Assessment report on *Orthosiphon aristatus* (Blume) Miq. var. *aristatus*, folium
Draft

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Orthosiphon aristatus* (Blume) Miq. var. *aristatus*, folium
Herbal preparation(s)	a) Comminuted herbal substance b) Powdered herbal substance c) Liquid extract (DER 1:1), extraction solvent: ethanol 25% m/m d) Dry extract (DER 5-7:1), extraction solvent: water e) Dry extract (DER 8-12:1), extraction solvent: ethanol 60% V/V f) Dry extract (DER 7-8:1), extraction solvent: ethanol 70% V/V g) Dry extract (DER 5-7:1), extraction solvent: ethanol 30% V/V
Pharmaceutical form(s)	Herbal substance or comminuted herbal substance as herbal tea for oral use. Herbal preparations in liquid or solid dosage forms for oral use.
Rapporteur(s)	A Sawaya/J Viguet Poupelloz (first assessment), C Purdel (revision 1)
Peer-reviewer	J Wiesner

Note: This draft assessment report is published to support the public consultation of the draft revised European Union herbal monograph on *Orthosiphon aristatus (Blume)* Miq. var. *aristatus*, folium. It is a working document, not yet edited, and shall be further developed after the release for consultation of the monograph. Interested parties are welcome to submit comments to the HMPC secretariat, which will be taken into consideration but no 'overview of comments received during the public consultation' will be prepared on comments that will be received on this assessment report. The publication of this draft revised assessment report has been agreed to facilitate the understanding by Interested Parties of the assessment that has been carried out so far and led to the preparation of the draft revised monograph.

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Abbreviations

ARs	Adverse reactions
AUC	Area under the curve
Cmax	Maximum plasma concentration
CYP	Cytochrome P450 enzymes
ESCOP	European Scientific Cooperative on Phytotherapy
GFR	Glomerular filtration rate
ICSRs	Individual case safety reports
i.p.	intraperitoneal
i.v.	intravascular
MLM	Medical literature monitoring
SD	Sprague Dawley
$t_{1/2}$	Elimination half-time

Introduction

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

- Herbal substance(s)

The herbal substance is the whole or fragmented, dried leaf and top of stem of *Orthosiphon aristatus* (Blume) Miq. var. *aristatus.* L. According to the definition in the Ph. Eur., the herbal substance should contain a minimum 0.3% of rosmarinic acid ($C_{18}H_{16}O_8$; Mr 360.3) (dried drug) (European Pharmacopeia. monograph ref.: 1229).

In the first version of the assessment report, the herbal substance was described as the dried leaf and top of stem of *Orthosiphon stamineus* Benth. (*O. aristatus* Miq.; *O. spicatus* Bak.), containing not less than 0.05% of sinensetin ($C_{20}H_{20}O_7$; Mr 372.4) (dried drug).

- Herbal preparation(s)

The following herbal preparations have been reported as constituents of medicinal products on the market in the EU/EEA Member States (for further information see section 2 "Data on medicinal use"):

   a) Comminuted herbal substance

   b) Powdered herbal substance

   c) Liquid extract (DER 1:1), extraction solvent: ethanol 25% m/m

   d) Dry extract (DER 5-7:1), extraction solvent: water

   e) Dry extract (DER 8-12:1), extraction solvent: ethanol 60% V/V

   f) Dry extract (DER 7-8:1), extraction solvent: ethanol 70% V/V

   g) Dry extract (DER 5-7:1), extraction solvent: ethanol 30% V/V

- Constituents

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, 2014; Bruneton, 1998, Paris and Moyse, 1967)

- 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. Search and assessment methodology

Revision 1:

Scientific databases: PubMed (Using the Mesh term 'Orthosiphon folium' from 2010 to present, Search date: 10 June 2019, 32 hits; '*Ortosiphon stamineus*' 112 hits).

Medical databases: Cochrane Database of Systematic Reviews (Using the search terms 'Orthosiphon', Search date: 10 June 2019, 4 hits)

Toxicological databases: ToxNet (June 2020)

Pharmacovigilance resources: EudraVigilance 20 August 2019, active substance (high level): contain *Ortosiphon stamineus*.
MLM report was provided by EMA on 16-07-2019, indicating 24 new references but no ICSRs detected for the substance group 'ORTHOSIPHON'.

1.3. Major changes introduced in the first revision

The Ph. Eur. monograph on Java tea have been updated indicating another preferred botanical name of the plant and the content is expressed in rosmarinic acid (minimum 0.3%). In the previous version the content was expressed in sinensetin with another limit (minimum 0.05%). Accordingly, the herbal substance and herbal preparation in the EU herbal monograph on *O. aristatus* L., folium, have been updated in section 1.1. 'Description of the herbal substance(s), herbal preparation(s) or combinations thereof'.

According to the market overview, there are several new Java tea preparations for oral use that fulfils the criteria of medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA. These herbal preparations have been included in the monograph (see section 2 "Data on medicinal use").

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
Comminuted herbal substance	Flushing of the urinary tract drainage and in case of renal gravel.	Herbal tea SD: approx. 2 g DD: several times daily	Since 1986, DE; German Standard Marketing Authorisation

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
		If acute symptoms persist longer than 1 week or recur periodically, a doctor should be consulted.	
Comminuted herbal substance	Traditionally used in mild inflammations of urinary ways.	Herbal tea SD: 2-3 g DD: 6-12 g pour SD with 150ml of boiling water, stay under cover for 15 min, strain. If the symptoms persisted or in case of adverse events it is advised to consult with a doctor or inform health professionals.	Since 1999, Poland, TUR
Powdered herbal substance	Traditional herbal medicinal product used to increase the amount of urine. The product is a traditional herbal medicinal product for use in specified indication exclusively based on long-standing use.	Capsule, hard *Adults* SD: 500-750 mg DD: 1000-1500 mg Duration of use: 6 weeks	Since Nov 1991 by former registration scheme and since 2012, ES**, TUR
Powdered herbal substance	Traditional herbal medicinal product used to increase the amount of urine.	Capsule, hard SD: 650 mg DD: 1300 mg	Since 1997 in FR (< 30 years); TUR
Dry extract (DER 5-7-:1), extraction solvent water	For flushing in bacterial and inflammatory diseases of the urinary tract drainage and in renal gravel.	Capsule, soft *Adults:* SD: 500.4 mg DD: 1501.2 mg	Since 1995, DE, WEU

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
		No longer than 5 days without medical advice	
Dry extract (DER 5-7-:1), extraction solvent water	Traditional herbal medicinal product used to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary tract complaints.	Capsule, hard *Adults:* SD: 360 mg DD: 1080 mg 2 – 4 weeks If the symptoms persist longer than 5 days during the use of the medicinal product, a doctor should be consulted.	Since 1976, DE; WEU*, in 2015 registration as TUR
Dry extract (DER 8-12:1), extraction solvent ethanol 60% V/V	For flushing in bacterial and inflammatory diseases of the urinary tract drainage and in renal gravel.	Coated tablet *Adults:* SD: 200-400 mg DD: 600-1200 mg No longer than 5 days without medical advice, if the symptoms do not improve. Duration of use is not limited, however If complaints of symptoms such as fever, dysuria, spasms or blood in the urine occur during the use of the medicinal product, a doctor should be consulted.	From 1976 until 12/2016, DE, WEU (*MA expired because the company did not submit an application for prolongation*)
Dry extract (DER 7-8:1), extraction solvent ethanol 70% V/V	For flushing in bacterial and inflammatory diseases of the urinary tract drainage and in renal gravel.	Capsule, hard *Adults and adolescents:* SD: 277.5 mg	From 1976 until 03/2018, DE, WEU (*MA expired because the company did not*

Active substance	Indication	Pharmaceutical form Strength (where relevant) Posology Duration of use	Regulatory Status (date, Member State)
		DD: 832.5 mg No longer than 5 days without medical advice, if the symptoms do not improve. Duration of use is not limited, in principle, but has to be determined by the doctor based on the kind, gravity and course of the disease.	*use it for 3 consecutive years*)
Liquid extract (DER 1:1), extraction solvent ethanol 25% m/m	Traditional herbal medicinal product used to increase the amount of urine Traditionally herbal medicinal product used as adjuvant in slimming diet	2 g, 2 times daily Duration of use: 2 to 3 weeks	From 1952 until 2018, FR; in 2006, TUR
Dry extract_(DER 5-7:1), extraction solvent: ethanol 30% V/V	Traditionally used to promote the renal elimination of water, or as an adjuvant to slimming regimes.	Capsule, soft *Adults* SD: 200 mg DD: 400 mg	Since 1991, FR, TUR

* the old WEU products expired in 06/2020; for these products the daily dose is given with 3-4 times daily = 1080-1440 mg dry extract daily

** commercialization was suspended by the company in Nov 2018.

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

No information available.

2.1.2. Information on products on the market outside the EU/EEA

No information available.

2.2. Information on documented medicinal use and historical data from literature

Orthosiphon aristatus (Blume) Miq. var. *aristatus.* L. 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, 1967. 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.

Following 4 historical monographs were found:

The complete German Commission E Monographs (Blumenthal et al., 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: 6-12 g herb; equivalent preparation.
Method of administration: Cut herb for infusions and other galenical preparations for oral use.

European Scientific Cooperative on Phytoterapy (ESCOP) monograph
The first version of monograph on Java tea was published on 1996 and revised in 2003 and 2014.
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 2-3 g 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.

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".*

British Herbal Medicine Association (BHMA) (British Herbal Pharmacopoeia, 1996)
Therapeutic indication: "*Diuretic".* No further details on posology

Table 2: Overview of historical data

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
Comminuted herbal substance	Irrigation therapy for bacterial and inflammatory diseases of the lower urinary tract and renal gravel".	Oral use. Unless otherwise prescribed daily dosage: 6-12 g herb; for infusions and other galenical preparations for oral use.	C*Blumenthal et al.*, 1986
Herbal substance	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.	Oral use. Adults: An infusion of 2-3 g of dried material in 150ml of water, two to three times per day; equivalent preparations. *Duration of administration:* No restriction.	ESCOP, 2003
Comminuted herbal substance	As a diuretic in chronic or recurrent inflammation of the renal pelvis.	Herbal tea 1 teaspoon (2 g) with 150ml of boiling water, stay under cover for 15 min, strain.	Wichtl, 2004

2.3. Overall conclusions on medicinal use

Table 3: Overview of evidence on period of medicinal use

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
Comminuted herbal substance	Traditional herbal medicinal product used to increase the amount of urine to achieve	Herbal tea SD: 2-3 g DD: 6-12 g	Since 1986, German Standard Marketing Authorisation DE; Blumenthal *et al.*, 1998

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
	flushing of the urinary tract as an adjuvant in minor urinary tract complaints.		
Powdered herbal substance	Traditional herbal medicinal product used to increase the amount of urine.	Oral use *Adults:* SD: 500-750 mg DD: 1000-1500 mg	Since 1991, ES
Liquid extract (DER 1:1), extraction solvent ethanol 25% m/m	Traditional herbal medicinal product used to increase the amount of urine.	Oral use SD: 2000 mg DD: 2000-4000 mg	Since 1952, FR
Dry extract (DER 5-7:1), extraction solvent water	Traditional herbal medicinal product used to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary tract complaints.	Oral use *Adults:* SD: 360 mg DD: 1080-1440 mg	Since 1976, DE
Dry extract (DER 8-12:1), extraction solvent ethanol 60% V/V	For flushing in bacterial and inflammatory diseases of the urinary tract drainage and in renal gravel.	Oral use *Adults:* SD: 200-400 mg DD: 600-1200 mg	Since 1976, DE
Dry extract (DER 7-8:1), extraction solvent ethanol 70% V/V	For flushing in bacterial and inflammatory diseases of the urinary tract drainage and in renal gravel.	Oral use SD: 277.5 mg DD: 832.5 mg	Since 1976, DE
Dry extract (DER 5-7:1), extraction solvent: ethanol 30% V/V	Traditionally used to promote the renal elimination of water, or as an	Oral use SD: 200 mg DD: 400 mg	Since 1991, FR

Herbal preparation Pharmaceutical form	Indication	Strength Posology	Period of medicinal use
	adjuvant to slimming regimes.		

The indication reported in the medicinal use can be reworded as a general indication for a "traditional herbal medicinal product used to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary tract complaints." Long-standing medicinal use for at least 30 years within the European Union is therefore demonstrated in the above indication for the following preparations for oral administration which are proposed for the monograph on traditional use:

a) Comminuted dried leaves as herbal tea for oral use.

Traditional medicinal use of this preparation is substantiated by extensive bibliography and the presence on the German market for more than 30 years and in Poland since 1999. The posology is based to existing data: single dose: 2-3 g of the comminuted herbal substance in 150 ml of boiling water as a herbal infusion(according to the Polish product); as the German product corresponds to "approx. 2 g" is also fitting in the proposed range; daily dose: 6-12 g, in line with both products from the Polish and German market

The daily dose in adults and adolescents over 12 years is 1.5 g or up to 3 g of the comminuted herbal substance in 150 ml of boiling water as a herbal infusion daily (in Spain) as herbal tea for oral use.

b) Powdered dried leaves in solid dosage forms for oral use.

Traditional medicinal use of this preparation is substantiated by the presence of medicinal products in Spain since 1991. Single dose: 500-750 mg, daily dose: 1000-1500 mg.

c) Liquid extract (DER 1:1), extraction solvent ethanol 25% m/m in solid or liquid dosage forms for oral use.

Traditional medicinal use of this preparation is substantiated by the presence of medicinal products in France since 1952. Single dose: 2 g, daily dose: 2-4 g.

d) Dry extract (DER 5-7:1), extraction solvent water in solid dosage forms for oral use.

The medicinal use of this preparation is substantiated by the presence of medicinal products in Germany since 1976 as WEU and since 2015 as TUR. Single dose: 360 mg; daily dose: 1080-1440 mg (according to the broader range used for the older products and as are no known safety concerns to reduce it).

e) Dry extract (DER 8-12:1), extraction solvent ethanol 60% V/V in solid dosage forms for oral use.

The medicinal use of this preparation is substantiated by the presence of medicinal products in Germany since 1976. Single dose: 200-400 mg; daily dose: 600-1200 mg.

f) Dry extract (DER 7-8:1), extraction solvent ethanol 70% V/V in solid dosage forms for oral use.

The medicinal use of this preparation is substantiated by the presence of medicinal products in Germany since 1976. Single dose: 280 mg (rounded value); daily dose: 840 mg.

g) Dry extract (DER 5-7:1), extraction solvent ethanol 30% V/V in solid dosage forms for oral use.

The traditional medicinal use of this preparation is substantiated by the presence of medicinal products in France since 1976. Single dose: 200 mg; daily dose: 400 mg.

Duration of use:

The EU monograph adopted in 2010 did not include a duration of use. During the revision 1, HMPC decided, in line with the authorised products and EU monographs with similar/comparable indication, to limit the use to 2 weeks. Therefore, section 4.2. 'Posology and method of administration' was updated: *If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.*

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

Diuretic activity

To support the traditional use of herbal preparations obtained from *Orthosiphon aristatus*, 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.

Englert and Harnischfeger (1992):
To study the diuretic activity of an aqueous extract prepared from leaves of *Orthosiphon aristatus*, 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.

Assessors' 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. 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):

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):

Extracts of *Orthosiphon aristatus* (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.

Casadebaig-Lafon *et al*. (1989):

Two types of extract produced from *Orthosiphon aristatus* (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:

The diuretic activity of a commercial hydro-alcoholic extract of *Orthosiphon aristatus* 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:

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 aristatus in a non-rodent species. It showed that an ethanolic (50% V/V) extract of Orthosiphon aristatus 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 aristatus* 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. 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. In addition, both extracts significantly increased urinary sodium and potassium excretion from day 4 and 2, respectively. 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. d. 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 aristatus administered repeatedly.

Adam *et al*., 2009:

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. aristatus* 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 administered 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. x. 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:

Methylripariochromene A (MRC) was isolated from the chloroform-soluble fraction of the water decoction of *Orthosiphon aristatus* (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.

<u>Schut and Zwaving, 1993:</u>

The flavonoids sinensetin and 3-hydroxy-5,6,7,4-tetramethoxyflavone were isolated from the leaves of *Orthosiphon aristatus*. 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. 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 aristatus*.

Table 4: Overview of the main non-clinical data/conclusions

Herbal preparation tested	Strength Dosage Route of administration	Experimental model In vivo / In vitro	Reference Year of publication	Main non-clinical conclusions
Aqueous extract (no further detail)	Oral; single dose 5 and 10 mg/kg	In vivo male rats	Adam et al., 2009	Dose-dependent diuretic activity
Methanol and methanol-water (1:1) extracts (no further detail)	single (2000 mg/kg) or repeated (500 g/kg/day for 7 days)	In vivo male rats	Arafat et al., 2008	Diuretic effect after repeated dose
Hydro-alcoholic extract (no further detail)	Intraperitoneal; single dose 50 mg/kg	In vivo male rats	Beaux et al., 1999	Diuretic activity; increased K^+ excretion, but no effect on Na^+ or Cl^-
Aqueous extract or hydro-alcoholic (70%) extract (no further detail)	Oral; single dose 18 and 180 mg/kg, or 13.5 and 135 mg/kg	In vivo male rats	Casadebaig-Lafon et al., 1989	Increased urine volumes but the effect was not dose-dependent
Aqueous extract (no further detail)	Oral; single dose 125, 750 and 1000 mg/kg	In vivo male rats	Englert and Harnischfeger, 1992	Diuretic activity; enhanced Na^+, K^+, Cl^- excretion
Ethanolic extracts extraction solvent ethanol 50% V/V or ethanol 70% V/V (no further detail)	Oral; single dose 700 mg/kg	In vivo male rats	Olah et al., 2003	Only the extract obtained with ethanol 50% increased urine volume; the excretion of Na^+, K^+ was increased by both extracts
50% hydro-ethanolic extract of Orthosiphonis herba	i.v. infusion; 18.8 mg/kg/min	In vivo male dogs	Chow et al., 1979	increased urine volume Na^+, K^+, Cl excretion and reduced reabsorption of Na^+ and Cl^-

3.1.2. Secondary pharmacodynamics

Hypouricemic activity and effect on calcium oxalate crystals

Orthosiphon aristatus 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 aristatus* (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 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.

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.

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 aristatus* (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).

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 aristatus* (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

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

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.

<u>Inhibition of NO production</u>

A series of experiments were conducted to identify the biologically active components of *Orthosiphon aristatus* (Awale *et al*., 2003a, 2003b, 2003c, 2003d; Nguyen *et al*., 2004).

First, it was 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 IC_{50} reaching 42 µg/mL. Thereafter, the efforts were continued 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 supernatant. For each compound, IC_{50} were calculated. 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 aristatus and all inhibited NO-production by LPS-activated macrophage-like J774.1 cells with an IC_{50} 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 IC_{50} values. The same effect was also reported with a methanolic extract of Orthosiphon aristatus.

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.

<u>Effect on arachidonic acid metabolism: inhibition of lipoxygenase</u>

Lyckander and Malterud (1992) tested the effect of an ethyl acetate extract of *Orthosiphon aristatus* (leaves) and 8 lipophilic flavonoids 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 IC_{50} value amounting to 0.018% (w/v). The two main flavonoids sinensetin and tetramethylscutellarein showed dose-related inhibition with IC_{50} values of 114 µM and 110 µM. The IC_{50} 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 aristatus (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.

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 aristatus 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 aristatus leaf is not used traditionally. Therefore, it is considered reasonable not to take these results into consideration.

Other authors showed that an aqueous extract of *Orthosiphon aristatus* displayed an antibacterial effect (intermediate to strong) towards *Streptococcus mutans* responsible for dental caries. (Chen *et al*., 1989)

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 (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 $3.8.10^{-5}M$ and $1.2.10^{-4}M$ 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).

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. x.

Hypoglycaemic effects

Mariam *et al.*, 1996:
An aqueous extract of *Orthosiphon aristatus* (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. 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.
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.

Sriplang *et al.*, 2007:
An oral glucose tolerance test was performed by administering orally to either normal or streptozotocin-induced diabetic rats an aqueous extract of *Orthosiphon aristatus* (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.

Assessor's comment:
The authors mention that glicenclamide reduced glucose levels in normal rats, but the figures reported for glibenclamide (mean ± SEM) 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.
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. 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. 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). (Sriplang *et al.*, 2007)

Assessor's comment:

Two published articles dealing with the hypoglycaemic effect suggested for Orthosiphon aristatus were found in the literature. They were performed with aqueous extracts of the whole plant, whereas the plant part traditionally used 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). xIn addition, other authors reported increased blood glucose levels in rats treated with an aqueous extract of Orthosiphon aristatus leaves (Adam et al., 2009, see Diuretic activity).

Other effects

Antifungal activity:

Guérin and Réveillère (1985) tested a hydro-alcoholic extract of *Orthosiphon aristatus* (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 aristatus was only reported in this article. We did not find any other experimental study in the scientific literature to support these results.

ACytotoxic effects:

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 aristatus* (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 aristatus* (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 scytotoxic effects were reported for the ethanolic (50%) extract in animals bearing Harding Pasey melanoma. Some cytotoxicity 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, these results have limited value.

<u>Anti-pyretic activity:</u>

The anti-pyretic activity of a standardized methanol: water (50/50) extract of *Orthosiphon aristatus* 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 aristatus (not used traditionally).

<u>Antioxidant activity:</u>

Water extracts of *Orthosiphon aristatus* (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 *et al*., 2003).

3.1.3. Safety pharmacology

No information available.

3.1.4. Pharmacodynamic interactions

No information available.

3.1.5. Conclusions

In view of the traditional use claimed for the leaves of Java tea, published data dealing with diuretic activity 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. x.

x x 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.

Also secondary pharmacodynamic effects as anti-inflammatory, hypoglycaemic, antibacterial activities and effects on uric acid level and calcium oxalate crystals were investigated.

None of the reported pharmacological studies constitute any cause for safety concern.

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

Six adult male Sprague-Dawley rats were administered either a Java tea extract (containing 15 mg/kg sinensetin, 21 mg/kg eupatorin and 5 mg/kg 3'-hydroxy-5,6,7,4'-tetramethoxyflavone) orally or a mixture containing 2.5 mg/kg of each of these flavonoids intravenously, in a cross-over study with a wash out period of one week. The calculated mean absolute oral bioavailability for sinensetin was 9.4%, for eupatorin 1.0% and for 3'-hydroxy-5,6,7,4'-tetramethoxyflavone 1.5% (Loon *et al*., 2005).

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

3.3.1. Single dose toxicity

The intraperitoneal LD_{50} of an ethanolic (50% V/V) extract of *Orthosiphonis herba* amounted to 19.6 g/kg in ICR mice (Chow *et al*., 1979).
The oral LD_{50} of aqueous, 50% aqueous ethanolic, and ethanolic extracts of Orthosiphonis leaves is greater than 5000 mg/kg in SD rats (Pariyani *et al*., 2015)

Mohamed *et al*. (2011) investigated the acute effects of an ethanolic extract 50% (declared "standardised) after single dose of 5000 mg/kg in rats. No acute effects were observed.

3.3.2. Repeat dose toxicity

Chin *et al* (2008) ғconducted a 14-day toxicity study with a methanolic extract of *Orthosiphon aristatus* (leaves). 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 LD_{50}, 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 LD_{50} 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.

Mohamed *et al*. (2011) investigated the effects of an ethanolic extract 50% (declared "standardised) after repeated-dose administration in rats. The extract was administered orally at doses of 1250, 2500 and 5000 mg/kg per day for 28 days to female and male SD rats, respectively. Authors concluded that no adverse effects were observed, and NOAEL corresponds to 5000 mg/kg/day.

3.3.3. Genotoxicity

During the revision 1 two new genotoxicity studies (Muhammad *et al*., 2011; Shafaei *et al*., 2015) conducted on two different preparations (aqueous and ethanolic extracts) were identified.
An aqueous extract was tested *in vitro* (Ames test) and *in vivo* (Mouse bone marrow micronucleus test) by Muhammad *et al*. (2011).

Ames test:
Ames test was conducted on *Salmonella typhimurium* strains TA100, TA 98, TA 97a and TA 1535 by the standard plate incorporation method with and without metabolic activation (S9 mixture). Java tea aqueous extract, tested in doses up to 5000 µg per plate, did not increase the number of his$^+$ revertant colonies over the negative control values. Results therefore indicated that Orthosiphonis aqueous extract was not mutagenic in the Salmonella/microsome assay.

Assessor's comment:
The tests were performed with an aqueous extract of Orthosiphon aristatus aerial parts (no information on DER); the phytochemical comparability with the leaves is not demonstrated. Any tester strain detecting DNA cross linking agents (e.g. Salmonella typhimurium TA102, Escherichia coli WPA2uvrA) were not used and this is required by 474 OECD guideline. In addition, the mutagenic potential of the extract was performed only by the standard plate incorporation method, whereas the pre-incubation method should also have been used at least with S9 mix.

Mouse bone marrow micronucleus test:
Swiss Webster mice were treated by gavage with *Orthosiphon aristatus* aqueous extract (0, 500, 2000 and 4000 mg/kg body weight/day) dissolved in distilled water for 3 days. A fourth group (positive control) was treated with a single i.p. injection of cyclophosphamide (CPA 25 mg/kg body weight) 24 h prior to euthanasia. Animals were killed by cervical dislocation and bone marrow cells were harvested 24 h after the last daily dose of *Orthosiphon stamineus* extract or CPA injection. The ratio of PCE:NCE remained unaltered in the treated groups, a finding that indicated that *Orthosiphon stamineus* aqueous extract was not toxic to the mouse bone marrow. The proportion of polychromatic erythrocytes with micronuclei (MNPCEs) noted in *Orthosiphon stamineus*-treated groups did not differ from the background incidence recorded in the vehicle-control group. The positive control drug with CPA however, markedly increased the frequency of MNPCEs over the background incidence thereby confirming that the assay was sensitive to detect genotoxic substances.

Assessor's comment:
As the evidence of the exposure to the target tissue (bone marrow) is missing (no plasma concentration tested or any signs of cytotoxicity/myelotoxicity) and the extract was obtained using aerial parts not covered by monograph, the results are not considered relevant.

Genotoxicity of an ethanolic extract included in nanoparticles was assessed using the Ames test on TA98 and TA100 *Salmonella typhimurium* strains only without metabolic activation (Shafaei *et al*., 2015). Results indicated that the extract, tested in doses up to 5000 µg per plate was not genotoxic.

Assessor's comment:
The ethanolic extract is not characterized (DER, strength of the extraction solvent) and is unclear if is comparable with the other preparations included in the monograph. The mutagenic potential of the

ethanolic extract was performed only on two strains and only without metabolic activation. In conclusion, these data have only limited value.

3.3.4. Carcinogenicity

No information available.

3.3.5. Reproductive and developmental toxicity

No data available.

3.3.6. Local tolerance

No information available.

3.3.7. Other special studies

No data available.

3.3.8. Conclusions

The available toxicological data is rather limited. Orthosiphonis preparations exhibited low acute toxicity by intraperitoneal or oral route in mice and rats.

A 14-day toxicity study was performed in rats by oral administration of a methanolic extract of *Orthosiphon aristatus* 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. Another repeated dose study (28 days, in rats) on an ethanolic extract revealed an NOAEL of 5000 mg/kg/day, indicating a low oral chronic toxicity.

Available tests on genotoxicity (aqueous and ethanolic extracts of the aerial parts and ethanolic extract included in nano particles) did not give any reason for concern, but as there are some methodological issues (non-compliance with OECD Guidelines, no extracts used which are covered by the monograph), it will not trigger the proposal for the List entry.

No carcinogenicity and reproduction toxicity studies are available.

3.4. Overall conclusions on non-clinical data

Results from in vitro and in vivo studies with extracts and isolated constituents, support the traditional use as diuretic. The mechanism of action of java tea preparations as diuretic agent is not known. Specific data on pharmacokinetics are limited

The available toxicological data is rather limited. Orthosiphonis preparations exhibited low acute toxicity by intraperitoneal or oral route in mice and rats. Also, the data on oral chronic toxicity with an ethanolic extract indicate a low toxicity.

Available tests on genotoxicity on aqueous and ethanolic extracts did not give any reason for concern, but as there are some methodological issues (non-compliance with OECD Guidelines, no extracts used which are covered by the monograph), it will not trigger the proposal for the List entry.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended. There are no published carcinogenicity studies.

4. Clinical Data

4.1. Clinical pharmacology

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

Early pharmacological studies (1927-1928) involving self-administration of aqueous extracts of Java tea, demonstrated increases in urine volume. 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 2014 citing Schumann (1927) and Westing (1928)).

In a placebo-controlled, double-blind crossover study, no influence on 12- or 24-hour urine output or sodium excretion was observed in 40 healthy volunteers after administration of 600 mL (3 times 200 mL at 4-hour intervals) of a decoction equivalent to 10 g of dried leaf (Doan *et al.*, 1992).

Six healthy male volunteers drank 4 times 250 mL of a decoction of Java tea (5.3 g leaves in one litre of water) at 6-hourly intervals during one day, for comparison they drank the same amounts of water on a separate control day. The acidity of the urine increased 6 hours after ingestion of the tea. There were no changes in urine volume or electrolytes (Nirdnoy *et al.*, 1991).

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

No data are available.

4.2. Clinical efficacy

4.2.1. Dose response studies

No information available.

4.2.2. Clinical studies (case studies and clinical trials)

The information on the clinical efficacy of *Orthosiphon aristatus* is limited.

Tiktinsky *et al*. (1983) investigated the effect of orthosiphon (Java tea) and *Equisetum arvense* on the course of uratic nephrolithiasis 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 (no further detail) and the second group (33 patients) consumed *Equisetum arvense* tea.
The endpoints investigated were 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.

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:

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 impossible to draw conclusions.

In a placebo-controlled study, patients suffering from nephrolithiasis with multiple health complaints (at least two active symptoms), and negative for urine white blood cells, received an aqueous dry extract at a daily dose corresponding to 3.2 to 3.6 g dried leaves (n=36) or placebo (n=40) for 14 days. Neither group was permitted to consume any of 25 purine-rich foods (PRFs) during treatment. The primary measure was the reduced sum of active severity of symptoms as recorded using the visual analogue scale before and after therapy (i.e. on day 7 and 14). The mean of the total symptom scores (95% CI) was decreased significantly ($p < 0.001$); 185.6 (153.3, 218.0) to 94.7 (58.2, 131.2) in the Orthosiphon group and 196.1 (164.4, 227.8) to 89.6 (62.8, 116.5) in the placebo group. When comparing between groups, no statistically significant difference was found. The mean consumption in PRFs was significantly decreased ($p < 0.001$) in both groups; however, Orthosiphon did not have additional benefit over placebo at 7 and 14 days of treatment during which they reduced these foods (Premgamone *et al*., 2009).

Table 5: Clinical studies on humans, in CVI

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
Tiktinsky *et al.*, 1983	Open study	Group 1 (n=34), java tea (no data on posology) Group 2 (n=33) *Equisetum arvense* tea 3 months	N=67 (age, sex? If not given, then to note that? No further detail on age or sex)	patients with uratic diathesis	. Both agents had a diuretic effect. 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%. The diuretic effect of java tea was smaller than the diuretic effect of *Equisetum arvense*. Long-term use of Java tea led to the alkalinization of the urine, while Equisetum arvense had acidifying effect. Both agents improved the plasma content and the	Student test	The relevance of results cannot be evaluated.

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
					excretion of inorganic phosphorus, 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.		
Premgamone *et al.*, 2009	placebo-controlled study	java tea group (aqueous dry extract at a daily dose corresponding to 3.2 to 3.6 g dried leaves) or placebo 14 days	N= 76 Placebo (n=40, mean age 55.6 years, 64.5% women) Java tea (n=36, mean age 53.7	patients suffering from nephrolithiasis	The main outcome measure was the sum of score of each patient's active symptoms (for myofascial pain, arthralgia, dyspepsia and back pain) on the visual analog scale (VAS). In the java tea group, the mean sum of VAS scores on day 7	Student test	No difference compared with placebo

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Outcomes (primary and secondary endpoints)	Statistical analysis (e.g. ITT yes/no, CI 95%) Quality score e.g. Jadad score	Comments on clinical relevance of results
			years; 67.5% women) (age, sex? If not given, then to note that?)		and 14 was 64.1 and 51% of the value on day 0. In the placebo group, the respective mean of the sum of VAS scores on day 7 and 14 was 66.1 and 45.7% of the value before treatment. No statistically significant difference for the java tea versus placebo group.		

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

No information available.

4.4. Overall conclusions on clinical pharmacology and efficacy

The pharmacological and clinical documentation available for Orthosiphonis folium is limited.

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 clinical studies are available related to the diuretic effect of Orthosiphonis folium. In the study by Tiktinsky *et al*. (1983), Java tea had a low diuretic effect compared with Equisetum arvense based on the increased glomerular filtration rate and diuresis. In the second study by Premgamone *et al*. (2009) Java tea aqueous extract had no statistically significant difference compared with the placebo. These data are not supporting an well-established use indication. The traditional use indication to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary tract complaints is based on long standing use of java tea. This indication is acceptable as Orthosiphonis folium is safe for patients and can be used without any supervision of a medical practitioner.

Effect on renal gravel:
No valid clinical study was found, although a long-lasting usage in this area is partially documented. In the study performed by Tiktinsky *et al*. (1983) in patients with uratic diathesis 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 on healthy volunteers by Nirdnoy *et 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.

5. Clinical Safety/Pharmacovigilance

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

In a study with nephrolithiasis patients receiving an aqueous dry extract at a daily dose corresponding to 3.2 – 3.6 g dried leaves (n=36) or placebo (n=40) for 14 days, the adverse effects in the first and second week of treatments were 27.8 and 2.8% for the first and the second week in the java tea group, and 17.5 and 17.5% in the placebo group for the first and the second week, respectively. When compared within the same group at the first and second week, the reported adverse effects were reduced significantly in the java tea group. The adverse effects reported during the treatments included myofascial, fatigue, back pain, abdominal pain, arthritis, gastrointestinal disturbance and headache (Premgamone *et al*., 2009).

Table 6: Clinical safety data from clinical trials

Type (aim) and objective(s) of Study Reference	Study Design and Type of Control Study duration (if available)	Test Product(s): herbal preparation, pharmaceutical form; Dosage Regimen; Route of Administration Duration of treatment	Number of Subjects (including age, sex, drop out)	Healthy Subjects or Diagnosis of Patients (inclusion criteria)	Adverse reactions(ARs)	Comments on clinical relevance of results
Premgamone *et al.*, 2009	placebo-controlled study	java tea group (aqueous dry extract at a daily dose corresponding to 3.2 to 3.6 g dried leaves) or placebo 14 days	N= 76 Placebo (n=40) Java tea (n=36)	patients suffering from nephrolithiasis	java tea group (week 1: ten ARs; week 2: one AR) placebo group(week 1: seven ARs; week 2:seven ARs) ARs: myofascial, fatigue, back pain, abdominal pain, arthritis, gastrointestinal disturbance and headache	Not correlated with java tea consumption

5.2. Patient exposure

Aside from market presence and data from studies, there are no concrete data concerning patient exposure 3

5.3. Adverse events, serious adverse events and deaths

In the first version of the assessment report, only one case report involving *Orthosiphon stamineus* has been retrieved (Garcia-Moran *et al*., 2004). 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 comment:
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 aristatus 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.

During the first review MLM report was provided by EMA on 16-07-2019, indicating 24 new references but no ICSRs detected for the substance group 'ORTHOSIPHON'.
In addition to literature reports, a screening for adverse reactions in the EV database was conducted in August 2019, and 7 ICSRs reports were found for the reference period, but the causality between exposure to java tea and adverse reactions reported was not confirmed.

5.4. Laboratory findings

No data available.

5.5. Safety in special populations and situations

No information available.

5.5.1. Use in children and adolescents

No information available.

In the absence of safety data, the use can only be limited to the adults and elderly (see section "special warnings and precautions for use" in the monograph).

5.5.2. Contraindications

For safety reasons the use is contraindicated in case of hypersensitivity to the active substance and in conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal disease)

5.5.3. Special Warnings and precautions for use

Following information was found in some monographs:

The Complete German Commission E Monographs (Blumenthal *et al.*,1998):
"Warning: No irrigation therapy in case of oedema due to limited heart and kidney function."

The ESCOP monographs (2003; 2014):
"Special warnings and special precautions for use: Java tea should not be used in patients with oedema due to impaired heart and kidney function."

In the EU monograph adopted in 2010, a warning regarding patients with oedema due to impaired heart and kidney function was added in the section 4.4 "special warnings and precautions for use", as a logical precautionary measure. During the revision 1, HMPC considered that for the clinical safety of patients suffering from conditions where a reduced fluid intake is recommended (e.g. severe cardiac or renal disease) as also done for other herbal preparations in the same therapeutic field, Orthosiphon tea use is contraindicated.

5.5.4. Drug interactions and other forms of interaction

No data available.

5.5.5. Fertility, pregnancy and lactation

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

5.5.6. Overdose

No information available.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

No information available.

5.6. Overall conclusions on clinical safety

The oral use of preparations of Orthosiphonis folium can be regarded as safe, especially at therapeutic doses as there are no reported adverse events.

Orthosiphonis folium preparations are contraindicated in cases of hypersensitivity to the active substance and in patients with severe cardiac or renal disease, conditions where a reduced fluid intake is recommended.

There are no data regarding interactions with other medicines.

As there is no information on safety during pregnancy and lactation and taking into account nonclinical data on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

During the longstanding use as a medicinal product in the European Union no serious side effects have been reported. Therefore, the oral use of Orthosiphonis preparations can be regarded as safe under the conditions of use described in the monograph.

6. Overall conclusions (benefit-risk assessment)

According to the market overview and literature, Java tea preparations for oral use fulfils the criteria of medicinal use throughout a period of at least 30 years, including at least 15 years within the EU/EEA, i.e. traditional medicinal use according to Directive 2004/24/EC for the following herbal preparations:

a) Comminuted herbal substance

b) Powdered herbal substance

c) Liquid extract (DER 1:1), extraction solvent: ethanol 25% m/m

d) Dry extract (DER 5-7:1), extraction solvent: water

e) Dry extract (DER 8-12:1), extraction solvent: ethanol 60% V/V

f) Dry extract (DER 7-8:1), extraction solvent: ethanol 70% V/V

g) Dry extract (DER 5-7:1), extraction solvent: ethanol 30% V/V

The clinical data cannot be considered sufficient to fulfil the criteria required for "well-established medicinal use", according to Directive 2001/83/EC. The positive effects of Orthosiphonis folium preparations as diuretic have long been recognised empirically. The traditional use indication and duration of use included in the monograph are: Traditional herbal medicinal product used to increase the amount of urine to achieve flushing of the urinary tract as an adjuvant in minor urinary tract complaints.

If the symptoms persist longer than 2 weeks during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

Oral administration of preparations of Orthosiphonis folium can be regarded as safe at traditionally used doses with the exception of patients with severe renal or cardiac disease (e.g. renal and heart failure).

No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

Data regarding genotoxicity are incomplete. Tests on reproductive toxicity and carcinogenicity have not been performed. Therefore, the use during pregnancy and lactation cannot be recommended. A European Union list entry cannot be supported due to lack of adequate data

Annex

List of references