity is acceptable as it is covered by human experience. Interestingly, an increased blood glucose level is noted; this result is in contradiction with that reported by other authors (Hypoglycaemic effects).

Matsubara et al, 1999 – see Table 3

Methylripariochromene A (MRC) was isolated from the chloroform-soluble fraction of the water decoction of *Orthosiphon stamineus* (leaves). According to Matsubara et al, MRC was a major component of the aforementioned decoction (yield: 2.3%).

Rats were treated orally with MRC (25, 50 and 100 mg/kg); controls received vehicle (0.5% Tween 80 solution), and hydrochlorothiazide (25 mg/kg) was used as reference compound. Urines were collected for 3 hours after administration of the test article.

No effect was noted up to 50 mg/kg MRC. The results obtained showed a significant 3-fold increase in urine volume in the high dose group, and in hydrochlorothiazide-treated rats. The quantity of ions (Na^+, K^+, Cl^-) excreted in the urine was also significantly increased at 100 mg/kg MRC. The intensity of the effect was half that reported for hydrochlorothiazide regarding the excretion of sodium and chloride ions, while the quantity of potassium urinary excreted was twice that of controls for both high dosed and hydrochlorothiazide-treated rats. The urinary concentration of each ion was not modified by MRC treatment, whereas hydrochlorothiazide significantly increased the urinary concentration of sodium and chloride ions. The authors conclude from the latter observation that the mechanism underlying the diuretic activity of MRC may not be the same as that of hydrochlorothiazide.

Assessor's comment

MRC was shown to possess diuretic activity in rats at the oral dose of 100 mg/kg. At this dose level, urine volume increased 3-fold similarly to what is observed with the reference compound hydrochlorothiazide. The quantity of sodium and chloride ions recovered in urine also increased but to a lesser extent to what is observed in hydrochlorothiazide-treated rats. The quantity of potassium excreted was similar in high-dosed rats and in rats treated with the reference compound. Contrary to hydrochlorothiazide, the urinary concentration of each ion was not modified in animals undergoing MRC treatment whatever the dose.

The diuretic activity of MRC was demonstrated at the oral dose of 100 mg/kg, but not at lower dose levels (25 and 50 mg/kg). Therefore, it can be concluded that this compound may be part of the diuretic effect of Orthosiphon stamineus, but that other compounds may also be involved.

Schut and Zwaving, 1993 – see Table 3

The flavonoids sinensetin and 3-hydroxy-5,6,7,4-tetramethoxyflavone were isolated from the leaves of *Orthosiphon stamineus*. They were intravenously administered to anaesthetized male rats at 10 mg/kg. In a second experiment on the same experimental model, doses of 1 mg/kg of each compound were compared to the reference compound hydrochlorothiazide (1 mg/kg).

For both compounds, the dose of 10 mg/kg induced a diuretic effect. The dose of 1 mg/kg also produced a diuretic effect, but it was shown that hydrochlorothiazide acts faster and produces a larger

quantity of urine in a shorter time (see Figure 2). The authors suggest that the longer lag time of the flavones might be attributed to an action via metabolites, whereas hydrochlorothiazide is known to act directly on the kidney which explains the shorter lag time.

The authors also state that the total diuretic activity of the leaves may not be attributed to these compounds because only some tenths of milligrams are extracted by hot water from the leaves during preparation of herbal tea. Therefore, they do not seem to be the main active constituents of *Orthosiphon stamineus*.

Assessor's comment

The relevance of this experiment to the use of herbal preparations in humans is questioned in view of the route of administration used in rats. In addition, it seems that no negative control group was included in the study so that any definitive conclusion cannot be drawn from the results obtained. No statistical test was performed.

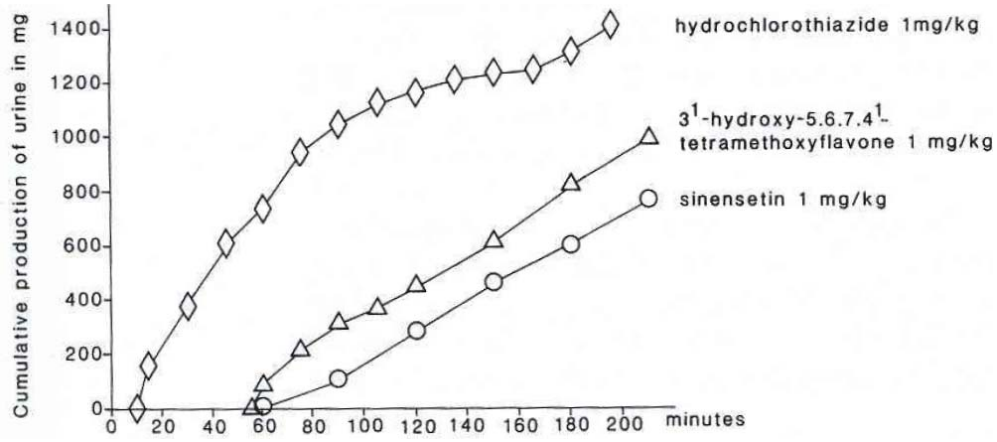
Table 3: studies performed to test the diuretic activity of isolated compounds

Reference	Substance	Species	Route	Duration of urine collection	Doses (mg/kg)	Urinary parameters (compared to controls)						
						Urine volume	Ion concentration in urine			Ion quantity in urine		
							$[Na^+]_u$	$[K^+]_u$	$[Cl^-]_u$	Na^+_u	K^+_u	Cl^-_u
Matsubara et al, 1999	MRC	Rat, sex unknown	Oral	3 hours	25 50 100 HCTZ (25)	x0.9 x1.4 x3.0 x2.9	x0.9 x0.8 x1.0 x2.0	x0.9 x1.0 x0.8 x0.7	x1.0 x0.9 x0.8 x1.6	x0.8 x1.2 x2.9 x5.8	x0.8 x1.5 x2.0 x2.0	x0.9 x1.4 x2.4 x4.7
Schut and Zwaving, 1993	Sinensetin	Rat, males	IV	4 hours	1 10 HCTZ (1)	↑ ↑ ↑						
Schut and Zwaving, 1993	3-hydroxy-5,6,7,4-tetramethoxyflavone	Rat, males	IV	4 hours	1 10 HCTZ (1)	↑ ↑ ↑						

HCTZ : hydrochlorothiazide; MRC: methylripariochromene A

in bold: statistically significant

Figure 2: production of urine of two flavones from *Orthosiphon stamineus* compared with hydrochlorothiazide (Schut and Zwaving, 1993)



Hypouricemic activity and effect on calcium oxalate crystals

Orthosiphon stamineus being traditionally used for irrigation of the urinary tract in cases of renal gravel, some authors investigated its hypouricemic activity in rats, and its effect on the growth of oxalate crystals. It is also noted that diuretics have been used as prophylactic agents for urolithiasis due to their key role in regulating kidney function and alleviating the urinary risk factors for stone formation (Arafat et al, 2008).

Hypouricemic activity

Arafat et al (2008) investigated the effect of a methanol:water (1:1) extract of *Orthosiphon stamineus* (leaves) on uric acid level in hyperuricemic rats. Experimentally, hyperuricemia was induced by injecting potassium oxonate (uricase inhibitor) to groups of 6 male rats. The latter received the extract orally one hour later, at either 250, 500, 1000 or 2000 mg/kg. Negative and positive controls received saline and allopurinol (50 mg/kg), respectively. Uric acid concentration was then measured in samples collected at 0, 2, 4, 6 and 8 hours post-injection.

Results reported in Table 4 below show that the uric acid concentration was statistically decreased in rats treated with the extract at 500 mg/kg and above 6 hours after administration. The uric acid level was statistically decreased at all time points. The authors conclude that the extract showed a marked decrease in uric acid formation as late as 6 hours compared to the more effective allopurinol which may indicate a level of similarity between *Orthosiphon stamineus* and the standard been used.

Table 4: effect of allopurinol and methanol:water (1:1) extract of *Orthosiphon stamineus* on serum urate levels in hyperuricemic rats (Arafat et al, 2008)

Treatment	Time after administration (h)				
	0	2	4	6	8
KOn (250 mg/kg)	7.9 ± 1.2	16.6 ± 1.3	12.1 ± 1.3	13.6 ± 1.1	11.7 ± 2.2
KOn + allopurinol (50 mg/kg)	9.1 ± 1.0	**9.9 ± 2.2***	**3.2 ± 0.2***	**2.9 ± 0.5***	**4.2 ± 0.9***
KOn + MeOH:water 1:1 (2 g/kg)	9.5 ± 1.1	13.9 ± 1.6	9.2 ± 1.9	**7.4 ± 0.7***	9.8 ± 1.4
KOn + MeOH:water 1:1 (1 g/kg)	9.4 ± 1.5	13.4 ± 0.9	11.4 ± 2.1	**7.6 ± 1.1***	9.5 ± 2.1
KOn + MeOH:water 1:1 (0.5 g/kg)	9.9 ± 1.9	17.8 ± 1.9	11.5 ± 1.7	**7.9 ± 0.8***	9.7 ± 1.8
KOn + MeOH:water 1:1 (0.25 g/kg)	8.3 ± 0.9	20.3 ± 2	14.6 ± 3.8	9.5 ± 1.9	10.8 ± 1.8

Mean±SEM, n=6. KOn: potassium oxonate
*p<0.05 vs. KOn

Assessor's comment

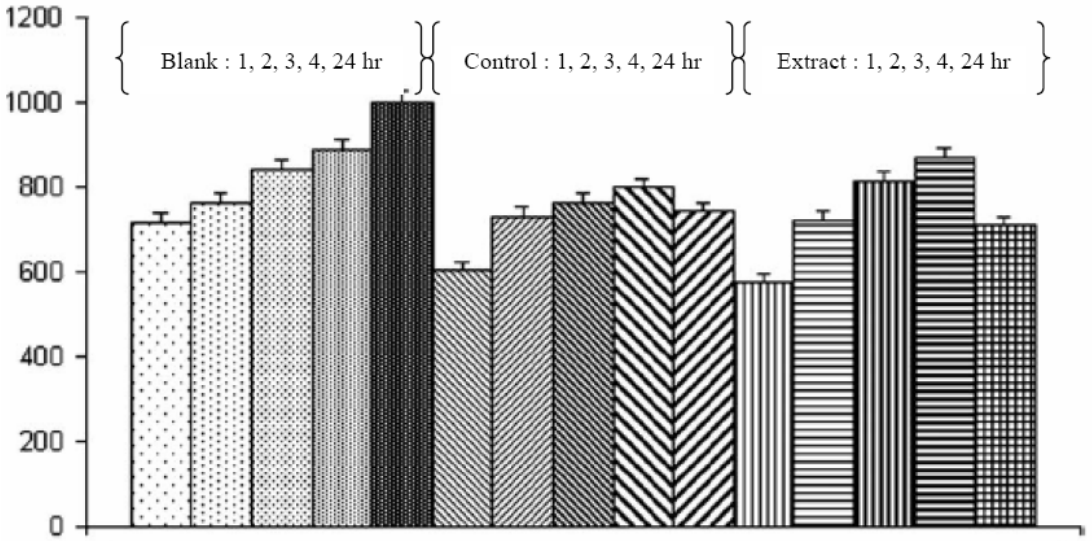
The effect on serum urate level obtained with the extract is slight compared to that obtained with a much lower dose of allopurinol (50 mg/kg vs. 500 mg/kg) in terms of intensity and duration. This is the only study found in the literature dealing with this issue which seems not sufficient to draw a firm conclusion. In addition, it is noticed that the extract tested is not used traditionally making the extrapolation to the clinical situation uncertain.

Effect on calcium oxalate crystals

Using a modified Schneider's gel slide method, Dharmaraj et al (2006) studied the inhibition of calcium oxalate crystal growth by a methanol (50%) extract of *Orthosiphon stamineus* (leaves) at the concentration of 5000 ppm. Sodium citrate (10 ppm) was included as a positive control, and the

experiment also included blank testing. It was concluded that both the extract and sodium citrate inhibited the growth of calcium oxalate crystals at 24 hours (statistically significant effect).

Figure 3: the growth profile for blank, control and sample (adapted from Dharmaraj et al, 2006)



Assessor's comment

This is the only study found in the literature dealing with this issue which seems not sufficient to draw a firm conclusion. Similar effects were observed with sodium citrate and the extract, but the latter was used at a considerably higher concentration (5000 ppm, vs. 10 ppm). It remains to know whether this effect would be observed in vivo. In addition, it is noticed that the extract tested is not used traditionally.

Anti-inflammatory activity

Effect on inflammation induced by TPA in mice

Masuda et al (1992) isolated orthosiphol A and B from a dichloromethane extract of *Orthosiphon stamineus* (leaves) and studied the anti-inflammatory effect of each compound in mice using a tumour promoter, TPA (12-O-tetradecanoylphorbol-13-acetate).

For each compound, a sample (200 µg)[1] and vehicle were applied to the inner part of the left and right ear, respectively, of the same mouse. After 30 minutes, TPA (2 µg)[2] was applied to the same part of both ears. After 6.5 hours, mice were killed, plugs of each ear obtained and weighed.

Each compound showed inhibitory activity, the ratio of which was 42% for orthosiphol A, and 50% for orthosiphol B.

Assessor's comment

Orthosiphol A and B were shown to decrease the inflammation induced by TPA applied on mouse ears. However, similar data obtained with a therapeutically-relevant extract of Orthosiphon stamineus is not available so that no conclusion can be drawn.

Inhibition of NO production

[1] dissolved in 20 µg acetone
[2] dissolved in 20 µg acetone

A team of Toyama Medical and Pharmaceutical University (Toyama, Japan) conducted a series of experiments to identify the biologically active components of *Orthosiphon stamineus* (Awale et al, 2003a, 2003b, 2003c, 2003d; Nguyen et al, 2004).

First, they found that a methanolic extract of aerial parts showed significant inhibition of NO production in lipopolysaccharide (LPS)-activated macrophage-like J774.1 cells with an IC50 reaching 42 µg/mL. Thereafter, they continued their efforts to characterize the NO production inhibitory activity of diterpenes isolated from the methanolic extract, and to elucidate the chemical structure of these compounds.

In these experiments, NO inhibitory assay was performed with cultures of J774.1 macrophage-like cells incubated with LPS and test compound for 24 hours. Then, NO production was determined by measuring the accumulation of nitrite in the culture supernatrant. For each compound, IC50 were calculated. The results are shown in Table 5 and Figure 4.

From the results obtained, it was concluded that 47 diterpenes isolated from *Orthosiphon stamineus* significantly inhibited NO-production in the experimental model used, i.e. LPS-activated macrophage-like J774.1 cells. The intensity of the effect depended notably on the chemical structure of each compound.

Assessor's comment

Forty seven diterpenes were isolated from methanolic extracts of Orthosiphon stamineus and all inhibited NO-production by LPS-activated macrophage-like J774.1 cells with an IC50 lower than that obtained with dexamethasone in the same experimental model. In addition, 15 were more potent than the most potent positive control used (L-NMMA) based on IC50 values. The same effect was also reported with a methanolic extract of Orthosiphon stamineus.

However, methanolic extracts are not reported to be used therapeutically. Therefore, it remains to be determined whether such effect would occur with herbal preparations for human use (although it is noted that diterpenes are involved in the NO production inhibitory effect). It would also have been interesting to have results from another experimental model, but such data were not found in the literature.

Table 5: inhibitory effects of constituents from *Orthosiphon stamineus* on NO production in LPS-activated J774.1 cells

Compounds		Inhibition (%)					IC50
		200 mM	100 mM	50 mM	20 mM	2 mM	
1	Orthosiphol A	99.662.0***	99.663.2***	91.763.7***	61.661.1***	13.568.2*	11.5
2	Orthosiphol B	98.662.7***	99.662.0***	88.064.7***	48.966.5***	25.068.7	20.5
3	Orthosiphol C	75.863.0***	97.563.2***	88.063.2***	55.867.8***	15.6610.0**	14.4
4		99.664.7***	92.263.6***	65.867.6***	26.767.0*	15.666.3**	34.5
5	Orthosiphol F	72.864.8***	50.568.4**	47.867.2***	41.367.6***	30.4610.7**	87.9
6		67.161.5***	30.063.6***	14.963.6**	9.664.8*	7.661.5**	145
7	Orthosiphol H	91.861.2***	98.861.5***	89.560.7***	39.964.9***	12.562.0***	24.1
8		88.363.1***	48.764.3***	21.963.4***	9.063.1**	5.064.9	102
9		97.163.5***	65.261.3***	39.664.7***	20.562.4***	20.563.6**	66.3
10	Orthosiphol K	94.561.6***	93.363.6***	86.161.2***	31.566.1***	1.560.6*	27.3
11	Orthosiphol L	94.561.6***	73.0616.9***	81.563.2***	37.663.1***	8.263.2**	25.1
12	Orthosiphol M	43.063.1***	9.164.3**	8.863.6**	5.562.6*	5.561.4***	>200
13	Orthosiphol N	100.960.6***	100.961.5***	67.063.3***	20.063.4**	6.762.6**	35.9
14		91.865.4***	95.762.0***	85.361.7***	30.468.3*	18.365.1*	27.7

15	Orthosiphol P	98.664.2***	99.162.0***	88.161.7***	43.662.7***	19.165.4*	22.8
16	Orthosiphol Q	101.662.3***	76.664.4***	35.564.9***	26.765.5**	23.164.7**	63.9
17	Orthosiphol R	101.666.0***	93.863.0***	59.166.3***	34.4610.0**	27.0610.3**	35.7
18		103.060.7***	100.761.1***	72.361.6***	29.860.7***	2.966.3	30.9
19		104.760.0***	100.162.5***	67.163.1***	19.664.1***	21.362.1	35.9
20	Orthosiphol U	85.761.1***	61.463.9***	46.165.0***	38.7618.5*	18.463.9*	59.7
21	Orthosiphol V	83.962.2***	65.063.0***	47.964.1***	29.466.5**	18.766.3*	54.5
22	Orthosiphol W	79.260.9***	65.065.3***	46.269.5***	22.564.3***	4.966.9*	57.6
23	Orthosiphol X	97.761.0***	101.560.9***	98.561.4***	77.966.4***	21.7610.6**	6.4
24	Orthosiphol Y	89.564.5***	82.268.5***	56.9618.0**	34.166.3**	21.764.9*	37.9
25	Siphonol A	10864.0***	98.361.6***	94.964.0***	61.466.7***	20.267.3**	10.8
26	Siphonol B	107.661.0***	107.161.1***	97.461.1***	52.262.9***	17.264.0*	17.3
27	Siphonol C	109.062.5***	108.060.0***	81.861.9***	44.461.0***	14.366.8*	22.9
28	Siphonol D	90.162.4***	69.263.2***	51.765.7***	29.966.0**	19.767.5**	46.5
29	Siphonol E	86.062.6***	97.861.9***	81.861.5***	44.262.4***	3.764.9**	23.0
30	3-O-deacetylorthosiphol I	91.964.9***	72.165.0***	48.963.2***	28.067.7**	29.965.5	66.3
31	2-O-deacetylorthosiphol J	93.661.1***	81.762.9***	69.3610.3***	39.3614.9**	27.669.2	24.1
32		96.563.2***	101.661.9***	79.365.1***	18.666.8*	4.4616.3*	32.1
33	7-O-deacetylorthosiphol B	102.161.3***	97.062.8***	88.062.8***	59.769.7***	34.2614.6*	102
34		98.363.2***	80.068.3***	32.2620.3**	22.666.2	29.169.5	64.7
35		101.860.0***	93.160.0***	59.260.0***	10.360.0*	27.260.0	42.1
36		78.160.0***	35.560.0***	15.260.0**	13.560.0**	14.260.0**	127
37		86.264.6***	55.967.0***	31.463.7**	9.569.9*	20.563.6*	84.6
38	Staminol A	79.769.5***	96.660.0***	82.361.3***	38.363.2***	3.862.4	25.5
39	Staminol B	101.660.7***	92.061.7***	16.762.3**	13.462.7**	24.862.3	67.9
40	Neoorthosiphol A	103.060.7***	92.660.7***	59.265.5***	18.064.2***	2.664.4	40.7
41	Neoorthosiphol B	105.060.7***	102.161.9***	92.667.7***	59.567.6***	22.063.1	14.0
42	Norstaminol A	101.261.3***	92.960.7***	55.862.9***	11.162.3*	27.8619.4	44.4
43	Norstaminol B	92.962.5***	88.061.3***	29.063.7**	13.462.0***	21.265.1	64.0
44	Norstaminol C	105.361.3***	106.061.8***	103.965.0***	84.767.8***	27.168.0*	5.0
45	Norstaminolactone A	97.562.2***	74.266.8***	31.463.9**	21.264.5**	8.469.3*	67.6
46	Staminolactone A	101.961.9***	90.062.5***	52.768.8***	12.166.9*	3.9618.1	47.1
47	Staminolactone B	79.361.6***	62.569.7***	53.563.1***	16.2611.7*	28.0613.9	45.9
	L-NMMA	101.663.1***	86.462.5***	67.963.8***	42.964.3***	4.368.2	26.0
	Polymixin B (mg/ml)	94.4611.5***	93.861.1***	73.966.3***	36.7615.4**	19.464.4**	27.8
	Dexamethasone	55.566.7***	32.365.7***	17.362.1***	2.763.5*	29.767.7	170
	CAPE	122.864.4***	119.867.7***	116.265.1***	116.261.9***	34.467.1**	3.1

Each value represents the mean ± S.E.M. (n=4); significantly different from the control: *** p,0.001, ** p,0.01, * p,0.05.

<u>Figure 4:</u> chemical structure of isopimarane- and staminane-type diterpenes isolated from *Orthosiphon stamineus* methanolic extract and tested for NO-production inhibitory activity (Awale et al, 2003a, 2003b, 2003c, 2003d; Nguyen et al (2004).

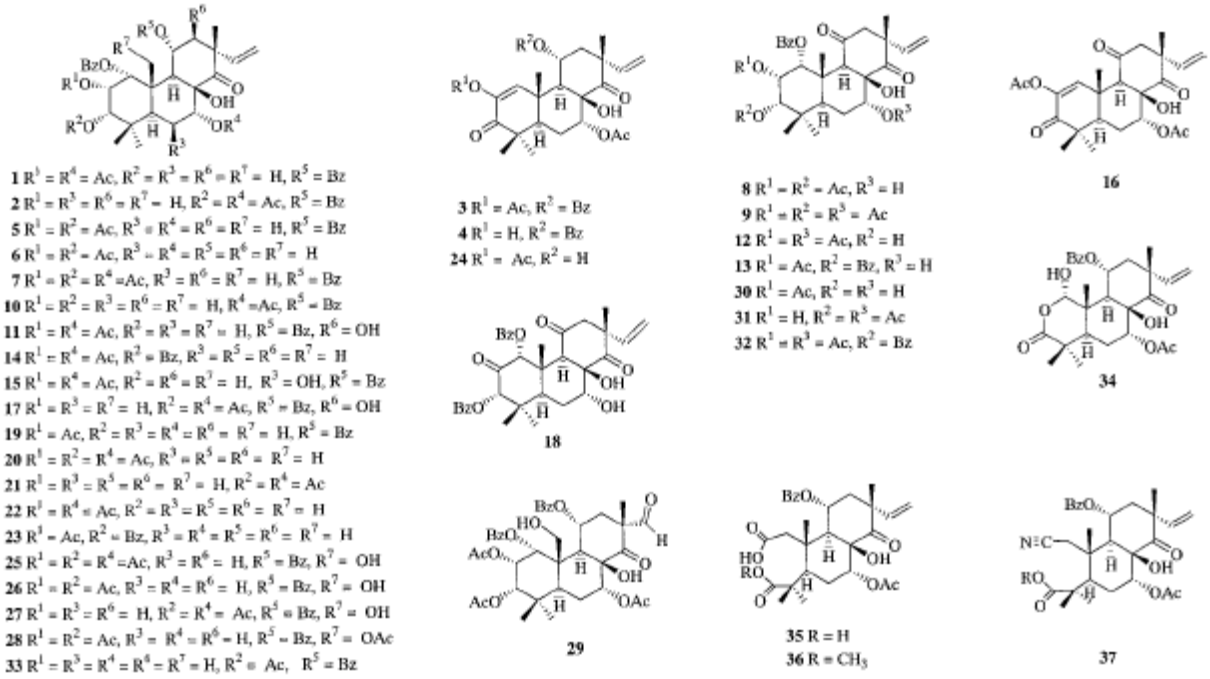


Chart 1. Isopimarane-Type Diterpenes from *Orthosiphon stamineus*

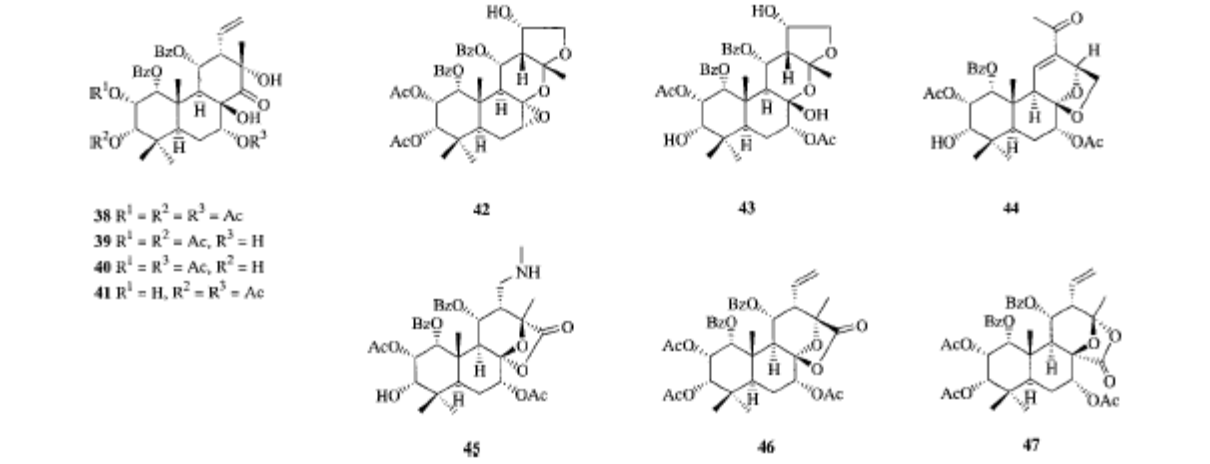


Chart 2. Staminane-Type Diterpenes from *Orthosiphon stamineus*

Effect on arachidonic acid metabolism: inhibition of lipoxygenase

Lyckander and Malterud (1992) tested the effect of an ethyl acetate extract of *Orthosiphon stamineus* (leaves) and 8 lipophilic flavonoids[3] isolated from this extract on the arachidonic acid oxidation catalysed by 15-lipoxygenase. Arachidonic acid or linoleic acid (less expensive and more stable) was used as enzyme substrate because results obtained with both compounds were comparable. Soybean lipoxygenase was used. To justify the use of this experimental model, it is specified that soybean lipoxygenase appears suitable for testing inhibitors of mammalian 15-lipoxygenase, but less so for 5- and 12-lipoxygenase inhibitors. In addition, it is also mentioned that soybean lipoxygenase is easily obtained, and in contrast to other lipoxygenases, inexpensive, fairly stable and easily assayed. According to Russel et al (2008), comparison of 3-dimensional structures of soybean and mammalian 15-lipoxygenase demonstrated that these enzymes have similar topology and analogous active sites.

Results obtained show that the crude ethyl acetate extract inhibited 15-lipoxygenase with an IC50 value amounting to 0.018% (w/v). The two main flavonoids sinensetin and tetramethylscutellarein showed dose-related inhibition with IC50 values of 114 µM and 110 µM. The IC50 of quercetin (positive control) was 98 µM. The other flavonoids tested were less efficient. The total inhibitory activity of flavonoids was found to be much lower than that of the crude extract so that it is hypothesized that synergism occurred between components of the extract, or that it contains other lipoxygenase inhibitors. Based on preliminary results of further work, the authors suggest that the second hypothesis is favoured.

Assessor's comment

Flavonoids isolated from an ethyl acetate extract of Orthosiphon stamineus (leaves) have been shown to inhibit soybean lipoxygenase. The inhibitory activity of the extract was much higher in this experimental model, but it is not used traditionally.

Antibacterial activity

The antibacterial activity of some isolated compounds or herbal preparations was tested by some authors. These experiments are reported in the primary pharmacodynamics section because herbal preparations of *Orthosiphon stamineus* are recommended by Commission E and ESCOP in case of bacterial infections of the urinary tract. Available studies were summarized in Table 6.

Assessor's comment

Only one study was performed with a range of bacterial strains involved in the occurrence of urinary tract infections. However none of the flavones tested showed an antibacterial activity in the experimental conditions used.

Another study investigated more precisely the antibacterial effect of a chloroform extract of Orthosiphon stamineus leaf against Staphylococcus aureus but the data available are scarce. For example, the concentration tested is unknown and no MIC was determined. In addition, it should be noted that the chloroform extract of Orthosiphon stamineus leaf is not used traditionally. Therefore, it is considered reasonable not to take these results into consideration. It is also mentioned that Staphylococcus aureus is not commonly isolated in patients suffering from lower urinary tract infections; it is isolated in urinary tract infections secondary to hematogenous renal infection.

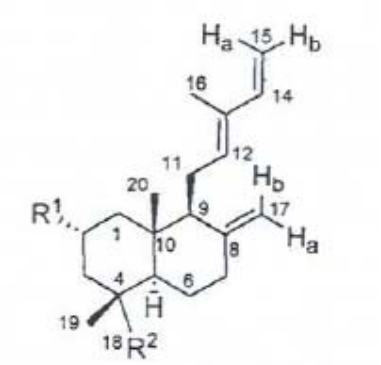
No therapeutically relevant extract was tested for antibacterial activity against bacteria known to cause urinary tract infections.

[3] Sinensetin, tetramethylscutellarein, eupatorin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone, 3'-hydroxy-5,6,7,4'-tetramethoxyflavone, salvigenin, trimethylapigenin, tetramethylluteolin

Other authors showed that an aqueous extract of *Orthosiphon stamineus* displayed an antibacterial effect (intermediate to strong) towards *Streptococcus mutans* responsible for dental caries.

Figure 5 for detailed chemical structure). However, as mentioned by the authors, other Orthosiphon species belonging to the flora of Southeast Asia (like Orthosiphon stamineus) possess staminane and isopimarane derivatives.

<u>Figure 5</u>: structure of the labdane diterpenoids isolated from an ethanolic extract of Orthosiphon labiatus by Hussein et al, 2007



1 $R^1 = OH, R^2 = CH_2OH$
2 $R^1 = OH, R^2 = COOH$

Table 6: antibacterial activity of herbal preparations or isolated compounds

Reference	Compound / herbal preparation		Bacterial strains tested		Results
	Name	Concentration	Name	Pathogeny	
Chen et al, 1989	Aqueous extract of *Orthosiphon stamineus* (whole plant)	0.5 to 250 mg/mL	*Streptococcus mutans* serotype c (MT5091) serotype d (OMS 176)	Dental caries	Due to the pathogeny of the bacterium in humans, experiments were performed in absence or presence of 5% sucrose in the culture medium. MIC (mg/mL) | - 5% sucrose | + 5% sucrose Serotype c | 7.8 | 7.8 Serotype d | 23.4 | 46.9 It was concluded that the extract has strong and intermediate antibacterial activity against *Streptococcus mutans* serotypes c and d, respectively.
Abdel Sattar et al, 1995	Chloroform extract (leaf)	?	*Staphylococcus aureus*	Superficial skin lesions, pneumonia, astitis, phlebitis, meningitis, urinary tract infections, osteomyelitis, endocarditis…	Diameter of the inhibitory zone = 12 mm
Schut and Zwaving, 1993	A – Sinensetin B – 3-hydroxy-5,6,7,4-tetramethoxyflavone C – Tetramethylscutellarein	10 and 100 µg/mL	*Escherichia coli* *Proteus mirabilis* *Pseudomonas aeruginosa* *Staphylococcus aureus* *Enterococcus*	Urinary tract infections	None of the compounds A, B or C showed any antibacterial activity.

Hussein et al, 2007	Two labdane diterpenoids[a] isolated from an ethanolic extract of Orthosiphon labiatus[b] (fresh aerial parts)	Up to 650 µM	Mycobacterium tuberculosis (strain H37RvATCC27294)	Tuberculosis	Compound 1: no activity up to the highest concentration tested Compound 2: inhibitory activity, MIC = 157 µM

MIC: minimal inhibitory concentration

[a] see structure in Figure 5; [b] a species growing in South Africa

Secondary pharmacodynamics

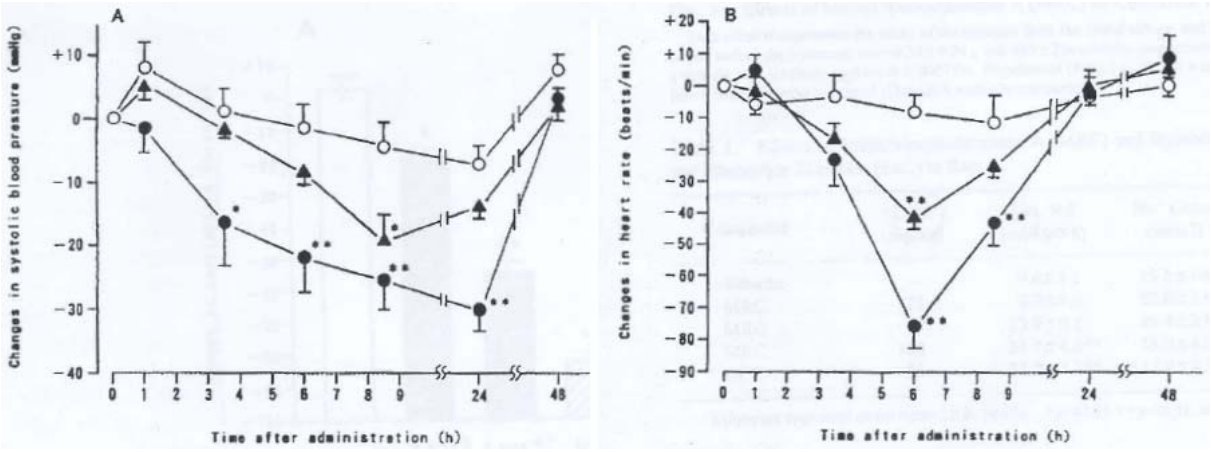
Antihypertensive effects

Methyl ripariochromene A (MRC) was administered subcutaneously at doses of 50 and 100 mg/kg to conscious, stroke-prone, spontaneously hypertensive male rats (SHRSP). A decrease of 15-30 mmHg in mean systolic blood pressure was observed from 3.5 to 24 hours with the higher dose whereas no change was noted in control animals. The lower dose caused a significant decrease only at 8 hours. In the same experiment, MRC caused significant decreases in heart rate in the high dosed group at 6 and 8.5 hours (-75 and -45 beats/min, respectively). The decrease noted in low dosed rats was slight but significant, and noted at 6 hours only. Heart rate figures returned to baseline values after 24 hours. Results are shown in Figure 6 (Matsubara et al, 1999; ESCOP, 2003).

MRC was also shown to suppress concentration-dependent contractions induced by high K+, phenylephrine or prostaglandin F2α in endothelium-denuded rat thoracic aorta (Matsubara et al, 1999). In the same *in vitro* model, neoorthosiphols A and B, MRC, acetovanillochromene, orthochromene A, sinensetin and tetramethylscutellarein were also shown to suppress concentration-dependent contractions induced by K^+.(Ohashi et al, 2000).

After cumulative applications at 3.8.10-5M and 1.2.10-4M to spontaneously beating isolated guinea pig atria (ex vivo), MRC was also shown to significantly suppress the contractile force (-18.8% and -54.7%, respectively) without significantly reducing the beating rate (Matsubara et al, 1999).

Figure 6: time courses of changes in systolic blood pressure (A) and heart rate (B) after subcutaneous administration of MRC in conscious SHRSP (Matsubara et al, 1999)



MRC was administered at doses of 50 mg/kg (▲) and 100 mg/kg (●) (8 animals per group). The vehicle was similarly given to 9 animals (○). Each point is expressed as the mean±S.E. of changes from the initial values. *p<0.05, **p<0.01, significantly different from the corresponding value in the vehicle control group on the respective time (Dunnett's multiple comparison).

Assessor's comment

Results obtained in vivo in conscious stroke-prone spontaneously hypertensive male rats, in vitro and ex vivo showed an antihypertensive effect for MRC, which is not completely unexpected in view of its diuretic property reported previously (see Table 3). However, no data is available with a therapeutically relevant extract so that the human relevance of these results remains unknown.

Hypoglycaemic effects

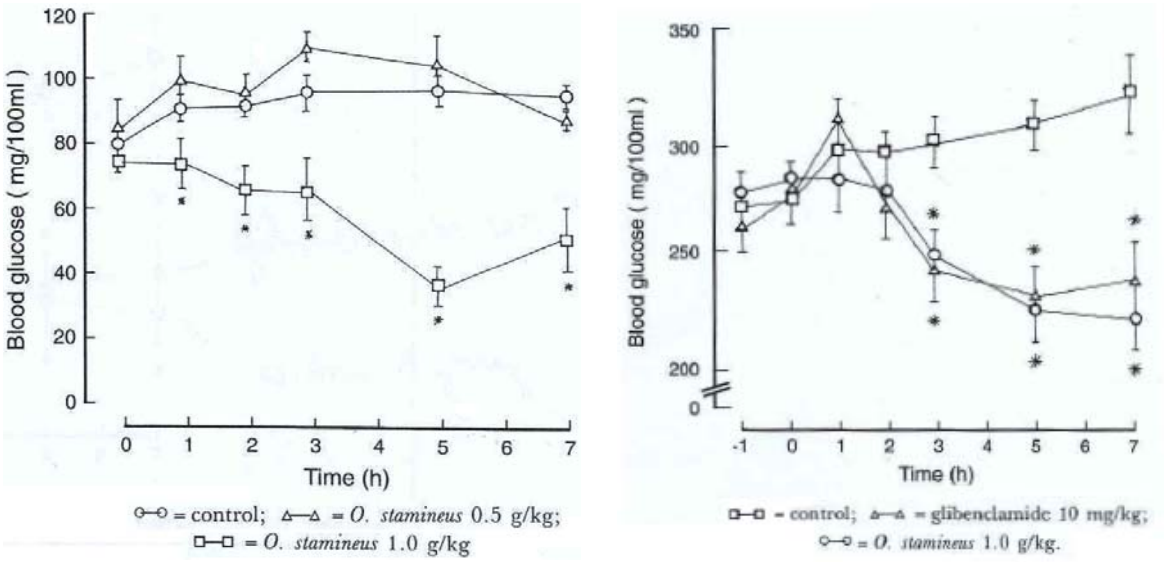
Mariam et al, 1996

An aqueous extract of *Orthosiphon stamineus* (whole plant) was administered to either normal or streptozotocin-induced diabetic rats by oral gavage at 0, 500 and 1000 mg/kg. Blood samples were collected up to 7 hours post-treatment and blood glucose levels were measured.

In normal rats, no significant effect was observed over 7 hours at 500 mg/kg, but a significant decrease was observed from 1 to 7 hours post-dose in animals treated with 1000 mg/kg compared to controls. In diabetic rats, blood glucose levels were significantly lower in animal groups treated with either orthosiphon extract (1000 mg/kg) or glibenclamide (10 mg/kg) than in controls. Effects of extract and glibenclamide on blood glucose were reported to be similar (see Figure 7).

Figure 7: Effect of the aqueous extract of *Orthosiphon stamineus* on blood glucose levels in normal and diabetic rats (Mariam et al, 1996)

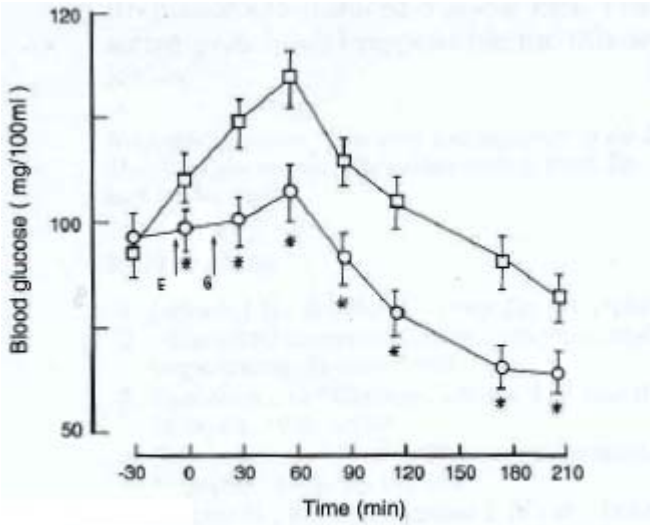
A- normal rats **B – diabetic rats**



An oral glucose tolerance test was then performed by administering orally to normal rats either the vehicle or extract (1000 mg/kg), followed after 15 minutes by an oral glucose load of 1500 mg/kg. Blood samples were collected 30 minutes before the test and every 30 minutes thereafter for 4 hours.

Compared to controls, blood glucose levels measured in rats treated with the extract were lowered over the whole observation period (see Figure 8).

<u>Figure 8:</u> Effect of the aqueous extract of *Orthosiphon stamineus* on oral glucose tolerance test in normal rats



□–□ = control; o–o – *O. stamineus* (E = oral administration of its aqueous extract, 1 g/kg; G – oral administration of glucose, 1.5 g/kg).

According to Mariam et al (1996), these results suggest that the aqueous extract tested possessed some hypoglycaemic activity in both normal and streptozotocin-induced diabetic rats. They indicate that further research is needed to identify the substance(s) responsible for this activity and evaluate the mechanism of action.

<u>Sriplang et al, 2007</u>

An oral glucose tolerance test was performed by administering orally to either normal or streptozotocin-induced diabetic rats an aqueous extract of *Orthosiphon stamineus* (whole plant) at 0, 200, 500 and 1000 mg/kg. A group was also treated with 5 mg/kg glibenclamide as positive control. After 30 minutes, an oral glucose load of 3000 mg/kg was given. Blood samples were collected 30 minutes before glucose loading and up to 210 minutes following glucose loading.

In normal rats, doses of 500 and 1000 mg/kg significantly reduced plasma glucose concentration by 18% and 25%, respectively, 30 min following glucose load. Those figures amounted to 15% and 34%, respectively, after 90 minutes of glucose load. The reduction in plasma glucose concentration was maintained up to the end of the experiment (210 minutes) in rats receiving 1000 mg/kg of extract (see <u>Table 7</u>).

Assessor's comment

The authors mention that glicenclamide reduced glucose levels in normal rats, but the figures reported for glibenclamide (mean ± SEM, <u>Table 7</u>) are exactly the same as those reported for control animals. This may be a typing error, but alters the conclusion that can be drawn from this experiment.

In diabetic rats, doses of 500 and 1000 mg/kg produced a significant reduction in plasma glucose concentrations 90 min following glucose administration. Maximum reduction in plasma glucose concentration amounted to 21% and 24% (210 min). As expected, glibenclamide also reduced glucose levels (see

<u>Table **8**</u>).

Table 7: effect of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration in normal rats (Sriplang et al, 2007)

Treatments	Time (min) before and after glucose administration					
	−30	0	30	90	150	210
Control (distilled water)	78.0 ± 3.2	82.5 ± 3.3	175.1 ± 2.6	137.3 ± 2.8	100.1 ± 6.0	87.1 ± 3.4
0.2 g/kg *Orthosiphon stamineus*	74.7 ± 3.3	71.6 ± 5.0*	176.6 ± 3.4	115.8 ± 4.4*	87.2 ± 5.9	81.1 ± 3.1
0.5 g/kg *Orthosiphon stamineus*	80.4 ± 3.6	72.3 ± 5.1	143.1 ± 0.8*	117.6 ± 2.5*	101.2 ± 4.3	84.5 ± 4.6
1.0 g/kg *Orthosiphon stamineus*	77.3 ± 1.6	72.3 ± 2.4	131.3 ± 2.1*	90.1 ± 2.8*	81.6 ± 2.0*	64.9 ± 4.6*
5 mg/kg Glibenclamide	78.0 ± 3.2	82.5 ± 3.3	175.1 ± 2.6	137.3 ± 2.8	100.1 ± 6.0	87.1 ± 3.4

Data are expressed as mean ± S.E.M.; $n = 6$ rats per group. *$P < 0.05$, compared to normal control group.

Table 8: effect of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration in diabetic rats (Sriplang et al, 2007)

Treatments	Time (min) before and after glucose administration					
	−30	0	30	90	150	210
Control (distilled water)	374.8 ± 15.6	367.8 ± 13.1	534.1 ± 12.4	425.3 ± 11.9	384.3 ± 11.6	384.2 ± 14.1
0.2 g/kg *Orthosiphon stamineus*	348.5 ± 14.9	356.4 ± 10.3	537.7 ± 12.4	391.2 ± 17.3*	353.1 ± 9.5	318.3 ± 11.4*
0.5 g/kg *Orthosiphon stamineus*	365.9 ± 11.9	343.0 ± 11.6	529.9 ± 13.0	385.4 ± 9.5*	352.1 ± 13.9	303.1 ± 11.9*
1.0 g/kg *Orthosiphon stamineus*	370.2 ± 9.6	364.5 ± 12.7	524.8 ± 10.6	380.9 ± 6.31*	342.5 ± 16.1*	291.8 ± 9.3*
5 mg/kg Glibenclamide	371.9 ± 14.1	370.4 ± 11.8	526.4 ± 12.3	343.9 ± 11.4*	333.0 ± 17.0*	284.4 ± 15.3*

Data are expressed as mean ± S.E.M.; $n = 6$ rats per group. *$P < 0.05$, compared to diabetic control group.

In another experiment, diabetic rats were treated orally for 14 days with the extract (500 mg/kg/day), distilled water (negative control), or glibenclamide (5 mg/kg/day, positive control). A group of normal rats treated with distilled water was also included in the study. The last day, fasting plasma glucose was measured, as well as total and HDL-cholesterol, and triglycerides. Histopathological examination of pancreas, kidneys and liver was also conducted.

Results are presented in Table 9. Significant reduction in plasma glucose levels were observed after 7 and 14 days of treatment with either the extract or glibenclamide, compared to diabetic controls. The overall histopathological picture of pancreas, kidney and liver is not reported to be modified between the groups.

Table 9: effect of oral administration of *Orthosiphon stamineus* aqueous extract on plasma glucose concentration, cholesterol, triglyceride and HDL for 14 days (Sriplang et al, 2007)

Treatment	Fasting plasma glucose (mg/dl)			Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-cholesterol (mg/dl)
	0 day	7 day	14 day			
Normal control ($n = 6$)	64.8 ± 17.9	91.92 ± 5.2	81.3 ± 5.9	93.3 ± 3.3	70.4 ± 7.1	44.2 ± 0.9
Diabetes alone ($n = 6$)	379. 7 ± 4.8	407.6 ± 9.5	395.7 ± 5.4	83.5 ± 5.4	110.8 ± 17.2	41.2 ± 2.2
Diabetes + *Orthosiphon stamineus* extract ($n = 6$)	392.7 ± 14.1	356.3 ± 12.5*	341.2 ± 7.1*	87.2 ± 3.5	64.0 ± 8.5*	48.9 ± 1.3*
Diabetes + glibenclamide ($n = 6$)	383.6 ± 8.0	344.8 ± 9.5*	326.8 ± 10.8*	84.2 ± 5.2	105.8 ± 15.6	48.5 ± 1.3*

Data are expressed as mean ± S.E.M.; $n = 6$ rats per group. *$P < 0.05$, compared to diabetic control group.

Further experiments in perfused rat pancreas showed that the extract did not increase insulin secretion in the presence of normal glucose concentration (5.5 mM). At a concentration of 100 µg/mL, the extract potentiated glucose-induced insulin secretion. This effect was not observed at the other concentration used (10 µg/mL).

Assessor's comment

Two published articles dealing with the hypoglycaemic effect suggested for Orthosiphon stamineus were found in the literature. They were performed with aqueous extracts of the whole plant, whereas

the plant part traditionally is the leaf. Normal and diabetic (streptozotocin-induced) rats were used and the route of administration was the same as that used in humans. It was shown in both normal and diabetic rats that the extract (≥ 500 mg/kg) could decrease the plasma glucose concentration following glucose load. In diabetic rats, repeated administrations of the extract at 500 mg/kg/day reduced plasma glucose levels after 7 and 14 days. Results of experiments performed on perfused rat pancreas suggest that the extract is able to potentiate glucose-induced insulin secretion when present at sufficient concentration (100 µg/mL). However, an extrapolation of these results to humans is uncertain in view of i) the limited number of articles dealing with this issue which were found in the literature ii) the considerable gap shown in rats between effective doses of aqueous extract of Orthosiphon stamineus (500 mg/kg/day) and positive control glibenclamide (5 mg/kg/day) iii) the lack of any adverse effect reported in patients that may be related to such an activity in humans (see monograph section 4.8). In addition, other authors reported increased blood glucose levels in rats treated with an aqueous extract of Orthosiphon stamineus leaves (Adam et al, 2009, see Diuretic activity).

Other effects

Antifungal activity:

Guérin and Réveillère (1985) tested a hydro-alcoholic extract of *Orthosiphon stamineus* (DER = 20%) against 9 fungal species. They showed it inhibited the spore germination in 6 fungal species (*Saccharomyces pastorius, Candida albicans, Rhizopus nigricans, Penicillium digitatum, Fusarium oxysporum, Trichophyton mentagrophytes*) and delayed the growth of remaining species (*Aspergillus fumigatus, Aspergillus niger, Botrytis cinerea*). The authors mentioned that the antifungal activity of *Orthosiphon stamineus* had not been established before. Consequently, further research on this plant had to be performed.

Assessor's comment

The antifungal activity of Orthosiphon stamineus was only reported in this article. We did not find any other experimental study in the scientific literature to support these results.

Antitumour acitivty:

Malterud et al (1989) isolated sinensetin and tetramethylscutellarein (the 2 most abundant lipophilic flavonoids found in the drug) from an ethyl acetate extract of *Orthosiphon stamineus* (leaves). They tested the activity of these compounds towards Ehrlich ascites tumour cells *in vitro* in suspension cultures. Both showed a concentration-dependent inhibitory effect with IC_{50} reaching 30 µg/mL and 15 µg/mL, respectively. The authors further indicate that that no cytostatic activity had been reported before for sinensetin. They also mention that tetramethylscutellarein had been previously tested on KB cells (ED50 = 27 µg/mL) but was not reported to be effective on 3 tumours *in vivo*.

Estevez-Nieto (1980) tested the antitumoral activity of *Orthosiphon stamineus* (leaves) dry extracts obtained with the following extraction solvents: ethanol (10%), ethanol (50%), ethanol (95%), water HCl (10%), and methanol HCl (10%). The experimental tumours tested were hepatoma 22 of C3Ha male mice (18-20 g), mammary adenocarcinoma 755 and Harding Pasey melanoma in C57BL male mice (18 g), and leukaemia 1210 in DBA/2 male mice (18 g). Animals were treated by IP route.

No extract showed activity against hepatoma 22 tumours. High toxicity and some antitumoural activity was reported for the ethanolic (50%) extract in animals bearing Harding Pasey melanoma. Some antitumoural activity was also reported for some extracts against mammary adenocarcinoma 755. No antitumoural activity against leukaemia was found for any extract.

Assessor's comment

Results obtained in vitro are not supported by in vivo studies for tetramethylscutellarein, and no in vivo study is available with sinensetin. In our opinion, in vitro / in vivo discrepancies may be explained in part by pharmacokinetic characteristics of each compound but data is lacking (see 3.2.). For example, a first approach to evaluate the influence of metabolism on the activity of these compounds towards Ehrlich ascites tumour cells could have consisted of adding a metabolic activation system in the culture medium.

The results obtained by Estevez-Nieto (1980) are reported to be preliminary results, but we did not find further articles dealing with this issue. The information provided is rather scarce, and there is little information about the extracts used (DER, manufacturing process are not described). Most of them are not used traditionally. In addition, the route of administration used is the IP route. Therefore, it seems reasonable not to take these isolated results into account.

Choleretic activity:

While they were studying the diuretic activity of two flavones (sinensetin and 3-hydroxy-5,6,7,4-tetramethoxyflavone) in rats, Schut and Zwaving (1993) also evaluated their choleretic activity by measuring bile production every 15 minutes for a period of 4 hours (general study design detailed previously). Doses of 10 mg/kg of these flavones did not increase the production of bile in the experimental conditions used.

Assessor's comment

Sinensetin and 3-hydroxy-5,6,7,4-tetramethoxyflavone (10 mg/kg, iv route) were not shown to possess choleretic activity in male rats.

Anti-pyretic activity:

The anti-pyretic activity of a standardized methanol:water (50/50) extract of *Orthosiphon stamineus* was investigated for its effect on normal body temperature and yeast-induced pyrexia in SD rats. The extract showed no effect on normal body temperature. Doses of 500 and 1000 mg/kg bw significantly reduced the yeast-induced elevation in body temperature. This effect persisted up to 4 h following the administration of the extract, and was comparable to that of paracetamol 150 mg/kg (p.o.), a standard anti-pyretic agent (Yam et al, 2009).

Assessor's comment

An anti-pyretic effect was reported in one study for a standardized methanol:water extract of Orthosiphon stamineus (not used traditionally).

Antioxidant activity

Water extracts of *Orthosiphon stamineus* (leaves) samples collected from different locations of Malaysia showed antioxidant activity based on β-carotene coupled with autoxidised linoleic acid system. The results of this study indicated that all extracts showed antioxidant activity comparable to that of quercetin and butylated hydroxylanisole (Akowuah, 2003).

Assessor's comment

Antioxidant activity was reported in one study for water extracts of Orthosiphon stamineus leaves.

Safety pharmacology

No data found in literature.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Overview of available data

No available data with an extract of *Orthosiphon stamineus* or isolated compounds were found.

Assessor's comment

A comprehensive research in the scientific literature did not find any study designed to evaluate the pharmacokinetics of neither any extract of Orthosiphon stamineus nor isolated compounds (orthosiphols, staminols, etc.).

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

Single dose toxicity

The intraperitoneal LD50 of an ethanolic (50% v/v) extract of *Orthosiphonis herba* amounted to 19.6 g/kg in ICR mice (Chow et al, 1979).

Assessor's comment

The acute toxicity of the extract tested by Chow et al (1979) is low. Unfortunately, the details of symptoms observed in animals remain unknown (article in Chinese / abstract in English).

Repeat-dose toxicity

Chin et al (2008) recently conducted a 14-day toxicity study with a methanolic extract of *Orthosiphon stamineus* (leaves). The study design and main results are presented in Table 10.

According to the authors, this study was undertaken to examine the possible toxicity effect of oral administration of methanol extract of *O. stamineus* in Sprague Dawley (SD) rats and hence to determine the LD50, no-observable effect level (NOEL) and no-observable adverse effect level (NOAEL). Fixed dose procedures (OECD guideline 420) were followed.

First, it is concluded that LD50 value could not be determined in this study as no mortality occurred at doses up to 5 g/kg. A test compound that causes no adverse effect at a dose exceeding 5 g/kg will be considered as 'practically non-toxic'.

Second, the authors conclude that the extract displayed beneficial rather than adverse effects on the liver, based on decreased serum AST and ALT levels observed at 1 and 3 g/kg/day, and 5 g/kg/day, respectively. Increased relative liver weight was reported at the two highest dose levels, and is suggested to be related to enhancement of activity of metabolizing enzymes. It is also mentioned that this effect was reversible.

Third, according to the authors and based on the results obtained after analysing serum urea, creatinine, total cholesterol and triacylglycerol, this study has demonstrated that repeated administration of the extract had no direct adverse effect on kidney function and also lipid metabolism in normal young female SD rats.

The NOAEL was determined at 5 g/kg/day, and the NOEL at 0.5 g/kg/day.

Table 10: repeat-dose toxicity study conducted with a methanolic extract of *Orthosiphon stamineus* (Chin et al, 2008)

Species	Route, duration, doses	Parameters monitored	Major findings
Rat (SD) 10F /group	Oral route 14 days 0*, 0**, 0.5, 1, 3, 5 g/kg	Clinical symptoms, food and water intake, body weight gain, hematocrit and clinical biochemistry at day 14 (AST, ALT, ALP, urea, creatinine, total cholesterol, triacylglycerol), 5F/group sacrificed at the end of administration period: macroscopic examination and weight of some organs (liver, kidneys, lungs, spleen) 5F/group selected for a 14-day recovery period: clinical symptoms, food and water intake, body weight gain, macroscopic examination and weight of some organs (liver, kidneys, lungs, spleen)	Clinical biochemistry ↓ serum AST (1 and 3 g/kg) ↓ serum ALT (5 g/kg) Organ weights ↑ relative liver weight (3 and 5 mg/kg) – not observed after treatment-free period

* untreated animals; ** vehicle-treated animals

Assessor's comment

The aim of the study is unclear, i.e. determination of endpoints related to both acute (LD50) and subacute/chronic toxicity (NOAEL, NOEL). Groups of animals were treated for 14 days with the same dose level, so that it seems to be rather a subacute toxicity study. In addition, it is pointed out that the number of animals used is insufficient because only females were used.

Based on decreased ALT and/or AST levels, it is concluded that the extracts possess beneficial effects on the liver. This statement cannot be supported, because the biological significance (e.g. dose-relationship) and the cause of these effects were not investigated. For example, potential causes of decreased serum activities of ALT and AST are reported to include: decreased hepatocellular production or release of the enzymes, inhibition or reduction of the enzyme's activity, interference with the enzyme assay (PSD, 2007). In addition, increased liver weight is suggested to be related to increased enzymatic activity. However, no definitive conclusion on liver effects can be drawn without histopathological examination. This is also true for other organs. The authors underline the need of histopathological examination.

Overall, it is considered that no definitive conclusion can be drawn from this study mainly due to the lack of histopathological examination. It is also noted that the extract administered to the animals is not used traditionally in humans so that the relevance of these data seems rather limited.

Genotoxic and carcinogenic potentials

No data available.

Assessor's comment

The monograph published by the ESCOP (2003) and the review made by Bradley (2006) present the results of a somatic segregation assay on Aspergillus nidulans D-30 (Ramos Ruiz et al, 1996). However, this test is not conventionally used for regulatory purposes in the evaluation of the genotoxic potential of medicinal products (either chemical or herbal). Therefore, this study cannot be taken into consideration.

According to nonclinical guidelines on herbal medicinal products (EMEA/HMPC/32116/05 and EMEA/HMPC/107079/07), at least one Ames test should be performed for herbal substances / preparations. As mentioned in the pharmacology part of this assessment report, some studies have reported or suggested an antibacterial activity for Orthosiphon stamineus extracts (or isolated compounds). ***When performing the Ames test with extracts for which registration will be sought, attention will have to be paid to a potential bacteriostatic effect of tested preparations in order to exclude any risk of false negative results****.*

Due to the lack of a carcinogenicity study, the duration of treatments with herbal medicinal products prepared from Orthosiphon stamineus should not exceed 6 months.

The lack of genotoxicity and carcinogenicity studies will be reported in monograph section 5.3.

Reproduction toxicity

No data available.

Assessor's comment

The lack of reproduction toxicity studies will be reported in section 5.3.

3.4. Overall conclusions on non-clinical data

Overall conclusions on non-clinical data

Pharmacology

Primary pharmacodynamics

In view of the traditional use claimed for the leaves of *Orthosiphon stamineus*, published data dealing with diuretic, anti-inflammatory, antibacterial activities and effects on uric acid level and calcium oxalate crystals were reviewed.

In rats, some authors reported a diuretic effect after oral administration of either aqueous or ethanolic (50% and 70%) extracts, as shown by increased urinary volumes compared to controls. However, a clear conclusion regarding the dose-effect relationship cannot be drawn. The oral effective doses are approximately 10-18 mg/kg for the aqueous extract, and 13.5 mg/kg for the 70% ethanolic extract. (Casadebeig-Lafon et al 1989, Adam et al 2009). However, the lack of effect of similar extracts administered at doses up to 1000 mg/kg was also noted. This discrepancy may be related to differences in the qualitative and quantitative composition of the extracts. In view of the results obtained by Arafat et al (2008), it could also be hypothesized that the lack of diuretic activity observed in some studies may be related to the fact that the extracts were administered only once and that a diuretic effect may have been reported upon repeated administrations.

Other authors demonstrated a diuretic activity of an ethanolic extract of *Orthosiphon stamineus* in male rats, but the clinical relevance of their results is questionable as the intraperitoneal route was used. Similarly, Chow et al (1979) showed in dogs that the administration of a 50% ethanolic extract

by the IV route induced an increase in urine volume. Although the route of administration is not that used traditionally in humans, it is interesting that the tubular reabsorption of sodium and chloride ions was reduced in treated animals.

According to the results published by Matsubara et al (1999), MRC is involved in the diuretic activity of *Orthosiphon stamineus* preparations. However, as the effective oral dose amounted to 100 mg/kg, it is concluded that other components may also be involved.

One study investigated the ability of a methanolic extract to decrease serum urate levels, and another one that of a similar extract to inhibit the growth of calcium oxalate crystals. Firm conclusions cannot be drawn, because the amount of data is not sufficient and the extracts used were not therapeutically relevant regarding the traditional use. For instance, it may be considered that the traditional use of *Orthosiphon stamineus* preparations in complaints of renal gravel is rather related to their diuretic activity.

The anti-inflammatory activity of orthosiphon extracts not used traditionally or isolated compounds was assessed. Their relevance to the administration to humans of extracts considered as traditionally used is therefore uncertain. First, it was shown that orthosiphol A and B decreased the inflammation induced by TPA applied on mouse ears. Second, it was also reported that a methanolic extract of *Orthosiphon stamineus*, as well as 47 diterpenes isolated from this extract inhibited NO-production by LPS-activated macrophage-like J774.1 cells. Third, flavonoids isolated from an ethyl acetate extract of *Orthosiphon stamineus* (leaves) were shown to inhibit soybean lipoxygenase which appears suitable for testing inhibitors of mammalian 15-lipoxygenase.

Antibacterial activity was not demonstrated against bacteria involved in urinary tract infections, for either a therapeutically-relevant extract or isolated compounds. The traditional use of *Orthosiphon stamineus* preparations in bacterial complaints of the lower urinary tract may therefore be related to their diuretic activity.

Secondary pharmacodynamics

Results obtained *in vivo* in conscious stroke-prone spontaneously hypertensive male rats, *in vitro* and *ex vivo* showed an antihypertensive effect for MRC, which is not completely unexpected in view of its diuretic properties. However, no data is available with a therapeutically relevant extract so that the relevance of these results to the clinical situation remains unknown.

Two publications dealing with the hypoglycaemic effect of oral aqueous extracts of *Orthosiphon stamineus* (whole plant) in normal and diabetic rats were found in the literature. However, an extrapolation of these results to humans is uncertain (see - Hypoglycaemic effects for more details).

Pharmacokinetics

A comprehensive research in the scientific literature did not allow finding any study designed to evaluate the pharmacokinetics of neither any extract of *Orthosiphon stamineus* nor isolated compounds (orthosiphols, staminols, etc.).

Toxicology

The available toxicological data is rather limited. The acute toxicity of an ethanolic extract of Orthosiphonis herba is low by intraperitoneal route in mice, as shown by the LD50 which amounted to 19.6 g/kg.

A 14-day toxicity study was performed in rats by oral administration of a methanolic extract of *Orthosiphon stamineus* leaves. However, this study is not considered relevant for risk assessment notably in view of the insufficient number of animals used, the lack of histopathological examination, and the lack of traditional use of the extract administered to animals.

No conventional genotoxicity, carcinogenicity and reproduction toxicity studies are available.

Monograph section 5.3

Conventional genotoxicity, carcinogenicity and reproduction toxicity studies were not performed.

4. Clinical Data

Clinical data on efficacy and safety of Orthosiphonis folium are very limited in the different indications as above listed.

4.1. Clinical Pharmacology

Clinical pharmacology on Orthosiphonis folium is not well documented.

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

In the literature, only data about the *diuretic and choleretic effects* of Orthosiphonis folium and its *effect on urinary stone* were found.

Diuretic effects and effects on urinary stone:

Early pharmacological studies (1927-1928) in three individuals, involving self-administration of aqueous extracts of Java tea, demonstrated increases in urine volume (Bradley P.R. British Herbal Compendium 2006). In these studies, increased diuresis was reported after oral administration of 400 ml/day of a 3.75% extract, 400 ml/day of a 15% extract and 500 ml/day of a 3.3% extract to healthy volunteers (ESCOP Monographs).

Assessor's comment:

These two publications Schumann R. 1927 and Westing J. 1928 which are the data source are not available. Thus, we can not assess these data.

Only two publications are available. These studies are further detailed below.

Studies on the individual and combined diuretic effects of four Vietnamese traditional herbal remedies (*Zea mays*, *Imperata cylindrica*, *Plantago major* and *Orthosiphon stamineus*) - Doan Du Dat and al. 1992.

Orthosiphon stamineus was tested in a placebo, controlled, double-blind, and crossover study.

Methodology:

Forty healthy volunteers aged 18 to 27 years were recruited in a 4-day trial. Because of space limitations, the subjects were divided up into two groups of 20, each of them subjected to a 4-day trial within a period of two consecutive weeks.

On the first day, the volunteers were given full information about the study and they were examined clinically. Blood samples were taken for measuring serum haemoglobin, creatinine, Na+ and K+.

On the second day, the volunteers received either the drug (decoction) or the placebo according to the randomization.

On the third day, a 'wash-out day', the subjects were given only standardized food and fluid amounting to the same quantity as during the treatment days.

On the fourth day, they were given the alternative decoction, placebo or herbal drug. Three other herbal remedies are also assessed on the same way (*Zea mays*, *Imperata cylindrica*, *Plantago major*).

The volunteers were not allowed to take any other medicines and smoking was forbidden.

A total volume of 1.2 L of fluids (water, soup, herbal drug or placebo) was consumed daily at fixed regular intervals. All intakes of food and liquids was carefully supervised and recorded.

Urine was collected every day at 8h before the administration of the first dose of drug/placebo and at 10, 12, 14, 16, 18, 20 and 22h and the volume was measured. Urine was collected every 24h for sodium and potassium determination.

Endpoints:

24 hours volume of urine output, 24h urine Na+ and 24h urine K+.

Extract:

For one daily dosage (600ml of water extract, 3 x 200 ml at 4 hour intervals), 10 g of the dried leaves of *Orthosiphon stamineus* were used.

Results:

Tables 11 and 12 below summarise the main results of this study.

Table 11: 24h volume of urine output in litres (+/- SD) during medication with *orthosiphon stamineus* and the placebo respectively:

Orthosiphon stamineus	Number	Remedy	Placebo	P value
Week 1	20	1.77 (0.48)	1.71 (0.32)	NS
Week 2	20	1.79 (0.31)	1.82 (0.35)	NS
Week 1 + 2	40	1.78 (0.40)	1.76 (0.34)	NS

Table 12: average 24h urine output of sodium and potassium before and after the herbal drug and the placebo respectively. (mmol/l of urine, average +/- S.D.)

Orthosiphon stamineus	Number	Intervention remedy		Placebo	
		Before	After	Before	After
24h urine Na+	20	136 (28.0)	106 (19.0)	136 (22.0)	105 (15.2)
24h urine K+	20	68.0 (2.7)	65.5 (1.3)	68.0 (3.6)	66.1 (1.3)

There was no statistically significant difference regarding the 24h urine output between *Orthosiphon stamineus* and placebo.

Furthermore, no differences were recorded when totalling the urine output during the first 12h of the day (1.16 (0.33) for *Orthosiphon stamineus* and 1.13 (0.24) for placebo - NS).

Comparing the output of urine sodium and potassium before and after the first day of treatment, there was a non-significant reduction in the total amount of salt.

Assessor's comment:

No influence was observed on 12- or 24-hour urine volume or excretion of sodium and potassium after administration of 600 ml (3 x 200 ml at 4 hour intervals) of an aqueous decoction of java tea to 40 healthy young volunteers. The 24h sodium and potassium urine output remained unchanged before and after the first treatment day.

However, this trial has a very short duration (one day of treatment) and cannot measure any late diuretic effect. The doses used might have been too low. Moreover, an impact of the environmental temperature was logged during the trial. The average temperature in the ward during the 2 weeks of trial was 2.1°C or more over that recorded during the other weeks of trial which might have influenced the results.

Effects of folia orthosiphonis on urinary stone promoters and inhibitors - Nirdnoy and al. 1991.

Methodology:

This study was carried out upon 6 healthy male volunteers who had no history of renal stone, renal bone and joint diseases.

On the control day, the volunteers drank 250 ml of water every 6 hours or 4 times per day. Urine samples were collected into 3 aliguots between 8.00-14.00, 14.00-20.00 and 20.00-8.00 hours.

On the treatment day, the volunteers drank tea 250 ml four times in one day at 6 hour intervals and the urine collection was done in the same moment as on the control day; at other times the volunteers could drink water as usual.

Endpoints:

Urine pH, calcium, sodium, potassium, chloride, citrate, titratable acidity, ammonia, osmolarity, magnesium, phosphorus, uric acid, oxalate, volume and creatinine were analysed.

Extract:

Decoction of Orthosiphonis folium (*Orthosiphon gradiflorus*) tea was prepared by boiling dry leaves and flowers of the herb weighing about 5.3 g in one litre of water.

Laboratory analysis of the tea revealed pH 5.730, phosphorus 0.220 mg/100 ml, potassium 11.70 mg/100 ml, calcium 3.37 mg/100 ml and citrate 4.69 mg/100 ml.

Results:

Table 13: urinary pH

Time	Control	Orthosiphon
08:00 – 14:00	6.088 +/- 0.266	6.477 +/- 0.185
14:00 – 20:00	6.138 +/- 0.250	6.202 +/- 0.266
20:00 – 08:00	5.868 +/- 0.102	5.844 +/- 0.126

An analysis of the urine showed an increase in the urinary pH of the first 6 hour period (8.00-14.00), from 6.088 +/- 0.266 to 6.477 +/- 0.185 and the titratable acidity decreased significantly from 25.64 +/- 2.18 (control) to 22.94 +/- 2.15 mEq/day ($p<0.05$).

Java tea produced no significant changes in urine volume, or in excretion of sodium, potassium or chloride compared to the control day. Osmolarity, creatinine, magnesium, phosphorus and ammonia were not changed significantly.

Citrate, which is known to be a potent stone inhibitor, showed an increase in the value from 341.51 +/- 10.89 (control) to 430.76 +/- 13.80 mg/day (Orthosiphon) but it was not significant.

Uric acid also showed a non significant increase from 591.96 +/- 50.37 (control) to 699.85 +/- 76.36 (orthosiphon) mg/day.

There was an increase of urinary calcium from 115.07 +/- 14.67 to 141.03 +/- 20.38 mg/day ($p<0.1$) but it was not significant and the urinary calcium level was still within normal limits.

Oxalate showed a significant increase from 22.91 +/- 1.80 (control) to 30.10 +/- 2.20 mg/day (Orthosiphon) ($p< 0.05$).

The increased level of uric acid may predispose to a higher risk of stone formation but the increased alkalinity may prevent the uric acid precipitation. Citrate was increased, which is in favour of stone prevention because citrate is a stone inhibitor. In the other hand, the increased excretion of oxalate in the urine may result in higher risk of stone formation. Otherwise, the majority of results are not statistically significant except for the titratable acidity and the urinary oxalate. In this study, which has been done only on healthy volunteers, all parameters were within normal limits after treatment. Thus, the authors concluded that Java tea may be beneficial in prevention of uric acid stone formation, primarily due to decreased acidity of the urine.

Assessor's comment:

The study of the effects of orthosiphon tea on healthy volunteers showed increased alkalinity of the urine 6 hours after ingestion. The titratable acidity was significantly decreased but there were no changes in the urine volume, creatinine and electrolytes. Only oxalate showed a statistically significant increase in the orthosiphon group.

These results indicate that orthosiphon has no diuretic effect but could be helpful for the prevention of recurrent uric acid stone due to its effect on the acidity.

However, due to the fact that this study has been carried out in a very limited sample size (6 healthy volunteers) and results were assessed after only one day of treatment, the clinical relevance of such results, whatever the parameters analysed, is limited and should be confirmed by additional clinical data.

Choleretic effect:

In early experiments (1935) on healthy volunteers, it was shown by means of duodenal probes and X-rays that intravenous administration of a Java tea preparation increased the production of bile and its liberation from the gall bladder (Bradley P.R. British Herbal Compendium 2006 – Rutenbeck H. 1935).

Assessor's comment:

The publication from Rutenbeck H. (1935) is a summary and experiments are not detailed.

Assessor's overall conclusions on pharmacodynamics

Diuretic effects

Only two publications are available and can be assessed (Doan Du Dat and al. 1992 – and Nirdnoy and al. 1991)

These two publications have methodological weaknesses although one has a double blind, placebo-controlled, crossover design (Doan, 1992). The first study is a 4-day trial with 40 healthy volunteers and the second study is a 1-day trial with only 6 volunteers.

In these two studies, Orthosiphonis folium produced no significant changes in urine volume or excretion of electrolytes. Further clinical data are required to establish a real diuretic effect of Orthosiphonis folium.

Effect on renal gravel

In the study by Nirdnoy and al. 1991, there was a decrease in acidity and an increase in urinary pH that was statistically significant. These results indicate that orthosiphon could be helpful for the prevention of recurrent uric acid stone which depend on urinary pH. However, due to the fact that this study has been carried out in a very limited sample size (6 healthy volunteers) and results were assessed after only one day of treatment, the clinical relevance of such results are limited, whatever the parameters analysed. The results should be confirmed by additional clinical data.

Choleretic effects

Only one publication is available (Rutenbeck, 1935). In healthy volunteers, Orthosiphonis folium increased the production of bile and its liberation from the gall bladder but data are very scarce.

Other activities of Orthosiphonis folium have been raised in the different monographs and in the literature. Moreover, Orthosiphonis folium has traditionally been used in Java for the treatment of *hypertension* and *diabetes*. It has also been used in folk medicine for *bladder* and *kidney disorders*, *gout* and *rheumatism*. After review of the documentation, no pharmacodynamic study was found to confirm the anti-inflammatory, antibacterial, antihypertensive, hypoglycaemic, and antifungal activities/properties in line with this traditional use.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data are available. The pharmacokinetics of Orthosiphonis folium extract has not been studied.

4.2. Clinical Efficacy

4.2.1. Dose response studies

According to the provided literature, no dose-finding studies have been conducted with Orthosiphonis folium.

Dosage recommendations found in available monographs are similar: 2-3 g of dried material in 150 ml of water in the ESCOP Monograph to 6-12 g daily in the German Commission E Monograph.

In the clinical trials, the posology used is 10 g daily in the Doan study and 5.3 g/litre of water in the Nirdnoy Study.

4.2.2. Clinical studies (case studies and clinical trials)

The information on the clinical efficacy of *Orthosiphon stamineus* Benth. is very limited. Only two publications about the diuretic effect and the effect on renal gravel of Orthosiphonis folium were found. These two studies are further detailed below.

Mercier F. and Mercier L.J. (L'Orthosiphon stamineus, médicament hépat-rénal. Stimulant de la dépuration urinaire. Le bulletin médical, 1936).

This publication is related to the diuretic activity of Orthosiphonis folium but unfortunately, it is not available. Only data were found from this article in two monographs (ESCOP Monograph and the British Herbal Compendium).

Methodology

This was an open study involving 14 patients with azotaemic uraemia associated with various other ailments. Patients were treated during 10 – 15 days with Java tea.

Endpoints

Urine volume, elimination of urea and chloride

Extract

For one daily dosage, 500ml of a 12% infusion of Java tea were used (5x100 ml daily).

Results

Java tea increased urine volumes substantially, in some cases more than two-fold within 4-5 days, together with increased elimination of urea and chloride.

Assessor's comment:

The study from Mercier F and al. (1936) is not available. The data were found in two monographs only. Thus, it is difficult to assess these data which are very scarce. However, even if not detailed particularly in quantitative terms (statistical analysis, results), an increase in the urine volume was observed in this study, which could partially sustain a diuretic activity of Orthosiphonis folium.

The therapeutic effect of Java tea and Equisetum arvense in patients with uratic diathesis, Tiktinsky and al, 1983

This publication is related to the diuretic activity of Orthosiphonis folium and its effect on renal gravel.

Methodology:

The effect of orthosiphon (Java tea) and *Equisetum arvense* on the course of uratic nephrolithiasis was studied in 67 patients with uratic diathesis throughout the three-month treatment course.

Patients were divided into two treatment groups. The first group (34 patients) was given Java tea and the second group (33 patients) consumed *Equisetum arvense* tea.

Extract:

The composition of Java tea and *Equisetum arvense* tea was not known.

Endpoints:

Diuresis, urine pH, glomerular filtration rate (GFR), osmotic urine concentration, plasma content and excretion of calcium, inorganic phosphorus and uric acid, renal clearance and daily urine volume.

Results:

The two groups were equivalent in terms of age (the majority of patients were between 41 and 60 years of age), length of time of disease, sex, metabolic disorders, urodynamic characteristics, functional condition of the kidneys and other parameters.

Table 14: baseline characteristics

	Orthosiphonis folium	**Equisetum arvense**
Diuresis	1244 +/- 191.9 ml	1184 +/- 140.7 ml
Glomerular filtration rate	72.5 +/- 0.93 ml/min	74.5 +/- 0.97 ml/min
Blood calcium content	2.487 +/- 0.241 mmol/l	2.393 +/- 0.165 mmol/l
Urine calcium	6.804 +/- 1.265 mmol/24h	5.97 +/- 0.736 mmol/24h

45.4% of patients had a plasma level of uric acid between 0.357 mmol/l and 0.422 mmol/l and the mean plasma level of uric acid was 0.27 mmol/l.

Table 15 below summarises the main results of this study.

Table 15: Diuresis at week 4 and 12, Glomerular filtration rate, blood calcium content and urinary calcium

Endpoints	Orthosiphonis folium	Equisetum arvense
Diuresis (week 4)	+ 10%	+ 18%
Diuresis (week 12)	+ 15%	+ 24%
Glomerular filtration rate GFR	88.4 +/- 1.2 ml/min = + 18%	87.8 +/- 1.1 ml/min = + 22%
Blood calcium content	2.58 +/- 0.22 (p<0.1)	2.52 +/- 0.17 mmol/l (p<0.05)
Urine calcium	0.49 +/- 0.06 mmol/l (p<0.01)	0.43 +/- 0.05 (p<0.001)
Blood phosphorus content (week 4)	-	1.29 +/- 0.16 mmol/l (p<0.05)
Urine phosphorus (week 12)	-	9.99 +/- 1.61 mmol/l (p<0.01)
Urine PH	7.69 +/- 0.228 (p<0.001)	5.35 +/- 0.241 (p<0.005)

Both agents increased diuresis and GFR. At week 12, Java tea increased diuresis by 15% and *Equisetum arvense* by 24%. For GFR, Java tea increased GFR by 18%, while *Equisetum arvense* by 22%. Both agents had a diuretic effect, even if the diuretic effect of Orthosiphonis folium was smaller than the diuretic effect of *Equisetum arvense*.

Long-term use of Java tea led to the alkalinization of the urine (up to pH 7.69 ± 0.228, p<0.001), while Equisetum arvense, in the opposite, had acidifying effect (down to pH 5.35 ± 0.241, p<0.005). This phenomenon was of paramount importance, since the low urinary pH during the long-term use of *Equisetum arvense* explained the continued crystalluria of urates and the consequent development of clinical symptoms.

There was a non-significant increase of the plasma level of calcium from 2.487 +/- 0.241 mmol/l to 2.58 +/- 0.22 (p<0.1) for Orthosiphonis folium. For *Equisetum arvense*, blood calcium content showed a statistically significant increase from 2.393 +/- 0.165 mmol/l to 2.52 +/- 0.17 mmol/l (p<0.05).

Both agents improved the plasma content and the excretion of inorganic phosphorus. Both preparations reduced osmotic urine concentration but had no effect on osmolarity of the blood.

Orthosiphon did not affect the plasma level and excretion of uric acid. *Equisetum arvense* reduced uricemia, increasing uric acid clearance and excretion rates.

Assessor's comment:

In the study from Tiktinsky OL and al. (1983), Java tea increased diuresis by 15% compared to 24% with Equisetum arvense and increased GFR by 18% compared to 22% with Equisetum arvense. Thus, we can conclude that Orthosiphonis folium has a low diuretic effect based on the increased diuresis and glomerular filtration rate.

Otherwise, Java tea led to the alkalinisation of the urine and there was an increase in the urinary pH, which was statistically significant. Thus, these findings indicate that Orthosiphonis folium could eventually be helpful for the prevention of recurrent uric acid stone due to its effect on the urinary pH.

Finally, Java tea did not affect plasma levels or the excretion of uric acid. Thus, a hypouricemic activity of Orthosiphonis folium is not demonstrated in this study.

Overall, the quality of this study cannot be evaluated. For example, the baseline characteristics of the patients are incomplete as well as the design of the study. Moreover, the characteristics of the Orthosiphonis folium extract are not specified. In addition, some parameters of interest have not been

assessed in this study such as the plasma content and excretion of oxalate and citrate. Some results are missing for Orthosiphonis folium such as blood phosphorus content at week 4 or urine phosphorus at week 12. Thus, it is difficult to draw conclusions. These results should be confirmed by other clinical studies.

4.2.3. Clinical studies in special populations (e.g. elderly and children)

After review of the provided literature, no clinical studies have been conducted with Orthosiphonis folium in elderly, children and in pregnant women.

Therefore, no recommendation of the use of Orthosiphonis folium can be given in these target populations.

4.3. Overall conclusions on clinical pharmacology and efficacy

The pharmacological and clinical documentation available for Orthosiphonis folium is very limited. Very few data are available.

Diuretic effect:

Regarding the pharmacological effects of Orthosiphonis folium, only two publications are available related to its diuretic effect. In these two studies, Orthosiphonis folium produced no significant changes in urine volume or excretion of electrolytes.

Regarding the clinical effects of Orthosiphonis folium, only two publications are available related to the diuretic effect of Orthosiphonis folium. In the study by Mercier F. and al (1936), an increase in the urine volume (in some cases more than two-fold within 4-5 days) was observed. In the second study by Tiktinsky Ol and al (1983), Java tea had a low diuretic effect based on the increased glomerular filtration rate and diuresis. From these data, we can conclude that Orthosiphonis folium has a low diuretic effect even if these results are not totally in line with results of the pharmacological studies. Despite the methodological weaknesses of the studies and the limited results in terms of benefit, an indication limited to the traditional use to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary tract complaints can be granted for Orthosiphonis folium. This indication is acceptable as Orthosiphonis folium is safe for patients and can be used without any supervision of a medical practitioner.

Choleretic effect:

Regarding the *choleretic effect* of Orthosiphonis folium, only one pharmacological publication is available. In this pharmacological study performed in healthy volunteers, an increase in the production of bile and its liberation from the gall bladder was observed; however, experiments are not detailed and these data are very limited in terms of effect.

Effect on renal gravel:

In a pharmacological study, an effect on the *acidity* and on the *urinary pH* was also observed (from Nirdnoy and al. 1991). In this study, there was a decrease in the acidity and an increase in the urinary pH that was statistically significant. However, due to the fact that this study has been carried out in a very limited sample size (6 healthy volunteers) and results were assessed after only one day of treatment, the clinical relevance of such results is limited, whatever the parameters analysed. The results should be confirmed by additional clinical data.

One clinical study was also found. In this clinical study performed by Tiktinsky Ol and al. (1983), Java tea led to the alkalinisation of the urine and an increase in the urinary pH which was statistically significant. These findings are in line with the results obtained in the pharmacological study performed

by Nirdnoy and al. (1991). However, the effect on urinary pH is insufficient from a medical point of view to recommend the use of this plant in this indication. Indeed, as the treatment of renal gravel requires the supervision of a medical practitioner to confirm the diagnosis, prescribe and monitor adequate treatments, such a traditional use indication without any supervision or medical examination before treatment could lead to disadvantages for patients. For all these reasons, a traditional use indication cannot be granted.

Hypouricemic activity:

Regarding its *hypouricemic activity* in the pharmacological study by Nirdnoy and al. (1991), uric acid showed a non significant increase and in the clinical study performed by Tiktinsky Ol and al. (1983), Java tea did not affect uric acid plasma levels, which limits the clinical relevance of such indication.

Other activities:

Other activities of Orthosiphonis folium have been observed in the different monographs and in the literature. Moreover, Orthosiphonis folium has traditionally been used in Java tea for the treatment of *hypertension* and *diabetes*. It has also been used in folk medicine for *bladder* and *kidney disorders*, *gout* and *rheumatism*. After review of the documentation, no pharmacodynamic study or clinical study were found to confirm the anti-inflammatory, antibacterial, antihypertensive, hypoglycaemic, and antifungal activities/properties in line with this traditional use.

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

See sections 4.1, 4.2 and 4.3.

5.2. Patient exposure

See sections 4.1, 4.2 and 4.3.

5.3. Adverse events and serious adverse events and deaths

According to the provided literature, only one case report involving *Orthosiphon stamineus* has been retrieved (Garcia-Moran S et al). This Spanish publication reports one case of hepatitis in a 25 year-old female patient. She had taken two herbal products (capsules composed of powder of Green tea leaves and capsules composed of *Orthosiphon stamineus* capsules) for two months before experiencing asthenia, icterus and pruritus. Investigations showed abnormal transaminases values (AST 1943 UI/l and ALA 2398 UI/l). Viral serologies were negative. The outcome was favourable after the discontinuation of both products.

***Assessor's comments*:**

The authors specify that some Green tea containing products have been associated with liver disorders. They remind that a product containing a hydroalcoholic extract of Green tea was withdrawn in 2003 in France and Spain due to cases of hepatitis. Cases of liver disorders have been spontaneously reported with products composed of powder of Green tea leaves. The responsibility or contribution of Orthosiphon stamineus in this case is rather doubtful but cannot be excluded. It should be noted that no other cases of hepatotoxicity involving this plant have been retrieved in the literature. There is no justification to mention these data in the monograph.

5.4. Laboratory findings

No relevant data available.

5.5. Safety in special populations and situations

Concerning section 4.4 "Special warnings and precautions for use", information was found in some monographs:

The Complete German Commission E Monographs (1998):

"Warning: No irrigation therapy in case of oedema due to limited heart and kidney function."

The ESCOP monographs (2003):

"Special warnings and special precautions for use: Java tea should not be used in patients with oedema due to impaired heart and kidney function."

This warning is not supported by clinical data and no clinical studies have been conducted with Orthosiphonis folium in patients with oedema due to impaired heart and kidney function. However, it is a logical precautionary measure because fluid intake is not recommended in this case. Therefore, this warning could be added in the monograph in section 4.4 "special warnings and precautions for use".

No data on the safe use in children and adolescents are available. Thus, it should be added in section 4.4 "Special warnings and precautions for use" that Orthosiphonis folium should not be used in this target population.

5.6. Overall conclusions on clinical safety

Clinical safety data are limited.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended

As no data on the use in children and adolescents are available, the use can only be limited to the adults and elderly.

No safety problems concerning the traditional use of java tea or its preparations have been reported. Java tea preparations are considered not harmful when used in the recommended dosages for specified preparations

6. Overall conclusions

In conclusion, due to its long-standing use and based on the available documentation, we are of the opinion that only a Traditional Use can be granted for Orthosiphonis folium. Only the preparations which have been used for at least 30 years will be described in the monograph.

To be in compliance with the wording validated in the other monographs (e.g. monographs on *Equisetum arvense* L., herba, *Taraxacum officinale* Weber ex Wigg., radix cum herba, *Betula pendula* ROTH, folium), the monograph information should remain limited to the traditional use to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary complaints.

As there is no clinical studies conducted with Orthosiphonis folium in children under the age of 18 years, Orthosiphonis folium should not be used in this target population and should be limited to adults.

Given that no reproductive toxicity studies have been conducted and there are no data from the use of Orthosiphonis folium in pregnant woman, section 4.6 of the monograph is adapted accordingly and in compliance with the wording validated in other monographs.

Annex

List of references