

Antibacterial effect of *Equisetum arvense* L.

AJAY KUMAR AND PURSHOTAM KAUSHIK

Department of Botany and Microbiology, Gurukul Kangri University, HARIDWAR (UTTRAKHAND) INDIA
E-mail : jkmr156@gmail.com

(Received: Jun, 2011; Revised: Jul., 2011; Accepted : Aug., 2011)

The present study intends to review antimicrobial investigation of the alcoholic and chloroform extracts of *Equisetum arvense* which is traditionally used as herbal medicine. Antibacterial activity of the alcoholic and chloroform extracts of *Equisetum arvense* were tested against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Seasonal sampling of the *Equisetum arvense* were also carried out in three different seasons summer, winter and rainy. The extracts of *Equisetum arvense* were prepared in alcohol and chloroform and compared with the 1 unit strength of antibiotic tetracycline. It was effective against *Bacillus subtilis* and more effective in summer season than rainy and winter seasons. Alcoholic extracts was found more effective than chloroform.

Key words : Antibacterial, *Equisetum arvense*

Kumar, Ajay and Kaushik , Purshotam . (2011). Antibacterial effect of *Equisetum arvense* L. *Asian J. Bio. Sci.*, **6** (2): 184-187.

INTRODUCTION

The herbal medicine is used for primary health care in present day. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurveda, homeopathic, naturopathic, traditional oriental, (Kumar and Kaushik 1999,2009). According to WHO around 119 plant-derived pharmaceutical medicines, about 74 per cent are used in modern medicine in ways that correlated directly with their traditional uses as plant medicines by native cultures. Major pharmaceutical companies are currently conducting extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value (Chandra, 2008)

Throughout the middle ages, home-grown botanicals were the only medicines readily available, and for centuries, no self-respecting household would be without a carefully tended and extensively used herb garden. For the most part, herbal healing lore was passed from generation to generation by word of mouth. Mother taught daughter; the village herbalist taught a promising apprentice (Kaushik and Chauhan, 2009).

Pteridophytes by virtue for their possesses great variety and fascinating foliage have drawn the attention and admiration of research worker. They reported around 305 genera and 10000 species all over the world, about

191 genera and 1000 species are reported from India. (Nema *et.al.*, 2008).

Equisetum is the only living genus in the *Equisetaceae*, family of vascular plants that reproduce by spores rather than seeds. which for over one hundred million years was much more diverse and dominated the understory of late Paleozoic forests. Some Equisetopsida were large trees reaching to 30 meters tall, the genus *Calamites* of family Calamitaceae for example is abundant in coal deposits from the Carboniferous period. A superficially similar but entirely unrelated flowering plant genus, mare's tail (*Hippuris*), is occasionally misidentified and misnamed as "horsetail". (Cetto *et al.*, 2000)

Equisetum contains high amounts of silica. Since at least the days of ancient Greece, physicians have relied on *Equisetum arvense*, and the other horsetail varieties as potent medicines. Horsetail's efficacy as a medicinal plant largely depends on its sterile stem (as opposed to the fertile stem), which contains high amounts of silica and magnesium. Extracts from the fresh plant are often used, but sometimes ashes from the burnt herb are prescribed. Seventeenth-century physicians frequently employed the herb, especially to treat bleeding. It is very powerful to stop bleeding, either inward or outward, the juice or the decoction being drunk, or the juice, decoction or distilled water applied outwardly (Nagai *et al.*, 2005).

Horsetail, an effective diuretic, is included in anti-

bloating PMS teas and cellulite creams. Their combinations of astringent and diuretic properties enable it to flush the kidneys and bladder, making it useful for various urinary tract infections. The high silica content of horsetail allows it to build connective tissue internally and externally. "It solders together the tops of wounds and cures all ruptures in children." The ashes of horsetail plant supposedly soothe stomach upset. It is recommended taking 3 to 10g. of the burnt herb as often as needed. (Santos *et al.*, 2005)

However, there is very less information is available about the pharmacological importance of *Equisetum arvense* and therefore, present study is designed to investigate the possible antibacterial action of the alcoholic and chloroform extracts of *Equisetum arvense* which is traditionally used as herbal medicine. Antibacterial activity of the alcoholic and chloroform extracts of *Equisetum arvense* against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*, was investigated. Seasonal sampling of the *Equisetum arvense* were also carried out in three different seasons summer, winter and rainy. The extracts of *Equisetum arvense* were prepared in alcohol and chloroform and compared with the 1 unit strength of antibiotic tetracycline.

RESEARCH METHODOLOGY

Equisetum arvense were collected from the bank of holly Ganga river from Rishikesh and washed with tap water, followed by a wash with sterilized distilled water, Screen and trim medicinal plants or fragments defined beforehand with care. At the same time, put aside a part of the fresh medicinal plant which after being weighed- was dried out at 50°C for 12 to 24 hours. Weighed the mixture again to get its dry weight, that generally corresponded to 20-30 per cent of the fresh weight; and thereafter 100 g of plant material was taken and macerated in 250 ml of absolute alcohol, on other hand 100 g of plant material was macerated in 250 ml of chloroform (lab. Reagent),

The macerate was preserved in a cool and dark place and kept in air-tight container for 3 weeks; shaken from time to time; At the end of the 3 weeks, macerated in ethyl alcohol 95 per cent; added to the blend the amount of distilled water necessary to get a degree of about 95 per cent alcohol and also retrieve the liquid from pressing strongly out the residue of the macerated plant; blended the whole mixture and these samples were stored at room temperature for 48-72hrs in dark away from direct sunlight. It was stirred at 12hr. intervals by means of sterile

glass rod. After 72 h of alcoholic and chloroform treatments, each extract was filtered through muslin cloth and re-filtered by passing through Whatman filter paper N. 42. This process of extraction was repeated with the same volume of alcohol. The filtrate was evaporated gently to get the requested ratio compared with the theoretical weight of dry matter.

The test organisms, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* were obtained from IMTECH, Chandigarh and inoculated evenly in uniform quantity in Petri plate containing suitable sterilized media. Whatman filter paper discs (0.5 cm dia) were used to test the antibacterial activity of alcoholic and chloroform extract (prepared as above) with its aqueous dilution (25 per cent -75 per cent). Each extract was also compared with 1 unit strength of tetracycline antibiotic. Distilled water, alcohol and chloroform were used as control.

RESULTS AND ANALYSIS

The present study clearly indicates that alcoholic and chloroform extract of *Equisetum arvense* possessed antibacterial activity. Data presented in Table 1-3 show the antibacterial effect of alcoholic and chloroform extract of *Equisetum arvense* in three different seasons summer, winter and rainy. It is clearly revealed that on increasing the concentration of plant extract the size of inhibition zone increased markedly.

The effective zones of inhibition of undiluted alcoholic extract of *Equisetum arvense* in summer season measured against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*, were 4.8 mm, 4.1 mm, 4.3 mm, 4.0 mm. and diluted alcoholic extract of 80 per cent dilution showed 3.5 mm, 2.3 mm, 2.5 mm, 2.7 mm. 50 per cent dilution show 2.2 mm, 1.8 mm, 1.5 mm, 1.7 mm, 20 per cent dilution showed 1.5 mm, 0.91 mm, 0.98 mm, 0.98 mm, respectively. Such zones formed by chloroform extract of *Equisetum arvense* in summer season were measured to be 4.7 mm 4.0 mm, 4.3 mm, 4.0 mm, and diluted chloroform extract of 80 per cent dilution showed 2.7 mm, 2.1 mm, 2.3 mm, 2.2 mm, 50 per cent dilution showed 1.8 mm, 1.4 mm, 1.2 mm, 1.1 mm. 20 per cent dilution showed 1.0 mm, 0.97 mm, 0.77 mm, 0.54 mm, respectively (Table 1).

The effective zones of inhibition of undiluted alcoholic extract of *Equisetum arvense* in winter season measured against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*, were 4.4 mm, 3.7 mm, 3.9 mm, 3.5 mm, respectively and diluted alcoholic

Table 1: Antibacterial effect of *Equisetum arvense* in summer season

Test organism	Diluted alcoholic extract in mm			Antibiotic tetracycline	Diluted chloroform extract		
	80%	50%	20%		80%	50%	20%
<i>Bacillus subtilis</i>	3.5	2.2	1.5	10.0	2.7	1.8	1.0
<i>Escherichia coli</i>	2.3	1.8	0.91	12.0	2.1	1.4	0.97
<i>Salmonella typhi</i>	2.5	1.5	0.98	15.0	2.3	1.2	0.77
<i>Staphylococcus aureus</i>	2.7	1.7	0.98	14.0	2.2	1.1	0.54
	Undiluted alcoholic extract replicates				Control alcohol	Control water	Effective inhi. zone
	1	2	3	Mean			
<i>Bacillus subtilis</i>	9.8	9.8	9.8	9.8	5.0	Nil	4.8
<i>Escherichia coli</i>	9.1	9.0	9.2	9.1	5.0	Nil	4.1
<i>Salmonella typhi</i>	9.3	9.5	9.2	9.3	5.0	Nil	4.3
<i>Staphylococcus aureus</i>	9.0	9.0	9.0	9.0	5.0	Nil	4.0
	Undiluted chloroform extract replicates				Control chloroform	Control water	Effective inhi. zone
	1	2	3	Mean			
<i>Bacillus subtilis</i>	9.9	9.8	9.4	9.7	5.0	Nil	4.7
<i>Escherichia coli</i>	9.0	9.0	9.0	9.0	5.0	Nil	4.0
<i>Salmonella typhi</i>	9.1	9.3	9.5	9.3	5.0	Nil	4.3
<i>Staphylococcus aureus</i>	9.0	8.9	9.1	9.0	5.0	Nil	4.0

extract of 80 per cent dilution showed 3.7 mm, 2.8 mm, 2.9 mm, 2.8 mm. 50 per cent dilution show 2.7 mm, 2.1 mm, 1.9 mm, 1.8 mm, 20 per cent dilution showed 1.8 mm, 1.1 mm, 1.0 mm, 1.0 mm, respectively. Such zones formed by chloroform extract of *Equisetum arvense* in winter season were measured to be 3.3 mm 3.5 mm, 3.8 mm, 3.5 mm, respectively and diluted chloroform extract of 80 per cent dilution showed 3.1 mm, 2.4 mm, 2.5 mm, 2.2 mm, 50 per cent dilution showed 2.1 mm, 1.7 mm, 1.7 mm, 1.4 mm. 20 per cent dilution showed 1.5 mm, 1.0 mm, 1.0 mm, 0.94mm,

respectively (Table 2).

The effective zones of inhibition of undiluted alcoholic extract of *Equisetum arvense* in rainy season measured against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*, were 3.7 mm, 3.2 mm, 3.5 mm, 3.0 mm, respectively and diluted alcoholic extract of 80 per cent dilution showed 3.5 mm, 2.3 mm, 2.7 mm, 2.5 mm. 50 per cent dilution show 2.2 mm, 1.8 mm, 1.7 mm, 1.5 mm, 20 per cent dilution showed 1.5 mm, 0.91 mm, 0.98 mm, 0.98 mm, respectively. Such zones

Table 2 : Antibacterial effect of *Equisetum arvense* in winter season

Test organism	Diluted alcoholic extract			Antibiotic tetracycline	Diluted chloroform extract		
	80%	50%	20%		80%	50%	20%
<i>Bacillus subtilis</i>	3.7	2.7	1.8	10.0	3.1	2.1	1.5
<i>Escherichia coli</i>	2.8	2.1	1.1	12.0	2.4	1.7	1.0
<i>Salmonella typhi</i>	2.9	1.9	1.0	15.0	2.5	1.7	1.0
<i>Staphylococcus aureus</i>	2.8	1.8	1.0	14.0	2.2	1.4	0.94
	Undiluted alcoholic extract replicates				Control alcohol	Control water	Effective inhi. zone
	1	2	3	Mean			
<i>Bacillus subtilis</i>	9.3	9.7	9.2	9.4	5.0	Nil	4.4
<i>Escherichia coli</i>	8.7	8.6	8.8	8.7	5.0	Nil	3.7
<i>Salmonella typhi</i>	8.9	8.9	8.9	8.9	5.0	Nil	3.9
<i>Staphylococcus aureus</i>	8.6	8.4	8.5	8.5	5.0	Nil	3.5
	Undiluted chloroform extract replicates				Control chloroform	Control water	Effective inhi. zone
	1	2	3	Mean			
<i>Bacillus subtilis</i>	8.3	8.4	8.2	8.3	5.0	Nil	3.3
<i>Escherichia coli</i>	8.5	8.7	8.3	8.5	5.0	Nil	3.5
<i>Salmonella typhi</i>	8.7	8.9	8.8	8.8	5.0	Nil	3.8
<i>Staphylococcus aureus</i>	8.5	8.7	8.3	8.5	5.0	Nil	3.5

Table 3: Antibacterial effect of *Equisetum arvense* rainy season

Test organism	Diluted alcoholic extract			Antibiotic tetracycline	Diluted chloroform extract		
	80%	50%	20%		80%	50%	20%
<i>Bacillus subtilis</i>	3.5	2.2	1.5	10.0	2.7	1.8	1.0
<i>Escherichia coli</i>	2.3	1.8	0.91	12.0	2.1	1.4	0.77
<i>Salmonella typhi</i>	2.7	1.7	0.98	15.0	2.3	1.2	0.72
<i>Staphylococcus aureus</i>	2.5	1.5	0.98	14.0	2.2	1.1	0.54
	Undiluted alcoholic extract replicates				Control alcohol	Control water	Effective inhi.zone
	1	2	3	Mean			
<i>Bacillus subtilis</i>	8.7	8.5	8.9	8.7	5.0	Nil	3.7
<i>Escherichia coli</i>	8.2	8.3	8.1	8.2	5.0	Nil	3.2
<i>Salmonella typhi</i>	8.4	8.3	8.8	8.5	5.0	Nil	3.5
<i>Staphylococcus aureus</i>	7.8	8.2	8.0	8.0	5.0	Nil	3.0
	Undiluted chloroform extract replicates				Control chloroform	Control water	Effective inhi.zone
	1	2	3	Mean			
<i>Bacillus subtilis</i>	8.8	8.3	8.1	8.4	5.0	Nil	3.4
<i>Escherichia coli</i>	8.0	7.8	8.2	8.0	5.0	Nil	3.0
<i>Salmonella typhi</i>	8.3	8.5	8.1	8.3	5.0	Nil	3.3
<i>Staphylococcus aureus</i>	8.1	8.2	8.0	8.1	5.0	Nil	3.1

formed by chloroform extract of *Equisetum arvense* in rainy season were measured to be 3.4 mm, 3.0 mm, 3.3 mm, 3.1 mm, respectively and diluted chloroform extract of 80 per cent dilution showed 2.7 mm, 2.1 mm, 2.3 mm, 2.0 mm, 50 per cent dilution showed 1.8 mm, 1.4 mm, 1.2 mm, 1.1 mm. 20 per cent dilution showed 1.0 mm, 0.77 mm, 0.72 mm, 0.54 mm, respectively (Table 3).

The antibacterial potential of *Equisetum arvense* was studied in present investigation against *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus* which was compared to 1 unit strength of antibiotic tetracycline . The extract of plants were made in alcohol, chloroform and both solvent were used as control as well. It was recorded that, *Equisetum arvense* was more effective against *Bacillus subtilis* as compared to *Escherichia coli*, *Staphylococcus aureus* and, *Salmonella typhi*. Seasonal sampling of the *Equisetum arvense* were also carried out in three different seasons summer, winter and rainy. The extract of *Equisetum arvense* was more effective in summer season than rainy and winter season. The alcoholic extract of plants was found more effective than chloroform extracts and both extracts of plants were found less effective than I unit strength of antibiotic tetracycline, however, these could be brought at par to the tetracycline by concentrating these extracts.

LITERATURE CITED

Cetto, A. A., Wiedenfeld, H., Revilla M. C., and Sergio, I. A. (2000). Hypoglycemic effect of *Equisetum myriochaetum* aerial parts on streptozotocin diabetic rats. *J. Ethnopharmacol.* **72**:129-133.

Chandra, S., Fraser-Jenkins, C.R., Kumari, Alka and Srivastava, Archana (2008). A summary of the status of threatened Pteridophytes of India. *Taiwania*, **53**(2): 170-209.

Kaushik, P. and Chauhan, A. (2009). Cyanobacteria : antibacterial Activity. New India Publishing Agency New Delhi pp XIV +1-198.

Kumar, A., and Kaushik, P. (1999). Antibacterial effect of *Adiantum capillus veneris* Linn. *Indian Fern J.*, **16** : 72-74.

Kumar A. and Kaushik, P. (2009). Antibacterial effect of two aquatic Pteridophytes. *Indian Fern J.*, **26** : 161-165.

Nagai, T., Myoda, T. and Nagashima T. (2005). Antioxidative activities of water extract and ethanol extract from field horsetail (tsukushi) *Equisetum arvense* L, *Food Chem.*, **91**(3): 389-394.

Nema, R.K., Meyyanathan, S.N. and Sharma, C.S. (2008). A practical approach to pharmaceutical analysis, 1st Edition, CBS Publishers & Distributors, New Delhi , pp. 89-90.

Santos, JGDos jr., Monte, FHMDo., Russi, M.,Lanziotti, VMNB., Leal, LKAM. and Cunha GM. (2005). Sedative and anticonvulsant effects of hydroalcoholic extract of *Equisetum arvense*, *Fitoterapia*, **6**(6): 508-513.

**** * ****