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THE ANTIBACTERIAL IMPACT OF EQUISETUM ARVENSE AND DETECTION OF ACTIVE COMPOUNDS

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Abstract

Background:

Methods: In this study, A different range of concentrations was applied (50, 25 and 12.5 %)w/v of methanolic and aquatic extract of Equisetum arvense L. for their antibacterial activity against five types of bacteria (Staphylococcus spp, Pseudomonas aeruginosa, and Proteus spp and E. coli). The antibacterial activity has been measured using the zone of inhibition and also performed an antibiotic test using erythromycin E, ciprofloxacin CIP, ampicillin-sulbactam SAM, cefoxitin FOX, oxytetracycline T50, and trimethoprim-sulphamethoxazole TS.

Results: The results showed that the methanolic extract had a broad-spectrum activity and was particularly effective against Staphylococcus spp (28 mm inhibition zone) and less effective against E. coli (27 mm inhibition zone). The aquatic extract had a weaker effect, with a 15 mm inhibition zone for Staphylococcus spp and a 14 mm inhibition zone for E. coli. Salmonella spp and Proteus spp were insensitive to the aquatic extract. Regarding antibiotics despite Pseudomonas aeruginosa being susceptible to all antibiotics, Salmonella spp was only resistant to all antibiotics except ciprofloxacin.

Keywords: Equisetum arvense L., antimicrobial activity, methanolic extract, antibiotic activity.

Introduction

Plant secondary products have gained more applications as alternative medicine and food supplements due to their bioactive ingredients. These phytochemicals' active compounds including many kinds of chemicals such as carotenoids, phenols and alkaloids were widely studied. Most of these studies were focused on their antioxidant activities. For example, phenolic compounds demonstrated strong antioxidant activity (Guimarães et al., 2009; Barros et al., 2010). The wide using of plant extracts is growing and applied extensively in traditional medicine (also called folk medicine) to treat a variety of human diseases. Folk medicine is a term used to describe healing methods used traditionally by people to cure various diseases with the using several plants including Equisetum arvense or by their own efforts (Yoder, 1975). Equisetum arvense L. usually called “field horsetail” is a species belonging to the family of Equisetaceae. The plant was utilized in the treatment of anemia, pulmonary tuberculosis, fistulas, peptic and other types of ulcers, hemorrhage, bleeding, inflammation, colon polyps, and kidney and bladder tuberculosis (Sandhu, Kaur, & Chopra, 2010). Many Recent studies achieve different biological impacts of the E. arvense extracts, such as antibacterial and antifungal agents and high antioxidant capacity activity (Dos Santos Jr et al., 2005; Milovanović et al., 2007; Garcia et al., 2011), Moreover, E. arvense has anticonvulsant, sedative, anti-inflammatory and anti-cancer effects (Radulović et al., 2006; Alexandru et al., 2007). The importance of E. arvense emerges from its high content of bioactive compounds such as phenols, oxalic acid, malic acid, tannins, flavonoids, aconite, saponins, resins, carotenoids, pectin, vitamin C and mineral substances (Wichtl, 1994; Cetojević-Simin et al., 2010 ; Dos Santos et al., 2012). This work aimed to focus on the antimicrobial ability of these substances extracted from E. arvense.

Material and methods

Plant Collection and Extracts Preparation

The Equisetum arvense plant was obtained from the local market. The whole plant was washed and dried in the air at room temperature. Then, the dry material (Leaves and stems) has been ground and turned into a powder by an electric grinder. The methanolic and aqueous extracts of E. arvense were prepared by dissolving 50g of powder in 250 ml methanol and/or distilled water successively in a soxhlet extractor for 24 hr. Extracts have been filtered with Watman paper 1, after that the combined methanolic and aqueous extracts were evaporated to dryness under a vacuum in a rotary evaporator at 40°C, and then crushed by the ceramic mortar. After obtaining

the extract powder, it was stored in a refrigerator for future use (Rashed & Butnariu, 2014).

Phytochemical screening (detection of the active compound)

The Equisetum arvense extracts were screened and chemical detection of some of the active compounds such as alkaloids, saponins, Resins, phenols, tannins, terpenoids, and flavonoids.

1- Detection of saponins

It was detected by adding 1 ml of mercury chloride to 5 ml of aqueous extract in a test tube, indicating the emergence White precipitate on the positive test (Jaffer, Mahmod, Jawad, Naji, & AL-Naib, 1983).

2- Detection of alkaloids

Meyer's detector was used and prepared as follows: The first solution was prepared by dissolving 35.1 gm of mercury chloride in 60 ml of distilled water. The second solution was prepared by dissolving 5 gm of potassium iodide in 10 ml of distilled water, then mixing the two solutions and completing the volume to 100 ml of distilled water. Alkaloids were detected by placing 1 ml of Mayer's reagent in the test tube and mixing it with 5 ml of aqueous extract, and the appearance of white sediment indicates that the test is positive(Jaffer, Mahmod, et al., 1983).

3 - Detection of tannins

The detection of tannins was performed using lead (II) acetate $Pb(ch_3coo)_2$ and the white precipitate is evidence of the presence of this component (Jaffer, Mohamed, Jawad, Naj, & Al-Naib, 1983).

4- Detection of flavonoids

This detection was performed by depending on the standard method (Jaffer, Mohamed, et al., 1983) as follows: The flavonoids were detected by mixing 10 ml of 50% ethyl alcohol with 10 ml of Potassium hydroxide at a concentration of 50%. Equal quantities of the aqueous extract of the plant were mixed with the solution described above. The appearance of a yellow precipitate indicates that the test is positive.

5- Screening for terpenes and steroids

The detection was performed by mixing 1 ml of an aqueous extract with 2 ml of chloroform and a drop of acetic acid and a drop of concentrated sulfuric acid were added. The appearance of a light brown ring indicates a positive test for terpenes.

While the appearance of the dark blue ring after leaving the mixture for 12 hours indicates a positive test for steroids (Al-Maisry, 1999).

Table (1): the results of the chemical detection of compounds in leaf extracts

Sequence	Active compound	Detection evidence result
1-	Saponins	white sediment
2-	Alkaloids	white sediment
3-	Tannins	white sediment
4-	Flavonoids	Yellow sediment
5-	Terpenes Steroids	light brown ring Dark blue ring after leaving the mixture for 12 hours

Bacterial Isolation and Identification

Five bacterial isolates collected from Baghdad city hospitals yielded from the cultivation of distinct clinical specimens, identified by colony morphology, Gram staining pattern, and a panel of biochemical tests, the isolate genus and species were confirmed by Vitek 2 system. After this, bacteria were preserved in brain heart infusion broth (BHIB) 80% with glycerol 20% at stored in a freezer at -20 °C for further work (Radulović, Stojanović and Palić, 2006).

Antibiotic sensitivity testing

The antibiotic test was performed by using the disk diffusion method(Atlas, Williams, & Huntington, 1995). Briefly, the physiological saline was inoculated with a number of pure bacterial colonies, and the tube was compared with McFarland 0.5 suspension, which equals ($1.5*10^8$) cells/ml, then bacterial suspension was spread with a cotton swab on Mueller Hinton's agar plate and the dishes were left to dry at the laboratory temperature, then the used antibiotic disks were placed on the surface of the Mueller-Hinton's agar including six antibiotics: Erythromycin E, ciprofloxacin CIP, ampicillin-sulbactam SAM, cefoxitin FOX, oxytetracycline T50 and trimethoprim-sulphamethoxazole TS), so that each disk was 30 mm away from the one next to it, were incubated at 37 °C for 24 h with ambient air, standard disks of were used. The diameters of the inhibition zones were measured in millimeters using a ruler and compared with Clinical and Laboratory Standards Institute reference standard tables for antibacterial susceptibility testing(Wayne, 2016). Each test was performed in

triplicate and repeated three times and results analyzed for statistical significance. Mean values were selected.

Results and discussion

Phytochemical screening

Table (2) shows the results of the chemical detection of the active compounds in both methanolic and aqueous extract of Equisetum arvense. The results showed that aqueous extract of horsetail plant contains (saponins, terpenes, tannins, steroids and flavonoids). The methanolic extract revealed the following active compounds (saponins, alkaloids, tannins and flavonoids).

Evaluation of antibiotic activity:

Five pathogenic bacterial isolates were obtained, four of them were Gram negative rods (E. coli, Proteus spp, Pseudomonas aeruginosa and Salmonella spp), while one of them was belonging to Gram positive cocci (Staphylococcus spp).
Determination of antibiotic activity against microorganism compared with the effect of the methanolic and aquatic Equisetum arvense extract were showed in table 3, the results showed that E. coli was the most sensitive isolate as it was susceptible for four antibiotics and esists only ampicillin-sulbactam, one antibiotic from betalactams group and pencillins subgroup,this

Table (2): the active compound in aquatic and methanolic extract of the sterile stems of Equisetum arvense

Active compound	methanolic extract	Aquatic extract
Terpenes and steroids	-	+
Flavonoids	+	+
Alkaloids	+	-
Saponins	+	+
Tannins	+	+

+ indicates presence of positive reactions, - indicates negative reactions
resistance probably due to betalactams enzymes, commonly produced in Gram negative bacteria (Authority & Control, 2022), then Proteus ssp and Staphylococcus ssp that each of them is susceptible to two antibiotics, Staphylococcus ssp was resistant to cefoxitin and erythromycin, cefoxitin resistance is a always associated with oxacillin resistance and due to betalactams enzymes production, erythromycin

resistance suggesting presence of the erm genes responsible for ribosomal methylases encoding which are the most common occurring erythromycin resistance-determining genes, Pseudomonas aeruginosa was sensitive for one used antibiotic, finally, Salmonella ssp Showed extensive resistance to all of them, this pan drug resistance must be mediated by several mechanisms such as extended spectrum betalactamases (ESBLs), efflux pumps and protein synthesis pathway modifications (Hamad and Mahmood Atiyea, 2021; Sinha, 2012; Haldorsen et al., 2014).

Table (3): Elucidate the antibacterial responses of clinical isolates against the antibiotic activity

	Antibiotics / Inhibition zone diameter (mm)*					
Microorganism	***E**	***T50**	***FOX**	***CIP**	***SAM**	***TS**
Pseudomonas aeruginosa	-	-	-	S	-	-
Proteus	-	R	S	R	S	R
E. coli	-	S	S	S	R	S
Salmonella	-	R	R	R	R	R
Staphylococcus	R	S	R	S	-	I

(E: erythromycin, CIP: ciprofloxacin, SAM: ampicillin-sulbactam, FOX: cefoxitin, T50: oxytetracycline, TS: trimethoprim-sulpamethoxazole, S: sensitive, I:intermediate and R: resistant)

Efficacy of methanolic and aqueous extract against pathogenic bacteria

It was found that the effect of the E. arvense methanol extract exhibits a significant antimicrobial activity, higher than the effect of the aqueous extract in inhibiting a wide and different range of bacteria, due to the alcohol extract's containing several effective compounds, including (saponins, alkaloids, tanins and flavonoids) which have been proven in previous studies to have a strong anti-bacterial action (Aldaas, 2011).

Table (4): illustrate clearly the inhibition zone of bacterial colonies due to the effect of methanolic and aqueous extracts in a concentration of 50%w/v.

Microorganism	Inhibition zone in methanolic extract, diameter (mm)*	Inhibition zone in aquatic extract, diameter (mm)*
Pseudomonas aeruginosa	28	15
Proteus spp	23	0
E. coli	23	14
Salmonella spp	23	0
Staphylococcus spp	26	15

Results indicated in table (4**)** showed that Pseudomonas aeruginosa had the highest inhibitory effect against the methanolic extract (inhibition zone 28 mm) and Slight effect with aquatic extract (inhibition zone 15 mm), then Staphylococcus spp reveal a moderate inhibition zone of 26 mm in diameter with methanolic extract and mild zone measured 15 mm in diameter with aqueous extract, finally, Proteus spp, E. coli and Salmonella spp appeared to have a moderate effect with methanolic extract (inhibition zone 23 mm) but the aqueous extract had a mild effect on E.coli as the inhibition zone measured 24 mm in diameter and did not have any effect against Salmonella spp and Proteus spp. The methanolic extract has affected upon a broad spectrum of these bacteria, this finding is similar to previous studied that mentioned the high antibacterial activity of Equisetum arvense leaf alcoholic compared with aquatic and chlorophormic extracts, on the other side, the activity was significantly different between seasons, extract prepared on summer has more activity than extract prepared on winter (Geetha, Lakshmi and Roy, 2011; Sinha, 2012). Other studies reveal a sufficient antibacterial activity of Equisetum arvense leaf and stem extracts (alcoholic extract and aqueous extract) against pathogenic bacteria: Klesiella pneumonia, Pseudomonas aeruginosa, Salmonella entritidis (Geetha et al., 2011), Proteus mirabilis, Staphylococcus aureus and Staphylococcus saprophyticus (Alavarce et al., 2015). Equisetum arvense alcoholic extract is rich in essential oil that denatures bacterial adhesive proteins, prevent protein transportation via cell membrane and disturb the cytoplasmic membrane(Oh, Kim, Cho, & Kim, 2004). Polyphenol in Equisetum arvense extract has been considered as responsible alternative antibiotic because of its bactericidal effect and antibiofilm effect. the Equisetum arvense extract may be taken with some antibiotics, as polyphenol has a synergistic effect with these antibiotics. The phenolic compounds in Equisetum arvense extracts decrease production of reactive oxygen species (ROS) caused by bacterial lipopolysaccharide (LPS), ROS damages human genetics and subsequently causes chromosomal instability, thereby provided a pathological basis for cancer development, so that, the Equisetum arvense leaf extract play a role in cancer prevention. The researchers suggest two mechanisms of how Equisetum arvense extract prevent ROS: the first mechanism is restriction of free radicals directly; the second mechanism is removing ROS by a reaction with antioxidant enzymes (Kelmanson, Jäger, & van Staden, 2000).

Antibacterial activity of methanolic extract

One of the goals of our research was to determine the antimicrobial effect of the Equisetum arvense L. extract. The results regarding the antimicrobial activity of Equisetum arvense L. extract evaluated by well diffusion method are summarized in

Figure (1). All of the five bacterial species found to be very susceptible to Equisetum arvense extract at all concentrations. The inhibition-mean zone of the extract against these bacteria increased with the increasing of the Equisetum arvense extract concentration. The highest inhibition-mean zone (28mm) was recorded against Pseudomonas aeruginosa. The control of dimethyl sulfoxide (DMSO) did not produce any zone of inhibition, while the lowest mean zone of inhibition (23mm) has been seen with (Salmonella spp, Proteus spp and E. coli). It was found that these bacteria were sensible indicating to the broad spectrum activity of the extract. The impact of E. arvense extract on all tested bacterial cultures depends on the exposure time and concentration. An increase in extract

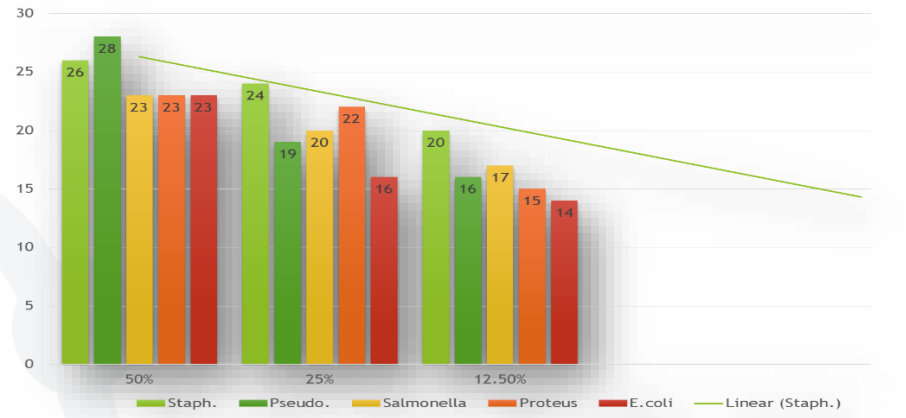

Figure (1): illustrate the inhibition zone of bacteria when using: 50%; 25% and 12.5% w/v of methanol extract concentration of Equisetum arvense.

* Mean zone of 3 assays

concentration slows down the growth of tested bacterial cultures and decreases the total number of bacteria (Kelmanson, Jäger and van Staden, 2000;Panthi and Chaudhary, 2006).By correlation of the obtained results, this study realizes that the inhibitory effect of E. arvense extract on experimented Gram positive and Gram negative bacteria is more effective than the results of inhibition obtained by means of antibiotics. The bactericidal effect of methanolic extract is positively correlated with the extract concentration and the optimal concentration was 50 %, this finding consistent with previous studies (Geetha et al., 2011). We conclude from this the plant is an effective, inexpensive and easy to obtain, in addition to that it does not contain any harmful side effects to human health.

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Conflict of Interests: Authors have declared that no competing interest.

No Ethical Issues Involved

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