Equisetum diffusum its Phytochemistry, Antimicrobial and Antifungal activity

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ABSTRACT

Equisetum diffusum was analysed for the presence of various phytochemicals and it had been found that the concerned plant is enriched with many phytochemicals with wide range of applications in daily life. In addition the plant was found to good antifungal activity against Aspergillus flavus, Aspergills Niger, Fusarium, Nigrospora oryzae. The concerned plant being anti fungal in nature is one of its best natures as least number of phytochemicals is antifungal in nature. In addition the plant extracts show antimicrobial activity against the concerned bacteria viz, E.coli, Bacillus cereus, Salmonella Typhi, Bacillus pumilus, Bacillus licheniformis, Micrococcous luteus, Streptococcus mutans. The plant extracts show a good range of Rf values where a good number of phytochemicals are isolable with potential medicinal properties.

Keywords
Bacteria, Chromatography, Equisetum diffusum, Fungi, Phytochemicals

Article Received: 10 August 2020, Revised: 25 October 2020, Accepted: 18 November 2020

Introduction

Medicinal plants have been considered and are mentioned from times immoral for treatment of various diseases. It had been known that about 250,000 to 500,000 plant species are under use (**Borris, R. P**. 1996). But a very little concentration (1 to 10%) of medicinal plants are being consumed as a feeding materials by humans and other animals, but a large portion of these plants are used medicinally (**Moerman, D. E.** 1996). Hippocrates have recognized nearly 300 to 400 plants which were medicinally important (**Schultes, R. E.** 1978). the degradation in the ancient civilizations in knowing the viability of medicinal plants, have been lost (**Stockwell, C.** 1988) many times. Medicinal plant usage in North America's history follows two strands, the indigenous cultures (Native Americans), dates back (**Weiner, M. A.** 1980), and an alternative movement among Americans in the 19th century. Use of medicinal plants in America have reviewed extensively as mentioned in many articles by (**Moerman, D. E.** 1996.). The plants are popularly known for their anti-inflammatory activity in Europe, Asia and America, and also in Turkey and America as antiseptic agents (**Klink, B.** 1997, **Holmes, O. W.** 1861, and **Lewis, W. H., and M. P. Elvin-Lewis.** 1995).

The genus *Equisetum* there are 30 species which are rush like, jointed conspicuously, and are perennial. *Equisetum* is the only living genus of the order *Equisetales* belonging to class Sphenopsida. *Equisetum arvense*, possesses tooth like sheath which cover its joints; and from the sheaths of central stem whorls arise, attaining a 60cm in height, but usually less (Clute W.N. 1928, Great Plains Flora Association. 1986.). Amny studies showed that the plant is hypoglycemic (Iyer H. 2006, Soleimani S., et.al 2007.) and diuretic (Mamedova K.T. et.al., 1996, Jung H.C., et.al., 1999.). The plant possesses an anti-inflammatory property and is being used for bathing purpose in Europe, Asia and America and is found to be antiseptic used in Turkey and America (Hoffman D. 1990, Ody P., and Kindersley D. 1993, Mineo S., et.al., 1993). The *Equisetum arvense* water and ethanolic extracts have the potential to neutralize the free radicles so is being considered to have an antioxidant property (Nagai T., et. Al., 2005). The hydro alcoholic extract have sedative effect (Santos et. al., 2005).

As already pointed that, all living horsetails are considered and have been kept in genus *Equisetum*. But few fossil species are not assignable to the modern genus.

Materials and methods

The plant ***Equisetum diffusum*** in fresh form have been collected from a local area bank of the river from the sandy soil at Dehradun. The authentication of the plant was done at BSI, Northern Regional Centre and Dehradun. The plant samples were submitted at B.S.I herbarium with specimen no. **113520**. The plant parts wer shade dried at room temperature and then were crushed into powder.

Extraction

50 Gms of plant powder was segregated shade dried, weighed and finally extraction was done by Soxhlet Apparatus. Various solvents with different polarity index (Petroleum ether, Chloroform, Ethanol and Water) were used as per their polarity index.

Equisetum diffusum was first extracted by using petroleum ether as a solvent. The respective extract was then filtratered and the obtained filtrate was evaporated to 1/4th its volume using water bath. The extract mixture after the solvent removal, was made a powder be keeping in an oven for a required time. The remained petroleum ether residue was then subjected to further extraction process using chloroform as solvent in a same way as used for extraction using petroleum ether. Similarly the extraction of the residue after extraction with chloroform was then extracted in a same manner with ethanol.

The residue after extraction with chloroform was then extracted using water as a solvent by decoction technique, in which 500ml of water was added to the residue. The complete mixture was heated using water bath and whole water was made to evaporate, and then more 500 ml of water was further added to the extract, the mixture was then further evaporated in which only 250ml of water was kept in the mixture. The extracted mixture was then filtered, and the obtained filtrate was evaporated completely to 1/4th of its volume. Finally the extracted filtrate was collected and made to dry by keeping in an oven at 30-50^{0}C temperature range.

Phytochemical Analysis (Schoendorfer, N., et.al. 2018, Al-Badri, HB. Et.al. 2016, Sola-Rabada et.al, 2016. **):**

The Phytochemicals like Terpenoids, Coumarins, Tannins, Alkaloids, Quinones, Flavonoids, Glycosides, Steroids, and Saponins etc have been analysed. These phytochemicals play an important role against various ailments of the body. as well and are used to cure various ailments.

Antimicrobial Assay (Kokate, CK., et.al. 2005**).**

An antimicrobial medicine is a drug that does posses the potential to neutralize and kill the microbes which include fungi, bacteria and viruses. The Antimicrobial medicines either directly kill the microbes so are called microbicidal or may pause the microbial growth so are called microbistatic. The term chemotherapy is used for the treatment of microbial infection

The Microorganisms Source

The antimicrobial assay was performed under the headings of antibacterial and antifungal assays.

Evaluation of the antimicrobial potential of plant extracts:

Five gram positive and three gram negative bacteria viz. *E.coli, Bacillus pumilus, Bacillus cereus, Bacillus licheniformis, Pseudomonas aeruginosa, Salmonella typhi,* and *Streptococcus mutans* were taken to evaluate antibacterial potential of the different extracts.

All the isolated extracts were analysed for the antimicrobial activity to know whether they are active against the particular bacteria or not. The extracts were dissolved in 30% DMSO, a highly polar, aprotic organic solvents. The food material which is being used as a substrate by microorganisms called culture medium and the growth is called culture.

Evaluation of the antifungal potential of plant extracts:

The extracts were also analysed as antifungal agents against, *Aspergillus flavus, Aspergillus Niger, Fusarium and Nigrospora oryza.*

Observations and Results

The extraction results of *Equisetum diffusum* shows the highest percentage yield for aqueous medium providing 13.39% and the Ethanol extract was found 4.99%. The percentage yield of petroleum ether extract was found 2.67% and the lowest yield was found in chloroform extract i.e. 1.28%.

Table 1: Showing various Phytochemicals qualitatively in various extracts

Chemical constituent	Test	P.E	C.E	E.E	A.E
Carbohydrates test	Molisch's test	+	+	+	+
	Benedict's test	+	+	+	+
	Fehling's test	+	+	+	+
Alkaloids	Mayer's test	-	+	-	-
	Wagner's test	-	+	-	-
	Hager's test	-	+	-	-
Glycoside	Modified Bortrager's	-	-	-	-
Saponins	Foam test	-	-	+	-
	Salkowski test	-	-	+	-
Phenols	Ferric chloride test	+	+	+	+
Proteins	Xanthoproteic test	-	-	+	+
	Biuret test	-	-	-	-

P.E (Petroleum ether extract) C.E: (Chloroform Extract) E.E: (Ethanol Extract) A.E: (Aqueous extract).

Table 2: Analysis of Antibacterial activity of Equisetum diffusum Extract against different bacteria

Extract/Bacteria	P.E ZoI (mm)	C.E ZoI (mm)	E.E ZoI (mm)	A.E ZoI (mm)	Standard antibiotic (Ampicillin) ZoI (mm)
Escherichia coli	0	13	12	13	18
Micrococcus luteus	0	14	0	12	18
Pseudomonas aeruginosa	11	13	14	13	10
B. pumilus	11	12	0	13	11
B. cereus	13	12	19	13	17
B. licheniformis	0	13	0	13	12
Salmonella typhi	0	12	12	14	14
Streptococcus mutans	0	0	11	12	12

ZoI= Zone of Inhibition

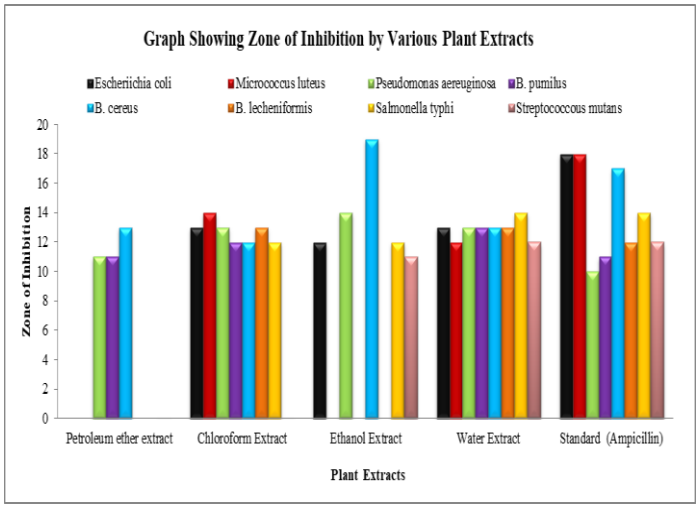

Fig 1: Graphical representation of Antibacterial activity of Equisetum diffusum extract

Table 3: Analysis of Antifungal activity of Equisetum diffusum extract against different fungus

Extract/ Fungal	P.E **ZoI (mm)**	C.E **ZoI (mm)**	E.E **ZoI (mm)**	A.E **ZoI (mm)**	Standard antibiotic (Ketoconazole) **ZoI (mm)**
Aspergillus Niger	12	13	15	12	24
Aspergillus flavus	13	12	14	12	21
Nigrospora oryza	14	14	15	12	19
Fusarium	13	15	0	0	22

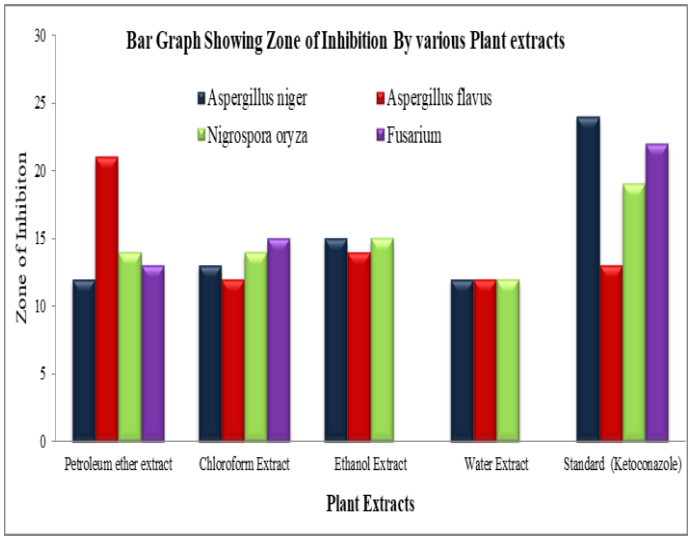

Fig 2: Graphical representation of anti-fungal activity of Equisetum diffusum Extract

Thin layer chromatography

Thin Layer Chromatography (TLC) is also known as open column or spread layer chromatography. It is based on the difference in the extent to which the component of a mixture gets adsorbed on a given adsorbent. The spots obtained on the thin layer chromatogram and their corresponding R_f values are mentioned in the following table.

Table 4:T.L.C. of Equisetum diffusum in Petroleum Ether Extract

Ratio of solvent Petroleum ether: Ethyl acetate	**No of T.L.C**	**Running solvent(cm)**	**Compound running (cm)**	**Rf value (cm)**
60:40	1	5.8	1.1	0.94
	2	5.9	5.4	0.19
70:30	1	5.1	4.8	0.95
	2	5.5	4.9	0.89
80.20	1	5.9	3.1	0.52
		5.9	2.4	0.40
	2	6.9	2.5	0.37
		6.9	1.9	0.27
90.10	1	7.1	0	0
	2	7	0	0

Table 5:T.L.C. of plates Equisetum diffusum in Chloroform Extract

Ratio of solvent Petroleum ether : Ethyl acetate	**No of T.L.C**	**Running solvent (cm)**	**Compound running (cm)**	**Rf value (cm)**
60:40	1	5.6	0	0
	2	5.4	0	0
70:30	1	5.5	4.4	0.8
		5.5	3.0	0.55
		5.5	1.4	0.25
	2	5.5	3.9	0.70
		5.5	2.8	0.50
80:20	1	5.1	3.7	0.72
		5.1	1.9	0.37
	2	4.9	3.9	0.79
		4.9	2.2	0.44
90:10	1	5	0	0
	2	5.1	0	0

Table 6: T.L.C. plates Equisetum diffusum in Ethanolic Extract

Ratio of solvent Petroleum ether : Ethyl acetate	**No of T.L.C**	**Running solvent(cm)**	**Compound running (cm)**	**Rf value (cm)**
60:40	1	5.2	1.6	0.30
	2	4.9	0	0
70:30	1	5.6	1.5	0.26
	2	4.4	3	0.68
		4.4	0.9	0.20
		4.4	0.4	0.09
80:20	1	3.9	3.1	0.79
		3.9	0.8	0.20
		3.9	0.5	0.12
	2	5.0	0.8	0.16
		5.0	0.5	0.1
90:10	1	5.5	1.1	0.2
		5.5	0.7	0.12
	2	5.1	1.2	0.23

Thin layer chromatography of different extract shows that the best separated compound of Petroleum ether extract was

found in Petroleum ether: ethyl acetate in the ratio of 70:30, 80:20. The similar result was found in Chloroform extract & Ethanol extract with the solvent system petroleum ether: Ethyl acetate and in the 70:30, 80:20. Another solvent system Petroleum ether : Ethyl acetate also used for Chloroform extract which shows the best separation in the ratio of 70:30 & 80:20 for Ethanolic extract solvent system Petroleum ether : Ethyl acetate which show best separation in 70:30 & 80:20.

Discussion

The phytochemical results of the extracts of *Equisetum diffusum* depicted Carbohydrate and Phenols in Petroleum ether extract. Also in chloroform extract the Carbohydrate, Phenols and Alkaloids were also found present. The Ethanolic extract was found to show the presence of Carbohydrate, Phenols, Saponins and Proteins. Carbohydrate, Phenols, and Proteins are present in Water extract (Qureshi, MN, et.al. 2016, Kotwal, SD. et. al., 2016)

The obtained extracts were analysed for antibacterial activity against both gram (+) and gram (-) bacteria. The obtained results showed the aqueous extract most effective in inhibiting the growth of all bacteria e.g. *Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, B. pumilus, B. cereus, B. licheniformis Salmonella typhi, Streptococcus mutans*. The Chloroform extract showed inhibition against all the selected bacteria except *Streptococcus mutans*. The Ethanolic extract showed maximum zone of inhibition against *B. cereus* (Chouitah, O. et al., 2017). The results of inhibition by Petroleum ether against *Pseudomonas aeruginosa, B. cereus, B. pumilus* were encouraging. The obtained antimicrobial results run parallel with the results obtained by (Vesna M, et. al., 2007). Also the polar solvent extracts like ethanol and water posses more antimicrobial activity as compared to less polar solvent extracts gets verified by the results of (Radulovi, N. et.al, 2006)

Analysis of antifungal activity mentioned that the chloroform extracts showed inhibition against all the fungi species in with maximum zone of in 15mm against the *fusarium*. The Petroleum ether extract was found more effective against all fungi showing max zone of inhibition of 14mm against *Nigrospora oryza*.

The Ethanolic extract also records highest zone of inhibition of 15mm against *Aspergillus Niger* and *Nigrospora oryza* and not effective against *Fusarium*. The Aqueous extract shows minimum zone of inhibition in comparison to all extracts and unveils the growth inhibition of *Fusarium*. Although the antifungal activity of *Equisetum diffusum* has been explored yet, but few species of equisetum does posses the antifungal property as per the studies carried out by (Sandhu, NS. et. al., 2010). The antifungal activities of *Equisetum diffusum* run parallel to the antifungal activity of *Equisetum arvense* as per the resulted mentioned by (Asgarpanah. J, 2012).

As per the thin layer chromatography a good no of R_f values were obtained which corresponds to a particular component. So the study under analysis showed that the extracts are enriched profoundly with wide no of phytochemicals.

Conclusion

The plant in reference shows good antimicrobial and antifungal activity as per the results is taken into consideration. Plants are the ultimate source of all types of chemicals which do have preferential activity against all types of diseases which visit human life from time to time. The concerned plant as per the observation may provide a hope for its use as a wide spread use in treating various diseases. Also further work is required to isolate the bio active compound(s) which may have good antimicrobial properties and have lesser side effect as compared to the market available synthetic drug.

Acknowledgement

We want to acknowledge the Prince Mohammad Bin Fahd University Al Khobar Kingdom of Saudi Arabia for given a chance to carry out such a research.

Conflict of Interest

There is no conflict of interest between the authors nor outside.

Funding Support

The authors declared that they have no funding support for this study.

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