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Research Article

EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *DIOSCOREA VILLOSA* TUBERS- AN *IN VITRO* STUDY

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ABSTRACT

The aim of the present study was to evaluate the anti bacterial activity of the ethanolic extract of *Dioscorea villosa* (wild yam) tubers. *Dioscorea villosa* (dioscoraceae) is used for centuries as a medicinal herb for a wide range of ailments. It is a tuberous, twining vine with pale- brown, knotty, wood and cylindrical tubers. Wild yam is sometimes suggested as an alternative to hormone replacement therapy. The ethanolic extract of *Dioscorea villosa* tubers was screened for its antibacterial activity against *Shigella dysenteriae, E.coli, Vibrio cholera*, *Klebsiella pneumonia, Psuedomonas aerugenosa, Staphylococcus aureus*, using disc diffusion technique .The results of this study showed good anti bacterial activity against the bacterial species tested, at different concentrations of the extract.

Keywords: Wild yam, Tubers, Antibacterial activity, Zone of inhibition**,** Disc diffusion technique.

INTRODUCTION

Dioscorea villosa (dioscoraceae) also called Wild Yam Root, Colic Root and Rheumatism Root [1] , China root, Mexican yam, colic root, Yuma, devil's bones.[2] It is edible and is used for centuries as a medicinal herb for a wide range of ailments. Wild yam is sometimes suggested as an alternative to hormone replacement therapy. The components of wild yam are chemically manufactured into the hormones progesterone or estrogen since the body is unable to use wild yam in the same way[2]. Amongst the medicinal yams, *Dioscorea villosa* is mentioned in English pharmacopoeias .[3]

D.villosa is a tuberous, twining vine with pale- brown, knotty, wood and cylindrical tubers. The tubers are crooked and bear horizontal branches.[4] Many species of Dioscorea have many organic acids and polyphenols present in them and have antioxidant property.[5] Some species of *Dioscorea* like *D. sylvatica* and *D. dregeana* show antibacterial activity against Gram-positive and Gram-negative bacteria.[6]

D.villosa have five steroidal saponins; Dioscin, Protodioscin, Meprotodioscin, Perrisaponin and Progenin II. It also has a spirostanol glycoside.[4] Other constituents include phytosterols (beta-sitosterol), alkaloids and tannins make this plant useful as an antiinflammatory, diuretic, antispasmodic, cholagogue, diaphoretic and vasodilator. A decoction of the root is used to alleviate many of the symptoms of menopause and PMS such as hot flashes, night sweats, mood changes, and vaginal dryness. It is also used to treat irritable bowel syndrome, gastritis, painful menstruation, and in small doses is especially helpful in treating the nausea of pregnant women.[7] Study shows that Wild yam is used in the management of the symptoms of menopause.[8] In this present study, an attempt was made to evaluate the antibacterial activity of the ethanolic extract of *Dioscorea villosa* tubers.

MATERIALS AND METHODS

Plant material

The ethanolic extract of *Dioscorea villosa* tubers was obtained from Green Chem Herbal Extract & Formulations. Bangalore.

Test microorganisms

Bacterial strains used were *E.coli* [Gram negative bacilli-GNB], *Klebsiella pneumoniae*[GNB], *Pseudomonas aeruginosa*[GNB],*and Staphylococcus aureus,*[Gram positive cocci], *Bacillus subtilus*[Gram positive bacillus]. The organisms were obtained from department of Microbiology, Saveetha Dental College and maintained in nutrient agar slope at 4° C

Methodology

Different concentrations of the ethanolic extract like 2mg/ml, 4mg/ml and 6mg/ml were prepared in sterile water and 50µl of the extract at different concentrations were loaded on sterile filter paper discs measuring 6mm in diameter, so that the concentration of the extract on each disc was 100µg, 200µg and 300 µg respectively. The discs were dried and kept aseptically.

Screening of Antibacterial activity [Disc diffusion technique] [9,10]

Broth cultures of the bacterial strains compared to Mac Farland's standard 0.5,[11,12] were prepared. Lawn culture of the test organisms were made on the Muller Hinton agar [MHA-Hi media M1084] plates using sterile cotton swab and the plates were dried for 15 minutes**.** Filter paper discs loaded with different concentrations of the extract were placed on the respective plates. The plates were incubated at 37 °C overnight and the zone of inhibition of growth was measured in millimeters.[13, 14] Standard antibiotic discs of amoxicillin (30mcg/disc) and Penicillin G (30mcg/disc) were used as positive control. All the tests were done in triplicate to minimize the test error.

Determination of Minimum Inhibitory Concentration (MIC)

Macro broth dilution or tube dilution method was done to determine the Minimum inhibitory concentration (MIC) of the extracts. A series of two fold dilution of the extract ranging from 8mg/ml to 0.125mg/ml was made in Muller Hinton broth as specified by National Committee for Clinical Laboratory Standards (NCCLS, 1990). 100µl of standard inoculum of the bacterial strains matched to 0.5 Mc Farland's standard was seeded into each dilution. Two control tubes were maintained for each test batch. These included antibiotic control (tube containing extract and growth media without inoculum) and organism control (tube containing the growth medium and the inoculum) .The tubes were incubated at 37°C for 24 hours and checked for turbidity. MIC was determined as the highest dilution (that is, lowest concentration) of the extract that showed no visible growth.

Determination of Minimum Bactericidal Concentration (MBC)

The MBCs were determined by selecting tubes that showed no visible growth during MIC determination; a loop full from each tube was sub cultured onto Muller Hinton agar plates and incubated for further 24 hours at 37°C. The least concentration, at which no growth was observed, was noted as the MBC.

RESULT AND DISCUSSION

The antibacterial activity of the extracts at different concentrations was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter. The results are given in the Table 1 and Figure 1. The minimum inhibitory concentration [MIC] and minimum bactericidal concentration [MBC] were also determined for the extracts and the results are given in Table 2 and Figure 2.

The extracts at different concentration exhibited antibacterial activity against all bacterial strains tested. The ethanolic extract was more effective against *E.coli* and *Staphylococcus aureus* with a zone of inhibition of 20mm and 18 mm diameter (at conc. 300 µg.) respectively and was less effective against *Klebsiella pneumoniae* with zone of inhibition of 12mm (at conc. 300 µg.) Among the other bacterial species studied *Pseudomonas aeruginosa* and *Bacillus subtilus* showed a zone of inhibition of 14mm and 15mm diameter (at conc. 300 µg.) respectively.

The ethanolic extract was found to have Low MIC and MBC values of 2mg/ml and 2mg/ml for *E.coli* and for *Staphylococcus aureus,* it was 1mg/ml and 4mg/ml. With *Klebsiella pneumoniae* ethanolic extract showed a higher MIC and MBC value of 8mg/ml & 8mg/ml and for *Pseudomonas aeruginosa* and *Bacillus subtilus* it was 4mg/ml & 8mg/ml and 4mg/ml & 4mg/ml respectively. The lower MIC and MBC value is an indication of high effectiveness of the extract whereas higher MIC and MBC indicates the less effectiveness of the extract.

Herbs play a significant role in Pharmaceutical industries as natural sources of life saving drugs. [15,16] Plant extracts are able to restrict the growth of bacteria due to the presence of active principles in it. These active principles may inhibit protein synthesis of bacterial cell wall or alter the membrane function, inhibit protein synthesis or synthesis of purine and pyrimidines, hinder respiration or antagonize the metabolic pathways of microorganism leading to retardation of growth of bacteria. These active principles in these plants could be used as potent antibiotics. [17]

Table 1: Antibacterial activity of the Ethanolic extract of *D.villosa* tubers

Extract	Concentration [µg]	Zone of inhibition[in mm diameter]				
		B1	B2	B3	B4	B5
Ethanolic	100	10	12	7	-	11
Extract	200	13	16	9	8	13
	300	18	20	12	14	15
Penicillin G	30mcg/disc	20	23	20	21	20
Amoxicillin	30mcg/disc	22	21	18	19	21

B1-*Staphylococcus aureus,* B2- *E.coli*, B3- *Klebsiella pneumoniae* B4- *Psuedomonas aerugenosa*, B5-*Bacillus subtilus*

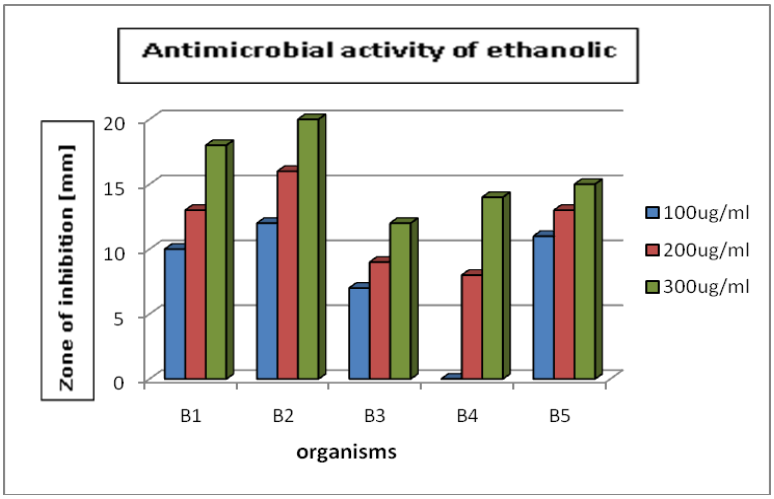

Fig. 1: Graph showing antimicrobial activity of the Ethanolic extract of *D.villosa* tubers

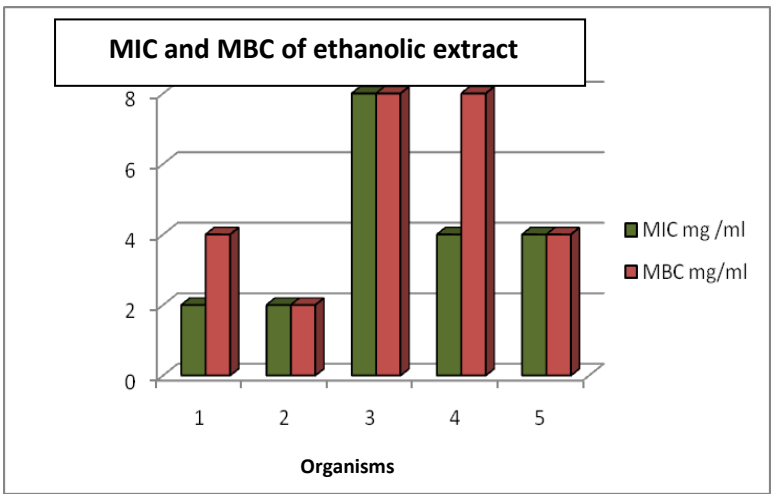

Fig. 2: Graph showing the MIC and MBC of the Ethanolic extract of *D.villosa* tubers

Table 2: MIC and MBC values of the Ethanolic extract of *D.villosa* tubers

Test Organisms	MIC mg /ml	MBC mg/ml
B1-*Staphylococcus aureus*	2	4
B2- *E.coli*	2	2
B3- *Klebsiella pneumoniae*	8	8
B4- *Psuedomonas aerugenosa*	4	8
B5-*Bacillus subtilus*	4	4

CONCLUSION

The present study shows the antibacterial activity of *Dioscorea villosa* (wild yam) tubers against the microorganisms tested in this *in vitro* evaluation. This research provides information, which could trigger further research in the direction of partial or full isolation and characterization of the constituents of *Dioscorea villosa* (wild yam) tubers in order to decipher the specific phytochemical constituent(s) responsible for the antibacterial activity of the plant.

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