IJBPAS, August, 2013, 2(8): 1579-1596

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EQUISETUM ARVENSE: SCIENTIFIC EVIDENCES FOR CLINICAL USE

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ABSTRACT

Equisetum arvense L. (horsetail) is a medicinal plant that is native to Europe and also commonly found in the Americas, Northern Africa and Asia. It has been traditionally indicated as a mild diuretic, antiedematous, anti-inflammatory and remineralizing treatment. The aim of this study was to review the literature concerning horsetail to provide support to phytotherapy researchers and policymakers interested in data on this medicinal plant. The literature review was conducted using the PubMed, LILACS, SciELO, Virtual Health Library (VHL), Cochrane and Scopus databases, from July 2011 to July 2012, using the uniterm *"Equisetum arvense"*. The study included 58 articles involving pharmacognostic, pharmacological, pharmacokinetic, *in vivo* toxicological and clinical studies focusing on *E. arvense* as an herbal drug or an extract. The results of preclinical pharmacological studies demonstrate several important *in vitro* and *in vivo* biological activities, including antioxidant, sedative, antimicrobial, antiplatelet, cytotoxic, vasorelaxant, hepatoprotective, antidiabetic, analgesic, anti-inflammatory, wound-healing, remineralizing, antilithiasic and diuretic activities. No clinical studies supporting the use of horsetail as a diuretic or studies on the mechanism of action of horsetail extracts were identified. Horsetail has been classified as a

traditional- and popular-use drug whose uses must be regulated and whose research needs to be encouraged.

Keywords: Phytotherapy, Medicinal Plants, *Equisetum arvense*, Traditional Medicine, Ethnopharmacology

INTRODUCTION

Equisetum arvense L. is a medicinal plant that is native to Europe and also commonly found in the Americas, Northern Africa and Asia **[1]**. In Brazil, *Equisetum arvense* L. is found widespread throughout the country and is popularly known as "horsetail" **[2]**. Horsetail has been traditionally indicated for a wide range of health disorders as a mild diuretic, antiedematous, anti-inflammatory and remineralizing treatment **[1]**.

In Brazil, this plant is a member of the National List of Medicinal Plants of Interest to the Unified Health System (Relação Nacional de Plantas Medicinais de Interesse ao Sistema Único de Saúde - RENISUS), compiled by the Ministry of Health **[3]**. Through the regulation of RDC No. 10 (2010), which addresses the notification of herbal drugs to the National Health Surveillance Agency (Agência Nacional de Vigilância Sanitária - ANVISA), *Equisetum arvense* is marketed as a herbal drug, indicated for "edema (swelling) due to fluid retention", in the form of infusions and decoctions **[4]**. A pharmaceutical specialty based on *Equisetum arvense* dry extract is registered with the ANVISA under the category of herbal medicine **[5]**.

However, in its Evaluation of Medicines for Human Use program, the European Medicines Agency (EMEA) states that clinical data concerning the absorption, distribution and pharmacokinetics of *Equisetum arvense* remain insufficient **[1]**.

The aim of this study was to review the literature concerning *Equisetum arvense* (horsetail) to provide support to phytotherapy researchers and policymakers interested in data on this important medicinal plant.

MATERIAL AND METHODS

The literature review was conducted using the PubMed, LILACS, SciELO, Virtual Health Library (VHL) and Cochrane and Scopus databases, from July 2011 to July 2012, using the uniterm *"Equisetum arvense"*. The study included articles in Portuguese, English, French, Spanish, Japanese and Polish involving pharmacognostic, pharmacological, pharmacokinetic, *in vivo* toxicological and clinical studies focusing on *E. arvense* as an herbal drug or an extract. Studies concerning the agronomic aspects and details related to the elucidation of the chemical structure of compounds isolated

from *E. arvense* were excluded from this work.

The articles that met the scope of the study, regardless of the publication date, were selected for review.

RESULTS & DISCUSSION

The bibliographical research on *Equisetum arvense* obtained from the databases generated a total of 159 articles. In addition to official documents, 58 articles concerning various aspects of this plant were included in this review. The texts were distributed as follows: pharmacognostic aspects (4), *in vitro* (23) and *in vivo* (27) biological activities, review articles (2), clinical trials with *E. arvense* in association with other plants (1) and clinical trials using only *E. arvense* (1).

Pharmacognostic Aspects

The herbal drug is produced in the stems, which are primarily obtained from sterile dried branches **[1, 6]**. These stems have cortex, parenchyma, xylem, stomata and silica granules. The following herb drug characteristics have been reported for *Equisetum arvense*: water content (15.45%), total ash (22% w/w), acid-soluble (11% w/w) and insoluble ash (8% w/w) and foaming Index (100) **[7]**.

Equisetum arvense L. is rich in minerals, with a high silicon content in the form of SiO_2 (5-10%) and a water-soluble silicate. In addition to silicic acids and silicates, potassium ions, calcium, phosphorus and, to a lesser extent, sodium, magnesium, zinc, aluminum and manganese have also been identified **[6]**. Approximately, 0.2 to 0.9% flavonoids have been identified in this plant, primarily as the glycosides kaempferol, apigenin, luteolin, quercetin (quercetin 3-glucoside and malonyl esters), rutin and isoquercitrin **[6-8]**. Phenolic glycosides, such as equisetumoside A, B and C, were identified in the fertile strobilus **[9]**. The steroidal fraction is essentially composed of beta-sitosterol (60%), campesterol (32.9%), isofucosterol (5.9%) and cholesterol (trace amounts) **[8, 10]**. Chlorogenic acid, phenolic acids, such as di-E-caffeoyl-meso-tartaric acid (0.008%) and methyl esters, including protocatechuic and caffeic acids, have also been detected in *Equisetum arvense* plants **[6]**. Estirilpirone, polyene acids, dicarboxylic acids and traces of alkaloid (nicotine, palustrine and palustrinine) have been described **[6, 9, 11]**.

Gallo *et al*., **[12]** used 2 chromatographic methods, high-performance thin-layer chromatography and high-performance liquid chromatography (HPTLC and HPLC, respectively), to determine the chemical fingerprint of *E. arvense* and other related species. For *E. arvense*, HPLC can be useful to identify and assess the quality of the plant material originating from related species and to evaluate the presence of single

components. However, HPTLC has been the method of choice for the comprehensive assessment of the quality of herbal medicinal preparations due to the ease of sample preparation and the capacity to analyze multiple samples in a short time period **[13]**. Rutin, chlorogenic acid, kaempferol, caffeic acid and isoquercitrin were the suggested markers for use in thin-layer chromatography (TLC) with *E. arvense* **[8-13]**. Wagner and Bladt **[8]** developed standard thin-layer chromatograms using isoquercitrin, caffeic acid, galuteoline, rutin and brucine as markers to identify various species of *Equisetum*, including *E. arvense*.

Brune *et al*., **[14]** reported the application of "inter simple sequence repeats" (ISSR)-PCR for the taxonomic differentiation of species of *Equisetum*, with a special focus on the detection of hybrids. The authors state that the ISSR fragment patterns are fairly unique for each of the most common species of the genus *Equisetum* **[14]**.

Medicinal Uses, Preparations and Doses

In Ayurvedic medicine, *E. arvense* is traditionally used for the treatment of inflammation and prostate hypertrophy, urinary incontinence and nocturnal enuresis in children **[15]**.

The legislation-approved uses for *E. arvense* in different countries are based on its long history of use in traditional medicine, ethno pharmacological studies, phytochemical investigations and pharmacological studies. The Herbal Medicinal Products Committee (HMPC) of the European Medicines Agency (EMEA) published reports supporting the use of *E. arvense* for renal elimination, post-traumatic edema and stasis, as an irrigation therapy for bacterial and inflammatory diseases of the urinary tract and urinary calculi in the kidneys and bladder **[1]**.

The German E-Commission approved the use of *E. arvense* as an herbal medicine at an average daily dose of 3 to 6 grams, divided into 2 or 3 applications, to treat posttraumatic and static edemas and as a diuretic to treat bacterial and inflammatory diseases of the urinary tract with the presence of urine sediment. For external use, in the form of a decoction (50 g/L), *E. arvense* has been applied to bandages or baths, and the fluid extract has been recommended at a dose of 50 drops diluted in water as an adjunct in the treatment of poorly healing wounds **[16]**.

In Brazil, the RDC No. 10 of ANVISA recommends the use of *E. arvense* in the form of herbal medication for the preparation of infusions and decoctions, administered at a ratio of 3 g/150 mL of water at 2 to 4 times a day, for the treatment of edema (swelling) due to fluid retention **[4]**.

Toxicity, Contraindications and Adverse Effects

The LD50 of *E. arvense* extract administered intraperitoneally (i.p.) in rats was > 1,000 mg/kg [17]. Other studies have shown that the hydroalcoholic extract of *E. arvense*, at the doses of 2 and 5 g/kg (i.p.) in rats, induced mortality in 12% and 37.5% of animals, respectively. Because the LD50 was higher than 5 g/kg, the extract was practically considered nontoxic **[17, 18]**. No toxicity was detected in the clinical, hematological, urinary, serum biochemical, body mass and mass of internal organs when 0.03% to 3% hydroalcoholic *E. arvense* extract was added to the diet of male and female rats for 13 days **[19]**. Dos Santos *et al*., **[17]** observed no chronic toxicity (8 weeks) when treating rats with the hydroalcoholic extract from the branches of *E. arvense* at a dose of 50 mg/kg (i.p.). In the evaluation of acute hepatotoxicity in rats, no pathological changes in the liver tissue and no biochemical changes in the liver enzymes were detected **[2]**.

By analogy with most herbal medicines, whose official use does not require a prescription, *E. arvense* is contraindicated during pregnancy and lactation and for children less than 12 years due to lack of specific studies **[1]**. Pharmacological inferences suggest that the drug could cause vitamin B1 (thiamine) deficiency due to the presence of the enzyme thiaminase among its chemical constituents [4]. Pharmacological correlations indicate that people with renal and cardiac failure should not use this plant, as its diuretic effects could cause a loss of potassium, which could particularly affect heart failure patients treated with digitalis or other medications that decrease serum potassium concentrations **[4]**.

According to ANVISA, rare cases of allergy might occur in patients sensitive to the chemical constituents of *E. arvense*, and misuse could cause exudative erythema, dysphagia, headache, tenesmus, loss of appetite and, in high-dose cases, gastric and urinary tract irritation due to the presence of nicotine **[4]**. Pharmacologic interactions with lithium and digitalis have also been described **[20]**.

***In vitro* Pharmacological Activities**

Antioxidant Activity

The antioxidant activity of *E. arvense* extracts prepared using different solvents was evaluated using several methods, such as the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical, electron spin resonance (ESR) and the nitric oxide radical inhibition test. The results indicated that several extracts had significant antioxidant activity **[20-28]**, with the methanol extract

presenting the highest free radical scavenging capacity **[21]**.

Antimicrobial Activity

Several microbial agents (bacteria, fungi and viruses) were used to evaluate the *in vitro* antimicrobial activity of *E. arvense* extracts obtained using different solvents (water, ethanol, methanol and dichloromethane), with conflicting results. In a recent study conducted in Brazil, the glycolic extract of *E. arvense* exhibited effective antimicrobial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus mutans, Candida albicans, Candida tropicalis and Candida glabrata* **[29]**. Studies conducted in the past decade have reported antibacterial activity against various pathogens (*E. coli, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus saprophyticus* and *Enterococcus faecalis*) **[23, 30, 31]**. Moreover, Husson et al. [32] reported antiviral activity in cell culture. Other studies, conducted in the 1980s and 1990s, showed no antimicrobial, antifungal or antiviral activities **[14, 33-35]**. However, in 2012, a study reported that hydromethanolic extracts from the aerial parts of *E. arvense* exhibited antibacterial activity against *Staphylococcus aureus* **[36]**.

Antiplatelet Activity

Mekhfi *et al*., **[37]** reported that the aqueous extract of *E. arvense* inhibited thrombin and ADP-induced aggregation *in vivo*, indicating a slight inhibition of platelet aggregation. The authors suggest that this effect might be associated, in part, with the polyphenolic compounds present in the extracts **[37]**. In another study, 38% and 84% antithrombin activity was reported in ethanolic and dichloromethane extracts of *E. arvense*, respectively **[38]**.

Cytotoxic Activity

Different *E. arvense* extracts produce cell growth inhibition depending on the cell line and on the type and concentration of the extract used. An extract containing ethyl acetate showed a prominent antiproliferative effect, without inducing cell growth in human tumor cell lines (HeLa, HT-29 and MCF7) **[22]**. A dose-dependent cytotoxic effect on human leukemia cells (U937) was reported for the aqueous extract of this plant **[38]**. A protein obtained from the crude extract of *E. arvense* inhibited the growth of LI210 leukemia cells, HMV-I melanoma and 3T3 fibroblasts by 32-55%, 31-36% and 6-84%, respectively **[39]**. Studies conducted with rat leukemia cells (L1210) reported 38% cytotoxic activity for the ethanolic extract and 99% activity for the dichloromethane extract of *E. arvense* **[40]**.

Vasorelaxant Activity

The vasorelaxant activity of dicafeoil meso-tartaric acid extracted from *E. arvense* was investigated on isolated rat aorta tissue. This substance exhibited slow relaxant activity against norepinephrine (NE)-induced contractions in the aorta, with and without endothelium; however, the dicafeoil meso-tartaric acid extract did not affect high potassium (60 mM)-induced contractions, suggesting that the inhibition of NE-induced vasoconstriction resulted from reduced calcium influx from the extracellular space **[41]**.

Hepatoprotective Activity

The phenolic (onitin) and flavonoid (luteolin) compounds isolated from the methanolic extract of *E. arvense* exhibited hepatoprotective activity against tacrine-induced cytotoxicity in Hep G2 cells obtained from the human liver. The action of these 2 substances was superior to silybin, which was used as a control, and the luteolin-induced protection was superior to that of onitin. These compounds also demonstrated antioxidant activity. According to the authors, these results support the use of *E. arvense* for the treatment of hepatitis using traditional medicines **[42]**.

Bone Remineralizing Activity

A recent study concluded that the hydromethanolic extract of *E. arvense* stimulated the proliferation of MG63 bone cells *in vitro* **[43]**. In another study, the hydromethanolic extract of *E. arvense* increased the viability and proliferation of human osteoblastic cells *in vitro*, which, according to the authors, suggests a potentially interesting profile with regard to bone regeneration strategies **[36]**.

***In vivo* Pharmacological Activities**

Activity on the Central Nervous System

Extracts of *E. arvense* containing petroleum ether, chloroform, ethanol and water were evaluated for anxiolytic activity in rats. The ethanol extracts (50 and 100 mg/kg) exhibited anxiolytic effects with lower sedative activity when compared with diazepam **[44]**. Rezaie *et al*., **[11]** concluded that the hydroalcoholic extract of *E. arvense* caused a significant increase in ketamine-induced sleep and showed anxiolytic, sedative and preanesthetic effects at a dose of 200 mg/kg i.p.. According to the work of Santos *et al*., **[17]**, the hydroalcoholic extract of *E. arvense* showed anticonvulsant and sedative effects in rats at doses of 200 and 400 mg/kg. These researchers also observed that the administration of the hydroalcoholic extract of *E. arvense* (50 mg/kg, i.p.) to older rats (80 weeks old) reversed cognitive disorders associated with age, with no signs of toxicity **[19]**.

Antidiabetic Activity

Safiyeh *et al.*, **[45]** showed that the oral administration of methanolic, dichloromethane and hexane extracts of *E. arvense* promoted antihyperglycemic activity in diabetic rats (induced through streptozocin) at different doses (50, 100, 250 and 500 mg/kg). The rats treated with methanolic extract experienced an increase in body mass compared with the other groups **[45]**. Consistent with these results, Safiyeh et al. conducted histological studies, and the results revealed a significant regeneration of pancreatic beta cells in animals treated with the methanolic extract of *E. arvense* after streptozocin-induced cell damage **[46]**. Based on creatinine and urinary microalbumin levels, the researchers concluded that the methanolic extract of *E. arvense*, administered at different times for 5 weeks, showed renal protective effects **[47]**.

Analgesic and Anti-Inflammatory Activity

In the folk medicine of several countries, *E. arvense* is widely used for the relief of pain and inflammation **[6, 9]**. The anti-inflammatory and antinociceptive effects of the hydroalcoholic extract of *E. arvense* were detected in rats, using chemical models of nociception, particularly using the carrageenan-induced plantar edema test, which is not associated with the opioid system **[48]**.

Activity in Benign Prostate Hyperplasia

The extracts of *Chimaphila umbellata, Populus tremula, Pulsatilla pratensis* and *E. arvense* and a traditional formulation composed of these four plants, called Eviprostat, popularly used in Japan and Germany for the treatment of benign prostate hypertrophy (BPH), were tested *in vitro* and *in vivo* to evaluate their anti-inflammatory and antioxidant effects. The researchers observed anti-inflammatory activities in the formulation and in the pure extracts, justifying its therapeutic use in BPH **[49, 50]**.

Cicatrizing Activity

A 5% aqueous extract of *E. arvense* accelerated dermal wound contractions in rabbits, which was more effective than the control treatments (0.9% sodium chloride and 10% povidone-iodine solution) after 14 days of local administration **[51]**. In another study, ointments prepared with 5% and 10% powder from the stems of *E. arvense* showed significant dermal wound healing activity in rats, with 95.26% (5% ointment) and 99.96% (10% ointment) contraction of the wound area, suggesting higher dermal and epidermal regeneration, angiogenesis and increased thickness in the granulation tissue after 14 days of treatment compared with controls **[52]**.

Remineralizing Activity

A study evaluating the level and distribution of calcium, magnesium, iron and copper in 6 species of medicinal plants, including *E. arvense*, concluded that a small fraction of these minerals can be considered bioavailable when an infusion of the herbal drug is administered orally **[53]**. Another study showed that the concentration of silicon in *E. arvense* is approximately 5% w/w, whereas water-extractable silicon was 0.3% w/w. The authors concluded that this mineral does not contribute to the medicinal benefits of *E. arvense* **[54]**.

However, Law and Exley **[55]** demonstrated the presence of silicon in all parts of *E. arvense* plants. The deposition sites of this mineral mimicked the places and structures where beta-glucan hemicellulose is found, suggesting that this polysaccharide could be the basis for silicon deposition in *E. arvense*. Beta-glucan hemicellulose induced the formation and precipitation of silicon, suggesting that beta-glucan, and perhaps other similar carbohydrates, might be key molecules in biological silicification **[55]**.

Antilitiasic Activity

The effects of 7 plants, including *E. arvense*, commonly indicated for the prevention of renal calculi formation, were studied in rats. After the oral administration of an infusion prepared with 3 g/L of herbal medicine for 12 days, the authors concluded that the beneficial effects of these plants on urolithiasis might reflect the antiseptic actions and the presence of saponins **[56]**.

Diuretic Activity

A report from the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMEA) cited studies on the diuretic activity of *E. arvense*, conducted in 1930s and 1940s, which showed a mild diuretic effect of *E. arvense* in 3 species of animals: dogs, rats and rabbits **[1]**.

Rebuelta *et al*., **[57]** used female Wistar rats to evaluate the diuretic effects of the following preparations of *E. arvense*: infusions, flavonoid extracts in aqueous suspension, alcoholic ash suspension (to study the possible action of potassium salts) and methanolic extract supplemented with ash. The 2.5% preparations were administered intragastrically (2 mL/100 g body weight), and compared with theophylline (standard) and saline (placebo), *E. arvense* had a mild diuretic effect in all preparations tested **[58]**.

Preclinical Pharmacokinetics

No data concerning the *in vivo* pharmacokinetics of *E. arvense* was found.

Clinical studies

The efficacy of Eviprostat, which is the trade name of a formulation containing *Chimaphila umbellata, Populus tremula, Pulsatilla pratensis* and *Equisetum arvense*

extracts and wheat germ oil, was evaluated in the treatment of BPH. The clinical efficacy of this formulation was evaluated using IPSS (International Prostate Symptom Score) and QOL (Quality of Life) scores. The results were compared with those reported for other BPH treatments, and it was concluded that this formulation reduced nocturia **[49, 50]**.

Only one clinical study involving isolated *E. arvense* was identified, which analyzed the elimination of urinary flavonoid and hydroxycinnamic acid metabolites in 11 volunteers after ingesting a preparation containing the crude extract of *E. arvense* and consuming a flavonoid-free diet for 8 days. After 24 hours, urine samples were collected and analyzed using HPLC. The presumed quercetin metabolites (3, 4-dihydroxyphenylacetic acid and 3,4-dihydroxytoluene) were not detected. The amount of endogenous homovanillic acid, generally considered one of the primary quercetin metabolites, was also not significantly increased. However, hippuric acid and glycine-conjugated benzoic acid levels doubled after ingestion of the drug. The authors suggested the degradation of benzoic acid, rather than phenyl acetic acid, derivatives as the predominant metabolic pathway **[58]**.

Clinical Pharmacokinetics

With the exception of the Graefe and Veit study **[58]** mentioned above, there were no other studies concerning the pharmacokinetics of *E.* arvense.

E. arvense is an ancient medicinal plant used in traditional medicines in India, China and Greece and in the folk medicines of many countries. The data obtained from this traditional knowledge combined with the results of scientific studies conducted since the beginning of the 20th century have resulted in the approval of *E. arvense* as a medicine in several countries, including Brazil.

The EMEA, E-Commission and Brazilian Ministry of Health have supported the medicinal use of *E. arvense* based on the effects obtained form traditional use and the results of preclinical pharmacological studies, which demonstrate several important *in vitro* and *in vivo* biological activities, including antioxidant, antimicrobial, antiplatelet, cytotoxic, vasorelaxant, hepatoprotective, antidiabetic, analgesic, anti-inflammatory, wound-healing, remineralizing, antilithiasic and diuretic activities, along with effects in the central nervous system and as a treatment for benign prostatic hyperplasia.

The same criteria also apply to the evaluation of the toxicity and safety in the use of this plant. However, traditional

prolonged use does not necessarily exclude the possibility that there are unknown factors regarding the toxicity of the plant. The scarcity of toxicology studies in the literature is consistent with the final report of the HMPC/EMEA Adviser, who concluded that the toxicological data on *E. arvense* remain insufficient and recommended that the drug remain classified as a traditional use medicine in Europe. Moreover, due to the lack of safety data, the same reports contraindicate the use of this medicine in children under 12 years old, in pregnant and lactating women and in patients suffering from diseases requiring fluid restrictions (i.e., heart or kidney failure) **[1]**.

In reviewing the literature, no clinical studies supporting the use of *E. arvense* as a diuretic or studies on the mechanism of action of *E. arvense* extracts were identified. It is necessary to consider that diuretics are drugs that reduce the normal resorption of solutes from the glomerular ultrafiltrate along the nephron. They induce the loss of these solutes, with consequent water loss, reduce the volume of extracellular fluids and promote a negative extracellular fluid balance **[1, 59]**. Thus, it is reasonable to conclude that the data on the pharmacokinetic aspects and effectiveness of *E. arvense* are not sufficient to establish a mechanism of diuretic action concerning the negative extracellular fluid balance. The lack of clinical research on the diuretic efficacy of *E. arvense*-based phytotherapeutics in the literature is consistent with the restriction of the pharmaceutical industry in developing and registering new products containing this plant.

CONCLUSION

In conclusion, despite its long history and numerous studies concerning its *in vitro* and *in vivo* effects, *E. arvense*-based products do not meet the requirements for well-established medicinal use based on efficacy and acceptable safety levels. Nevertheless, due to its ancient and cosmopolitan use and existing research, *E. arvense* has been classified as a traditional- and popular-use drug whose uses must be regulated and whose research needs to be encouraged.

ACKNOWLEDGEMENT

We are thankful to the NEPET-UFG and ICF de Goiás, for providing necessary ambulatory and lab facilities. Special thanks to the HMA/SES-SUS de Goiás, for supporting the project. Financial support from FAPEG is gratefully acknowledged.

REFERENCES

[1] European Medical Agency – EMEA, Committee on Herbal Medicinal Products, List of References Supporting the Assessment Report on: Equisetum arvense, London:

European Medicines Agency Evaluation of Medicines for Human Use, 2008, 1-41.

[2] Baracho NC, Vicente BB, Arruda GD, Sanches BC and Brito J, Study of acute hepatotoxicity of *Equisetum arvense L*. in rats, Acta. Cir. Bras., 24 (6), 2009, 449-53.

[3] National List of Medicinal Plants of Interest to SUS/RENISUS 2009, Ministry of Health, Pharmaceutical Services Division / Office of Science Technology and Supplies, (Relação Nacional de Plantas Medicinais de Interesse ao SUS / RENISUS de 2009, Ministério da Saúde, Divisão de Assistência Farmacêutica / Secretaria de Ciência Tecnologia e Insumos) Especiais, Available from: URL: http://portal.saude.gov.br/portal/arquivos/pdf/RENISUS_2010.pdf

[4] Notifications of Herbal Drug with ANVISA, Ministry of Health National Health Surveillance Agency (ANVISA). Resolution RDC N°10, from March, 2010. [Notificações de Drogas Vegetais junto à ANVISA de 2010. Ministério da Saúde. Agência Nacional de Vigilância Sanitária (ANVISA)], 2010, Available from: URL: http://www.brasilsus.com.br/legislacoes/rdc/103202-10

[5] Electronic Bulary, Ministry of Health, National Health Surveillance Agency (ANVISA) [Bulário Eletrônico de 2012, Ministério da Saúde. Agência Nacional de Vigilância Sanitária (ANVISA)], 2012, Available from: URL: http://www4.anvisa.gov.br/BularioEletronico/default.asp.

[6] Sandhu NS, Kaur S and Chopra D, *Equietum arvense*: Pharmacology and phytochemistry - a review, Asia Pac. J. Pharmacol., 3, 2010, 146-150.

[7] Sandhu NS, Kaur S, and Chopra D, Pharmacognostic evaluation of *Equisetum arvense* L., Int. J. Pharm. Tech. Res., 2, 2010, 1460-1464.

[8] Wagner H and Bladt S, Plant Drug Analysis - a Thin Layer Chromatography Atlas, 2nd Ed., Berlin: Springer, 2001.

[9] Asgarpanah J and Roohi E, Phytochemistry and pharmacological properties of *Equisetum arvense* L, J. Med. *P*. Res., 6 (21), 2012, 3689-3693.

[10] D'Agostino M, Dini A, Pizza C, Senatore F, and Aquino R, Sterols from *Equisetum arvense*, Boll. Soc.

Ital. Biol. Sper., 60 (12), 1984, 2241-5.

[11] Rezaie A, Ahmadizadeh C, Mosavi G, Nazeri M, Jafari B and Ebadi R, Comparative study of sedative, pre-anesthetic and anti-anxiety effect of *Equisetum arvense* (horse Tail) extract with diazepam on rats, ABJAS J., 5, 2011, 786-789.

[12] Gallo FR, Multari G, Federici E, Palazzino G, Giambenedetti M, Petitto V, Poli F, and Nicoletti M, Chemical fingerprinting of *Equisetum arvense* L. using HPTLC densitometry and HPLC, Nat. Prod. Res., 25 (13), 2011, 1261-70.

[13] Duron R, Almaguer L, Garza-Juarez A, Luz M, Cavazos S, and Waksman-De-Torres N, Development and validation of thin-layer chromatographic methods for quality control of herbal products, *Acta Chromatographica*, 21 (2), 2009, 203-15.

[14] Brune T, Thiv M, and Haas K, Equisetum (Equisetaceae) species or hybrids? ISSR fingerprinting profiles help improve diagnoses based on morphology and anatomy, Plant Systematics and Evolution, 274 (1), 2008, 67-81.

[15] Jain R, Kosta S, and Tiwari A, Ayurveda and urinary tract infections, J. Young Pharm., 2 (3), 2010, 337.

[16] Aswal BS, Bhakuni DS, Goel AK, Kar K, and Mehrotra BN, Screening of Indian plants for biological activity--Part XI, Indian J. Exp. Biol., 22 (9), 1984, 487-504.

[17] Santos JGD Jr., Blanco MM, Monte FHD, Russi M, Lanziotti VM, Leal LK, and Cunha GM, Sedative and anticonvulsant effects of hydroalcoholic extract of *Equisetum arvense, Fitoterapia,* 76 (6), 2005, 508-13.

[18] Santos JGD Jr., Monte FHMD, Blanco MM, Lanziotti VMDNP, Maia FD and Leal LKDA, Cognitive enhancement in aged rats after chronic administration of *Equisetum arvense* L. with demonstrated antioxidant properties in vitro, Pharmacol. Biochem. Behav., 81 (3), 2005, 593-600.

[19] Tago Y, Wei M, Ishii N, Kakehashi A, and Wanibuchi H, Evaluation of the Subchronic Toxicity of Dietary Administered *Equisetum arvense* in F344 Rats, J. Toxicol. Pathol., 23 (4), 2010, 245-51.

[20] Pyevich D, and Bogenschutz MP, Herbal diuretics and lithium

toxicity, Am. J. Psychiatry, 158 (8), 2001, 1329.

[21] Cetojevic-Simin DD, Canadanovic-Brunet JM, Bogdanovic GM, Djilas SM, Cetkovic GS, Tumbas VT, and Stojiljkovic BT, Antioxidative and antiproliferative activities of different horsetail (Equisetum arvense L.) extracts, J. Med. Food., 13 (2), 2010, 452-9.

[22] Mimica-Dukic N, Simin N, Cvejic J, Jovin E, Orcic D, and Bozin B, Phenolic compounds in field horsetail (*Equisetum arvense* L.) as natural antioxidants, Molecules, 13 (7), 2008, 1455-1464.

[23] Milovanovic V, Radulovic N, Todorovic Z, Stankovic M, and Stojanovic G, Antioxidant, antimicrobial and genotoxicity screening of hydro-alcoholic extracts of five serbian Equisetum species, Plant Foods Hum. Nutr., 62 (3), 2007, 113-9.

[24] Stajner D, Popovic BM, Canadanovic-Brunet J, and Boza P, Free radical scavenging activity of three *Equisetum* species from Fruska gora mountain *Fitoterapia*, 77 (7-8), 2006, 601-4.

[25] Stajner D, Popovic BM, Canadanovic-Brunet J and Anackov G, Exploring Equisetum arvense L., *Equisetum ramosissimum* L. and *Equisetum telmateia* L. as sources of natural antioxidants, Phytother. Res., 23 (4), 2009, 546-50.

[26] Nagai T, Myoda T, and Nagashima T, Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) *Equisetum arvense* L., Food Chem., 91 (3), 2005, 389-94.

[27] Myagmar BE and Aniya Y, Free radical scavenging action of medicinal herbs from Mongolia, Phytomedicine, 7 (3), 2000, 221-9.

[28] Silva WSD and Carmo DRD Voltammetric behavior of ascorbic acid in the presence of the herb *Equisetum arvense* (Comportamento voltamétrico do ácido ascórbico em presença da erva *Equisetum arvense*.), 2009, Available from: URL: http://prope.unesp.br/xxi_cic/27_35011891844.pdf.

[29] Castro VC, Oliveira JR, Vilela PGF, Carvalho CAT, Rocha RF, Jorge AOC and De Oliveira LD, Microbicidal effect of glycolic extract of *Equisetum arvense* L. (horsetail) on *Staphylococcus* spp., *Streptococcus mutans* and *Candida* spp." (Efeito microbicida do extrato glicólico de *Equisetum arvense* L.

(cavalinha) sobre *Staphylococcus spp*., *Streptococcus mutans* e *Candida spp*.) In: XV Encontro Latino Americano de Iniciação Científica e XI Encontro Latino Americano de Pós-Graduação. Universidade do Vale do Paraíba 2011; São José dos Campos. São Paulo. Brasil.

[30] Geetha RV, Lakshmi T and Roy A, In vitro evaluation of anti bacterial activity of *Equisetum arvense* Linn on urinary tract pathogens, Int. J. Pharm. Sci., 3 (4), 2011, 323-325.

[31] Radulovic N, Stojanovic G and Palic R, Composition and antimicrobial activity of *Equisetum arvense* L. essential oil, Phytother Res., 20 (1), 2006, 85-8.

[32] Husson GP, Vilagines R and Delaveau P, Research into the antiviral properties of a few natural extracts, Ann. Pharm. Fr., 44 (1), 1986, 41-48.

[33] Heisey RM and Gorham BK, Antimicrobial Effects of Plant Extracts on *Streptococcus mutans*, *Candida albicans*, *Trichophyton rubrum* and other Micro-Organisms, Lett. Appl. Microbiol., 14 (4), 1992, 136-139.

[34] Guerin JC and Reveillere HP, Antifungal Activity of Plant Extracts in Therapy. I. Study of 41 Extracts against 9 Fungal Species, Ann. Pharm. Fr., 42 (6), 1984, 553-559.

[35] Suganda AG, Amoros M, Girre L, and Fauconnier B, Inhibitory Effects of Some Crude and Semi-Purified Extracts of Plants Indigenous to France on the Replication of Human Herpes Virus 1 and Human Poliovirus 2 in Cell Culture, J. Nat. Prod., 46, (5), 1983, 626-632.

[36] Pereira BC., Gomes PS, Costa-Rodrigues J, Almeida Palmas R, Vieira L, Ferraz MP, Lopes MA and Fernandes MH, Equisetum arvense hydromethanolic extracts in bone tissue regeneration: in vitro osteoblastic modulation and antibacterial activity, Cell Prolif., 45 (4), 2012, 386-96.

[37] Mekhfi H, Haouari ME, Legssyer A, Bnouham M, Aziz M, Atmani F, Remmal A and Ziyyat A, Latelet anti-
aggregant property of some
Moroccan medicinal plants, J. Ethnopharmacol., 94 (2-3), 2004, 317-322.

[38] Goun EA, Petrichenko VM, Solodnikov SU, Suhinina TV, Kline MA, Cunningham G, Nguyen C and

Miles H, Anticancer and antithrombin activity of Russian plants, J. Ethnopharmacol., 81 (3), 2002, 337-42.

[39] Yamamoto Y, Antitumor activity of crude protein extracted from *Equisetum arvense* L., J. Anal. Bio-Sci., 22 (5), 1999, 421-424.

[40] Alexandru V, Petrusca DN, Gille E, Investigation of pro-apoptotic activity of *Equisetum arvense* L. water extract on human leukemia U 937 cells, Rouman Biotech Lett, 12 (2), 2007, 3139-3147.

[41] Sakurai N, Iizuka T, Nakayama S, Funayama H, Noguchi M and Nagai M, Vasorelaxant activity of caffeic acid derivatives from Cichorium intybus and *Equisetum arvense*, *Yakugaku Zasshi*, 123 (7), 2003, 593-8.

[42] Oh H, Kim DH, Cho JH and Kim YC, Hepatoprotective and free radical scavenging activities of phenolic petrosins and flavonoids isolated from Equisetum arvense, J. Ethnopharmacol., 95 (2-3), 2004, 421-4.

[43] Ferraz MP, Pereira AC, Lopes MA and Fernandes MH, *Equisetum arvense*: Evaluation of possible applications in bone regeneration (Avaliação das possibilidades de aplicação na regeneração óssea.), Rev. Fac. Ciênc. Saúde., 5, 2008, 136-145.

[44] Singh N, Kaur S, Bedi PM and Kaur D, Anxiolytic effects of *Equisetum arvense* Linn. extracts in mice, Indian J. Exp. Biol., 49 (5), 2011, 352-6.

[45] Safiyeh S, Fathallah FB, Vahid N, Hossine N and Habib SS, Antidiabetic effect of *Equisetum arvense* L. (Equisetaceae) in streptozotocin-induced diabetes in male rats, Pak. J. Biol. Sci., 10 (10), 2007, 1661-6.

[46] Soleimani S, Azarbaizani FF and Nejati V, The effect of *Equisetum arvense* L. (Equisetaceae) in histological changes of pancreatic beta-cells in streptozotocin-induced diabetic in rats, Pak. J. Biol. Sci., 10 (23), 2007, 4236-40.

[47] Safiyeh S, Fathallah FB, Vahid N, Habib SS and Nabat N, Effect of *Equisetum arvense* L. (*Equisetaceae*) in microalbuminuria and creatinine excretion streptozotocin-induced diabetes in male rats, *Int. J. Phamacol.*, 3 (2), 2007, 155-159.

[48] Monte FHD, Santos JGD Jr., Russi M, Lanziotti VM, Leal LK and Cunha GM, Antinociceptive and

anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense* L. in mice, Pharmacol. Res., 49 (3), 2004, 239-43.

[49] Oka M, Tachibana M, Noda K, Inoue N, Tanaka M and Kuwabara K, Relevance of anti-reactive oxygen species activity to anti-inflammatory activity of components of eviprostat, a phytotherapeutic agent for benign prostatic hyperplasia, *Phytomedicine*, 14 (7-8), 2007, 465-72.

[50] Tamaki M, Nakashima M, Nishiyama R, Ikeda H, Hiura M, Kanaoka T, Nakano T, Hayashi T, and Ogawa O, Assessment of clinical usefulness of Eviprostat for benign prostatic hyperplasia--comparison of Eviprostat tablet with a formulation containing two-times more active ingredients, Hinyokika Kiyo, 54 (6), 2008, 435-45.

[51] Hayat A, Temamogullari F, Yilmaz R, and Karabulut O, Effect of *Equisetum arvense* on Wound Contraction of Full-Thickness Skin Wounds in Rabbits, J. Animal Veterinary Adv., 10, 2011, 81-83.

[52] Ozay Y, Ozyurt S, Guzel S, Cimbiz A, Olgun EG and Cayci MK, Effects of *Equisetum arvense* ointment on dermal wound healing in rats, Wounds, 22 (10), 2010, 261-267.

[53] Raczuk J, Biardzka E and Daruk J, The content of Ca, Mg, Fe and Cu in selected species of herbs and herb infusions, Rocz. Panstw. Zakl. Hig., 59 (1), 2008, 33-40.

[54] Bye R, Thingstad SF and Paulsen BS, Horsetail (*Equisetum spp*.) as a source of silicon supplement in human nutrition - a myth? J. Herb Spices Med. Plant, 16, (2), 2010, 119-125.

[55] Law C and Exley C, New insight into silica deposition in horsetail (*Equisetum arvense*), BMC Plant Biol., 11, 2011, 112.

[56] Grases F, Melero G, Costa-Bauza A, Prieto R and March JG, Urolithiasis and phytotherapy, Int. Urol. Nephrol., 26 (5), 1994, 507-11.

[57] Rebuelta M and San Roman L, Estudio Del Efecto Diurético de: *Equisetum arvense* L., *Bidens aurea* Aiton Sherff., *Microme fruticosa* L., *Spergularia rubra* L., *Cynod dactylon* L, Anal. Inst. Bot., 34, 1978, 703-714.

[58] Graefe EU and Veit M, Urinary metabolites of flavonoids and hydroxycinnamic acids in humans after application of a crude extract from *Equisetum arvense*, Phytomedicine, 6 (4), 1999, 239-246.

[59] Guyton AC and Hall JE, Treaty of Medical Physiology (Tratado de Fisiologia Médica), 11th Ed., Saunders Elsevier, 2006.