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Chemical composition and potential pharmacological properties of field horsetail extract based on GC-MS analysis

Abstract. The *Equisetaceae* family, particularly the species *Equisetum arvense*, has long attracted the attention of researchers due to its ancient origins and various applications in traditional medicine. Field *Equisetum arvense* has been used to treat inflammation, wounds, infections, and urinary tract diseases. In this study, a phytochemical analysis of the *Equisetum arvense* extract was conducted using gas chromatography-mass spectrometry (GC-MS) to examine its chemical composition and assess its wound-healing properties. The *Equisetum arvense* extract was obtained through percolation with ethanol, followed by treatment with diethyl ether and chloroform to isolate nonpolar compounds. Final purification of the extract was carried out using aluminum oxide. The GC-MS method revealed a variety of compounds, including terpenes and fatty acids. Among these were components with anti-inflammatory, antibacterial, and antioxidant properties that may aid in accelerating wound healing. The primary components of the extract associated with wound-healing effects include quercetin, kaempferol, and β-sitosterol. The phytochemical analysis confirmed significant antioxidant properties in the extract, which may help protect tissues from oxidative stress that occurs during wound healing. The identified antibacterial compounds may help prevent wound infections and accelerate tissue regeneration. Thus, the *Equisetum arvense* extract, processed with chloroform and diethyl ether, represents a promising source of bioactive compounds with potential for application in the development of wound-healing agents.
Key words: *Equisetum arvense*, quercetin, kaempferol, β-sitosterol, antioxidant properties, wound-healing effect.

Introduction

In the past decade, interest in natural plant extracts with therapeutic properties has significantly increased. This trend is driven by a growing number of studies focused on identifying safe and effective alternatives to synthetic drugs, which can cause side effects and possess high toxicity with prolonged use. One promising source of natural compounds is *Equisetum arvense*. *E. arvense* are perennial, spore-bearing, herbaceous plants and ancient vascular plants in the class *Equisetopsida*, represented in modern flora by the single genus *Equisetum*. There are over 30 species of horsetail, distributed worldwide except for Australia, New Zealand, and tropical Africa [1,2].

Field *Equisetum* is a widespread weed. It grows in fields, meadows, wastelands, ravines, roadside ditches, and on road slopes. The specific name *arvensis* refers to its habitat. Among all species of *Equisetum*, *E. arvense* is the officially recognized medicinal plant. It grows in meadows, spruce forests, light coniferous forests, lime, aspen, pine-birch, birch, and mixed forests. It prefers floodplain forests, riverbanks, and shrub thickets. As a weed, it frequently occurs in fields and gardens. It can also be found along roadsides, on railway embankment slopes, near ditches, and in sandy and clay pits and excavations. In crops, it is quite abundant and is considered one of the most difficult-to-eradicate rhizomatous weeds [3-5].

Equisetum arvense has been traditionally used to treat various conditions, including inflammation, infections, and urinary tract disorders. However, its potential to accelerate wound healing has garnered particular interest and requires detailed scientific investigation.

Traumatic skin injuries, such as wounds, burns, and ulcers, present a significant medical challenge, especially when the healing process is hindered by factors such as infections or chronic illnesses. Developing new plant-based treatments to promote tissue regeneration has both theoretical and practical importance. Bioactive plant components, such as flavonoids, phenolic acids, and terpenes, have demonstrated anti-inflammatory, antioxidant, and antimicrobial properties, which may aid the healing process.

Literature indicates that *Equisetum arvense* extracts contain several compounds with potential wound-healing properties. However, the chemical composition and mechanisms of action of these extracts in wound healing remain inadequately studied. Gas chromatography-mass spectrometry (GC-MS) offers a precise method for identifying the chemical compounds in the extract and assessing their biological activities. This opens up new possibilities for developing phytopreparations based on field horsetail that could effectively promote tissue regeneration.

The aim of this study is to investigate the chemical composition of *Equisetum arvense* extract, obtained through percolation and treated with chloroform, diethyl ether, and aluminum oxide, followed by an evaluation of its wound-healing properties.

Materials and methods

For this study, *Equisetum arvense* leaves were collected in the summer of 2023 from Shelek village, Almaty region, Kazakhstan, at coordinates [43.600817, 78.217464] (Figure 1). The plant material was identified by botanists and certified as corresponding to the *Equisetum arvense* species.

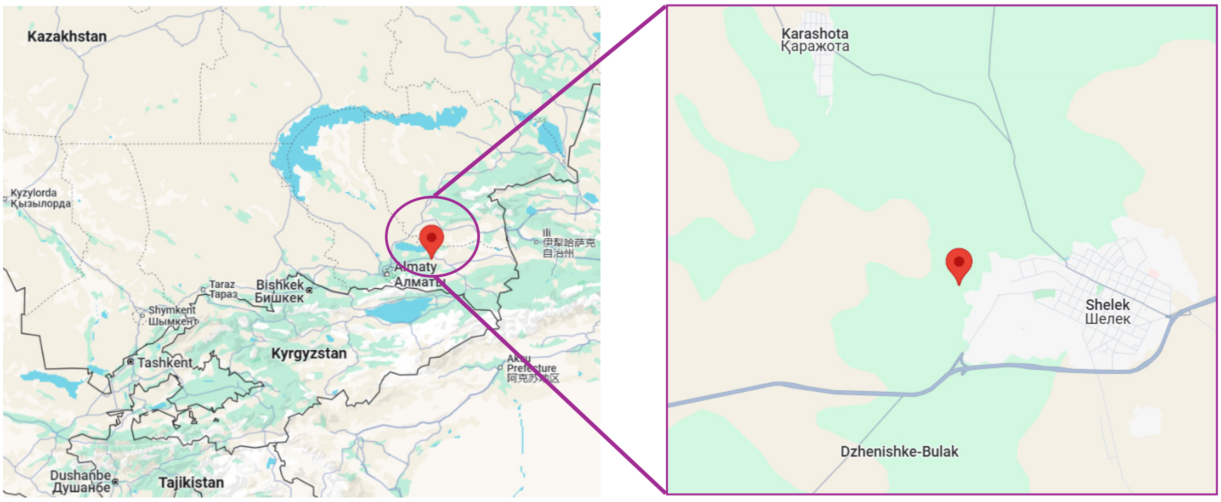

Figure 1 – The coordinates used for collecting field horsetail leaves

Extraction was conducted using ethanol (Talgar-Spirt, Kazakhstan) and other organic solvents, including chloroform (Baza 1 Khimreaktivov, Russia) and diethyl ether. Activated aluminum oxide was used for extract purification.

The component composition of the plant extract was analyzed on a TRACE 1310 gas chromatograph (Thermo Fisher Scientific, USA) equipped with a TSQ 8000 triple quadrupole mass spectrometric detector (Thermo Fisher Scientific, USA). Chromatographic separation was carried out using a TG-5SILMS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas with a constant flow of 1 mL/min. The initial temperature of the column thermostat was set at 70°C with a 2-minute hold, then increased to 200°C at a rate of 5°C/min with a 1-minute hold, and then to 285°C at 10°C/min with a 10-minute hold, followed by cooling back to the starting temperature of 70°C. The injector temperature was set at 250°C, and the mass spectrometric detector temperature at 260°C. The extract was injected in a volume of 1 μL in split mode (split ratio 1:10). The ionization mode was EI at 70 eV, with the ion source temperature at 230°C, and the scan range set to m/z 50-550 in Full scan mode. The chromatography process was controlled using XCalibur software. Bioactive compounds from two extractions were identified based on GC retention time using a library of reference mass spectra and NIST software (National Institute of Standards & Technology) for GC-MS data.

Results and discussion

To extract bioactive compounds from *Equisetum arvense*, solvents were selected, and extraction conditions optimized. To improve the extraction process, the effects of the raw material-to-solvent ratio, extraction time, and temperature were examined. The optimal extraction conditions were found to be 70% ethanol (1:8 raw material-to-extractant ratio, 2 hours, room temperature), yielding up to 60% bioactive compounds (biologically active complex).

The moisture content of the aerial part of *Equisetum arvense* was 5.44%, and ash content was 2.7%. The higher concentration of extractives at 70% ethanol is attributed to increased solubility of bioactive compounds at higher ethanol concentrations, enhancing extraction efficiency. This is influenced by various factors, including solubility of bioactive compounds, extraction intensity, and cell structure dissociation.

The most common biologically active complex groups in *Equisetum arvense* include coumarins (1.8%), free organic acids (1.83%), and saponins (1.2%), all essential components of the plant. *Equisetum arvense* contains coumarins, which have anti-inflammatory and anticoagulant properties and positively affect blood circulation. Free organic acids, such as malic and citric acids, aid digestion and possess antimicrobial properties. Saponins act as natural surfactants and have immunomodulatory and anti-inflammatory effects. Quantitative analysis revealed small amounts of flavonoids (0.62%) and alkaloids (0.5%).

Our chemical study revealed that *Equisetum* species vary in chemical composition, which may explain differences in their pharmacological properties. *Equisetum arvense* contains phenolic compounds (flavonoids, phenolcarboxylic acids) in quantities of 1.5% or higher [6]. More than 35 macro- and microelements were also detected, with maximum concentrations for manganese (30.39 μg/g), iron (16.5 μg/g), and zinc (11.4 μg/g). Iron, zinc, and manganese play significant roles in human health. Iron is essential for hemoglobin, necessary for oxygen transport throughout the body, and participates in metabolic processes and immune function. Zinc is vital for growth and development, immune system function, hormone formation, and the health of skin, hair, and nails. Manganese is part of many enzymes necessary for metabolism, anti-inflammatory processes, and antioxidant defense. All these elements are crucial for maintaining human health and proper body functioning.

Among the macroelements, potassium (701 μg/g), calcium (130 μg/g), and magnesium (700 μg/g) showed the highest concentrations. Potassium, calcium, and magnesium are also vital in the human body. Potassium is involved in regulating osmotic pressure, muscle and nerve function, maintaining a healthy heart rhythm, and controlling blood pressure. Calcium is a primary component of bones and teeth, necessary for normal muscle function, nerve impulse transmission, and blood clotting. Magnesium plays an essential role in over 300 biochemical reactions in the body, including protein and nucleic acid synthesis, muscle and nerve function, heart rhythm regulation, and maintaining healthy bone tissue metabolism. All these macronutrients are important for human health and proper body function [7].

The volatile components of *Equisetum arvense* were first studied using GC-MS, which identified twenty-five compounds. The primary components were cucurbitacin b, 25-desacetoxy-, rapamycin, Z,Z-2,5-Pentadecadien-1-ol, ergosta-5,22-dien-3-ol, acetate and geranyl isovalerate . The components of lipophilic extraction were identified by comparing the retention time and total mass spectra with the corresponding data from the Wiley275 and NIST98 libraries. The content of individual substances is calculated from the peak area, without the use of calibration coefficients with programmed automatic integration. Components with concentrations above 0.005% and a probability of matching mass spectra of more than 80% are taken into account. The repetition of the definitions is threefold.

The disc diffusion method was used to assess the antimicrobial activity of the oil against several microorganisms, including bacteria (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Salmonella enteritidis*) and fungi (*Aspergillus niger* and *Candida albicans*). Dilution of horsetail essential oil at a 1:10 ratio demonstrated high antimicrobial activity against all tested strains [8].

These compounds were isolated for their diuretic, antioxidant, vasorelaxant, germination-inhibiting, antinociceptive, and anti-inflammatory effects.

In the ethanolic extract of *Equisetum arvense*, 25 bioactive phytochemical compounds were identified and characterized based on peak area, retention time, molecular weight, and formula (Table 1, Figure 2). These chemical components may prove beneficial in various herbal medicines, such as anti-inflammatory, analgesic, antipyretic, cardiotonic, and anti-asthmatic drugs. Horsetail has traditionally been used for baths in treating rheumatic conditions, gout, as well as in the treatment of tumors and bone fractures in Europe. The plant is rich in sterols, ascorbic acid, phenolic acids, and flavonoids. Studies have shown that it possesses anti-inflammatory, antimicrobial, and analgesic properties.

Table 1 – Bioactive phytochemical compounds in the ethanol extract of field horsetail

No.	Compound	Molecular formula	RT	Content, %
1	Cucurbitacin b, 25-desacetoxy-	$C_{30}H_{44}O_6$	0.06	13.21
2	Rapamycin	$C_{51}H_{79}NO_{13}$	0.06	6.40
3	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	5.44	30.46
4	Ergosta-5,22-dien-3-ol, acetate	$C_{30}H_{48}O_2$	0.06	7.94
5	Geranyl isovalerate	$C_{15}H_{26}O_2$	4.78	6.35
6	5-(Prop-2-enoyloxy)pentadecane	$C_{18}H_{34}O_2$	5.01	5.96
7	4-Dimethyl(dichloromethyl)silyloxypentadecane	$C_{18}H_{38}Cl_2OSi$	5.01	5.38
8	5-Cyclopropylcarbonyloxypentadecane	$C_{19}H_{36}O_2$	5.01	21.85
9	Acetamide, 2,2,2-trifluoro-	$C_2H_2F_3NO$	3.42	82.43
10	DL-Homocystine	$C_8H_{16}N_2O_4S_2$	6.09	6.31
11	d-Mannose	$C_6H_{12}O_6$	6.90	7.70
12	Hydroxylamine	H_3NO	1.58	93.89
13	6-(1-Methylethyl)-4,4,6-trimethyltetrahydro-1,3-oxazin-2-thione	$C_{10}H_{19}NOS$	3.18	10.88
14	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	3.74	6.96
15	Z,Z-2,5-Pentadecadien-1-ol	$C_{15}H_{28}O$	5.44	30.46
16	d-Glucopyranose, 4-O-б-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	8.18	8.42
17	Melibiose	$C_{12}H_{22}O_{11}$	12.62	5.37
18	Desulphosinigrin	$C_{10}H_{17}NO_6S$	13.68	9.51
19	N-Hydroxymethyl fluoroacetamide	$C_3H_4F_3NO2$	3.42	11.99
20	Benz[e]azulen-3(3aH)-one	$C_{17}H_{24}O_5$	0.16	6.74
21	Trichloromethane	$CHCl_3$	2.11	27.81
22	Pregan-20-one,2-hydroxy-5,6-epoxy-15-methyl-	$C_{22}H_{34}O_3$	4.78	9.00
23	DL-Cystine	$C_6H_{12}N_2O_4S_2$	3.67	7.02
24	1-Nitro-б-d-arabinofuranose, tetraacetate	$C_{13}H_{17}NO_{11}$	6.09	6.56
25	Carbon dioxide	CO_2	1.52	27.93

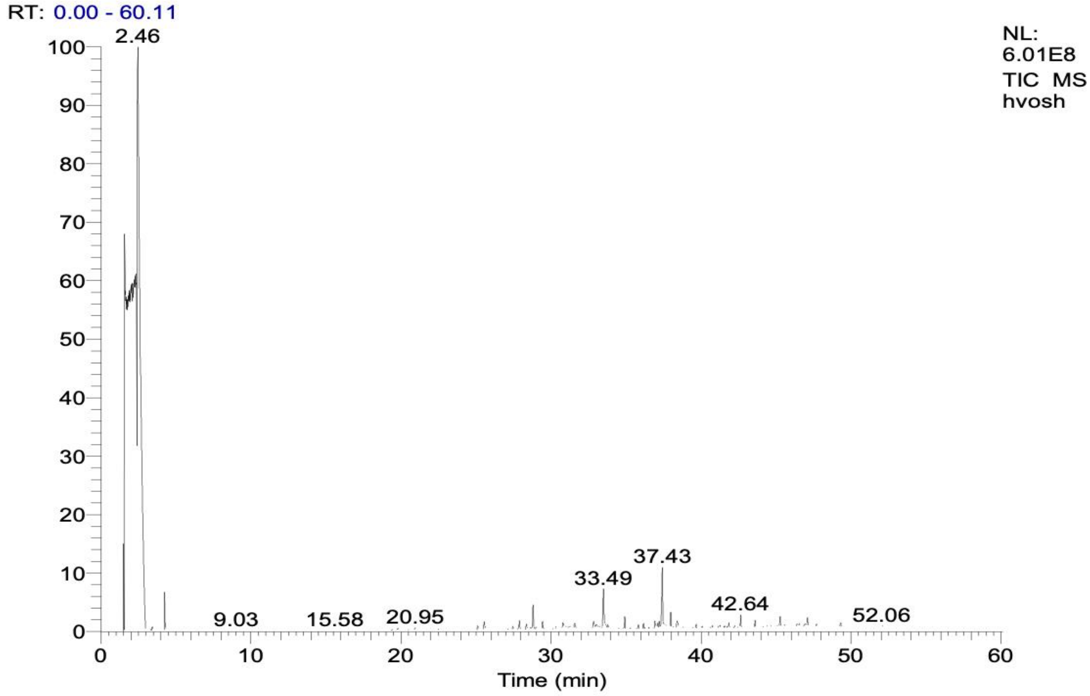

Figure 2 – Chromatogram of the GC-MS analysis of field horsetail extract *(Equisetum arvense)*

Equisetum arvense is particularly high in silicon, which is believed to significantly contribute to its medicinal properties, especially in bone diseases, while also providing diuretic, antioxidant, vasorelaxant, and sprouting-inhibitory effects, as well as antinociceptive and anti-inflammatory properties [9-15]. Acetylated flavonoid glycosides are common across all species of horsetail [16]. Studies on the antioxidant activity of field horsetail extracts have shown strong protective effects against free radicals, lipid peroxidation, and other oxidative agents [17].

Noteworthy phytochemicals found in horsetail include isobauerenol, taraxerol, germanicol, ursolic acid, oleanolic acid, and betulinic acid – the latter being a pentacyclic compound with known anticancer activity [18-20].

Conclusion

The findings of this study highlight the significance of *Equisetum arvense* extract in developing potential wound-healing agents based on natural components. Through phytochemical analysis using gas chromatography-mass spectrometry (GC-MS), a variety of bioactive compounds were identified, including phenolic acids and terpenes. These compounds demonstrated antioxidant, anti-inflammatory, and antibacterial properties, which contribute to wound healing by protecting tissues from oxidative stress and preventing infection.

Key components of the extract that support its wound-healing effects include quercetin, kaempferol, and β-sitosterol. Quercetin and kaempferol are known for their potent antioxidant properties, which help reduce oxidative stress and protect cells from damage caused by free radicals. In addition, β-sitosterol exhibits anti-inflammatory effects, reducing tissue inflammation and thereby accelerating regeneration. These bioactive compounds also play a role in modulating the immune response, promoting cell proliferation, and enhancing collagen synthesis, which are essential steps in wound healing.

The extract's additional applications include its use as a diuretic and vasorelaxant, underscoring its broad therapeutic potential. The plant's high silicon content is also believed to support bone health by promoting tissue mineralization and strengthening bone structure. This property is particularly important in the treatment of bone fractures and conditions such as osteoporosis. Furthermore, the anti-inflammatory and antioxidant properties of the extract may provide significant relief in conditions related to chronic inflammation, such as arthritis.

In conclusion, *Equisetum arvense* extract may be a promising raw material for creating agents that accelerate wound healing and have a general strengthening effect on the body. Given its wide spectrum of beneficial effects, including its role in improving skin regeneration, reducing inflammation, and supporting bone health, the extract shows substantial potential in both therapeutic and preventive healthcare. Further research is needed to investigate the biochemical mechanisms of the identified compounds and their potential application in clinical practice. Such studies will help refine the development of targeted, effective phototherapeutic products for wound care and broader health applications.

Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

References

1. Botirov E.K., Bonacheva V.M., Kolomiets N.E. (2021). Chemical composition and biological activity of metabolites of the *Equisetum* L. genus plants. *Chemistry of Plant Raw Materials*, 1, pp. 5-26.

2. Buzuk G.N., & Elyashevich E.G. (2010). Pharmacognostic characteristics of field horsetail *Equisetum arvense* L. Literature review. *Pharmacy Bulletin*, 2(48), pp. 65-72.

3. Kolomiyets N.E. (2006) Plants of the Genus Equisetum. *Pharmacy*, 3, pp. 46-48.

4. Kurkin, V.A. (2007) Pharmacognosy: Textbook for the students of pharmaceutical universities. Samara: Samara State Medical University and Ofort, pp. 827-832.

5. Goncharova T.A. (2004) Encyclopedia of medicinal plants. Herbal treatments, 1, M., pp. 88-90.

6. Kolomiets N. E., Kalinkina G. I., & Bondarchuk R. A. (2008). Plants of the horsetail genus (*Equisetum* L.): promising sources of new medicinal drugs. *Educ. Bull. Consciousness*, 10(9), pp. 392-393.

7. Kolomiets N.E., Ageeva L.D., Abramets N.Yu. (2014). Elemental composition of species of the *Equisetum* L. genus. *Fundamental Research*, 8-6, pp. 1418-1421.

8. Radulović N, Stojanović G, Palić R. (2006) Composition and antimicrobial activity of *Equisetum arvense* L. essential oil. *Phytother Res*., 20(1), pp. 85-8. https://doi.org/10.1002/ptr.1815.

9. Altameme H. J., Hameed I. H., Abu-Serag N. A. (2015) Analysis of bioactive phytochemical compounds of two medicinal plants, *Equisetum arvense* and *Alchemila vulgaris* seeds using gas chromatography-mass spectrometry and Fourier-transform infrared spectroscopy. *Malaysian Applied Biology Journal*, 44 (4), pp. 47–58.

10. D'Agostino M., Dini A., Pizza C., Senatore F., Aquino R. (1984) Sterols from *Equisetum arvense*. *Boll Soc Ital Biol Sper*., 60(12), pp. 2241-5.

11. Broudiscou L.P., Papon Y., & Broudiscou A.F. (2000). Effects of dry plant extracts on fermentation and methanogenesis in continuous culture of rumen microbes. *Animal Feed Science and Technology*, 87, pp. 263-277.

12. Dos Santos J.G. Jr., Blanco M.M., Do Monte F.H., Russi M., Lanziotti V.M., Leal L.K., Cunha G.M. (2005) Sedative and anticonvulsant effects of hydroalcoholic extract of *Equisetum arvense*. *Fitoterapia*, 76(6), pp. 508-13. https://doi.org/10.1016/j.fitote.2005.04.017.

13. Aramwit P., Sangcakul A. (2007) The effects of sericin cream on wound healing in rats. *Biosci Biotechnol Biochem*., 71(10), pp. 2473-2477. https://doi.org/10.1271/bbb.70243.

14. Duke J., Bogenschutz J., du Cellier J., Duke P. (2002) Handbook of medicinal herbs, 2nd ed. Boca Raton, FL: CRC Press, 896 p. ISBN 9780429126581.

15. Do Monte F.H., dos Santos J.G. Jr., Russi M., Lanziotti V.M., Leal L.K., Cunha G.M. (2004) Antinociceptive and anti-inflammatory properties of the hydroalcoholic extract of stems from *Equisetum arvense* L. in mice. *Pharmacol Res*., 49(3), pp. 239-243. https://doi.org/10.1016/j.phrs.2003.10.002.

16. Nosrati Gazafroudi K., Mailänder L.K., Daniels R., Kammerer D.R., Stintzing F.C. (2024). From stem to spectrum: phytochemical characterization of five *Equisetum* species and evaluation of their antioxidant potential. *Molecules*, 29(12), p. 2821. https://doi.org/10.3390/molecules29122821.

17. Mimica-Dukic N., Simin N., Cvejic J., Jovin E., Orcic D., Bozin B. (2008). Phenolic compounds in field horsetail (*Equisetum arvense* L.) as natural antioxidants. *Molecules*, 13(7), pp. 1455-1464. https://doi.org/10.3390/molecules13071455.

18. Raghda Makia, Khulood W. Al-Sammarrae, Mohammad M.F. Al-Halbosiy and Mohammed H. Al-Mashhadani. (2022) Phytochemistry of the *Genus Equisetum* (*Equisetum arvense*). *GSC Biological and Pharmaceutical Sciences*, 18(02), pp. 283-289. https://doi.org/10.30574/gscbps.2022.18.2.0059.

19. Weber R. (2005). Equisetites aequecaliginosus sp. nov., ein Riesenschachtelhalm aus der spättriassischen Formation Santa Clara, Sonora, Mexico. *Revue de paléobiologie*, 24(1), pp. 331-364.

20. Sandhu N.S., Kaur Sarabjit, Chopra Divneet (2010). *Equisetum arvense*: pharmacology and phytochemistry-a review. *Asian journal of pharmaceutical and clinical research*, 3(3), pp. 146-150.

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