"Antimicrobial activity of Leaves of *Artemisia vulgaris* L"

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Abstract

Objective: To screen the antimicrobial activity of different extracts of leaves of *Artemisia vulgaris.* Materials and Methods: To detect the *in vitro* antibacterial activity, 10 bacterial strains were selected. These bacteria are both gram +ve and gram -ve. Leaves were extracted with a petroleum ether, chloroform, ethyl acetate, ethanol and aqueous . In the present work the antibacterial activity was done by cup plate method. The antibacterial activity was expressed as zone diameter in millimeters. Different extracts from leaves of the plant was compared with standards like benzyl penicillin for gram +ve bacteria and streptomycin for gram –ve bacteria using DMF as control. The readymade media for inoculum and culture was obtained from Himedia labs. For antifungal activity four fungal organisms were selected and Griseofulvin was used as standard. Results: Herbal extracts prepared from the leaves of the plant were screened against bacteria and fungal organisms at the concentration range between 50 µg and 300 µg/0.1ml. The results of antimicrobial activity revealed that the extract exhibited activity against both gram +ve, gram –ve and fungal organisms. Conclusion: The present investigation reveals that the aqueous, chloroform and ethyl acetate extracts and in some cases petroleum ether extract showed significant antimicrobial activity when compared with standard.

Key words:, Artemisia species, antibacterial, soxhlet extraction, streptomycin, benzyl penicillin,

Introduction

Artemisia vulgaris is a tall aromatic perennial shrub, often gregarious, pubescent or villous[1]. It is used in asthma emmenagogue, anthelmintic, and stomachic also used as febrifuge antilithic, alexipharmic[2]**.** Flowers with leaves yield an essential oil for flavouring, roots are used as tonic and antiseptic, useful in the preparation of ointments for wounds[3]. The essential oil obtained from the leaves of *Artemisia vulgaris* exhibited significant antimicrobial activity[4]. Twenty known flavonoids were isolated from *Artemisia vulgaris* a plant used as an emmenagogue in traditional medicine[5] The present study aimed evaluation of antimicrobial activity of leaves extract of *Artemisia vulgaris* L.

Materials and Methods

Bacteria selected for the study.

1. *Bacillus subtilis (+)*
2. *Bacillus cerius (+)*
3. *Staphylococcus aureus (+)*
4. *Salmonella typhi (+)*
5. *Pseudomonas aerogenosa (-)*
6. *Escherichia coli (-)*
7. *Klebsiella pneumoniae (-)*
8. *Vibrio cholerae (-)*
9. *Proteus mirabilis (-)*
10. *Serratia marsupium (-).*

Fungal organisms selected for the study

1. *Aspergillus fumigatus*
2. *Candida albicans*
3. *Rhizopus japonicun*
4. *Candida tropicallis.*

Preparations of plant extracts

The leaves of *Artemisia vulgaris* were collected from local areas of Belgaum, Karnataka and authenticated by Dr.P.S.N.Rao, Joint Director, at Botanical Survey of India (BSI), Govt. of India, Ministry of Environment and Forests, Pune, India. The air-dried leaves of *Artemisia vulgaris* Linn. belonging to family Asteraceae were reduced to fine powder (40 size mesh) and around 100 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, ethanol and ethyl acetate. Another batch of powdered drug was macerated with chloroform-water I.P. (Each time before extracting with next solvent the powdered material was dried at room temperature). After the effective extraction, solvent were concentrated using rotary flash evaporator[6].

Sterilization

Sterilization of the medium, tubes for slants, borers was done by autoclaving at 15 lbs/inch^2 for 20 minutes. The glasswares like syringes, petridishes, pipettes, and empty test tubes were sterilized by dry heat in an oven at a temperature of 150ºC for 1 hr.

Antibacterial activity [7].

In the present work to know the antibacterial activity cup-plate method is employed. The antibacterial activity is expressed as zone diameter in millimeters, which is

measured with a divider. Different extracts of leaves of the plant was compared with standards and DiMethyl Formamide (DMF) as control for antimicrobial activity.

Standard used

1.Benzyl penicillin for gram +ve bacteria

2.Streptomycin for gram-ve bacteria

Preparation of sample solution

Different concentration of extracts equivalent to 50 μg, 100 μg, 150 μg, 200 μg and 300 μg/o.1ml by using DMF were prepared.

Preparation of standard solutions

Standard benzyl penicillin injection IP 1,00,000 units.

As per IP 1mg of benzyl penicillin=1500-1750 IU.

Benzyl penicillin injection (IP) 1,00,000 units manufactured by IDPL a streptomycin sulphate (ambistyn 1.0 gm) manufactured by Sarabhai chemicals were used. Different concentrations of standards equivalent to 50 μg, 100 μg, 150 μg, 200 μg and 300 μg/0.1ml of benzyl penicillin and streptomycin were prepared.

Preparation of inoculum

Nutrient agar medium (Himedia labs) of the following composition was used for preparation of slants.

Peptone----------5.0 gm

Beef extract----------1.5 gm

Sodium chloride----------5.0 gm

Agar----------15.0 gm

Yeast extract ---------15.0 gm

Distilled water to make ---1000 ml

About 28 gm of prepared medium was taken in 1000 ml distilled water and boiled to dissolve completely. The microorganisms were streaked under aseptic conditions, and the slants were incubated at 37±1ºC for 24 hrs. These 24 hrs cultures were used for preparation of inoculum. The suspension of the microorganisms was prepared in 10 ml of sterile water and 0.5 ml of this suspension was added to 100 ml of the agar medium.

Culture medium

In the present investigation antibiotic medium (nutrient agar –Himedia) was employed possessing the following composition (Ready made medium).

Peptone----------6.0 gm

Beef extract----------1.5 gm

Agar----------15.0 gm

Yeast extract –----------3.0 gm

Distilled water to make --1000 ml.

About 27 gm of above readymade medium was dissolved in freshly prepared distilled water (in 1000 ml) by gentle heating.

Preparation of agar plates

The sterilized medium was cooled at 40ºC and 0.5 ml of inoculum per 100 ml of medium was added to the conical flask. This was shaken gently to avoid the formation of air bubbles and then transferred into petridishes so as to obtain 6 mm thickness of medium. The medium in the plate was allowed to solidify at room temperature.

Experimental procedure

The sterile borer was used to prepare 4 cups of 8 mm diameter in the medium of each petridish. An accurately measured 0.1 ml solution of each concentration of solution of extracts and standard samples were added to the cups in the medium with the help of micropipette. All the plates were kept at room temperature for effecting diffusion of drug extracts and standards later they were incubated at 37±1ºC for 24 hrs. The presence of definite zones around the cup of any size indicated antibacterial activity. The control was run simultaneously to assess the activity of DMF, which was used as vehicle for extract and fractions. The diameter of the zone of inhibition was measured and recorded.

Antifungal activity of extracts of the plant[8]

In the present study the antimicrobial screening was done by cup-plate method here the antifungal extract is diffused from the cup through an agar layer in a petridish or plate to an extent such that the growth of added fungus is restricted entirely in circular area or zone around the cavity containing the solution of an antifungal substances. The antifungal activity is expressed as zone diameter in millimeters, which is measured with a divider.

Different extracts of the plant were screened for antifungal activity against wide spectrum of fungus and the activity was compared with appropriate standard Griseofulvin. DMF was used as control and as vehicle for various extracts.

Preparation of sample solution

Different concentration of extract equivalent to 50 µg, 100 µg, 150 µg, 200 µg and 300 µg/0.1ml by using DMF were prepared.

Preparation of standard solution

Different concentration of standard equivalent to 50 µg, 100 µg, 150 µg, 200 µg and 300 µg / 0.1 ml Griseofulvin were prepared.

Preparation of inoculum

Nutrient agar medium (Himedia labs, Pvt, Ltd) of the following composition was used for preparation of slants.

Peptone----------5.0 gm

Beef extract----------1.5 gm

Sodium chloride----------5.0 gm

Agar----------15.0 gm

Yeast extract ----------1.5 gm

Distilled water to make ---1000 ml

About 28 gm of prepared medium was taken in 1000 ml distilled water and boiled to dissolve the medium completely. After being streaked with fungus under aseptic condition the slants were incubated at 37±1ºC for 24 hr in an incubator, these 24 hrs cultures were then used for preparation of inoculum the suspension of the fungus was prepared in 10 ml of sterile water and 0.5 ml of this suspension was added to 100 ml of the agar medium.

Culture medium

In the present investigation antibiotic medium (Nutrient agar himedia) was employed this nutrient agar medium has the following composition (Readymade medium).

Agar----------15.0 gm

Peptone----------6.0 gm

Beef extract----------1.5 gm

Yeast extract ----------3.0 gm

Distilled water to make –1000 ml

About 27 gm of above readymade medium was dissolved in freshly prepared distilled water (1000 ml) by gentle heating.

Preparation of agar plates

The sterilized medium was cooled at 40ºC and 0.5 ml of inoculum /100 ml of medium was shaken gently to avoid the formation of air bubbles and then transferred into petridishes so as to obtain 6 mm thickness of medium. The medium in the plate was allowed to solidify at room temperature.

Experimental procedure

The sterile borer was used to prepare four cups of 8mm diameter in the medium of each petridish. An accurately measured 0.1 ml of solution of each concentration of extracts and standard samples were added to the cups with the help of micropipette. All the plates were kept at room temperature for a period of 2 hrs, later they were incubated at 37±1ºC for 24 hrs. The presence of definite zones around the cup of any size indicated antifungal activity. The controls were run simultaneously to assess the activity of DMF. The diameter of the zone of inhibition was measured and recorded.

Results and Discussion

Herbal extracts prepared from the leaves of the plant were screened against ten bacterial strains and four fungal organisms for the purpose of in vitro qualitative evaluation in the concentration range between 50 µg and 300 µg/0.1ml.

Along with petroleum ether extract the ethanolic, ethyl acetate, chloroform and aqueous extracts were subjected for antimicrobial activity. In these extracts aqueous, ethyl

acetate and chloroform extracts showed pronounced antibacterial and antifungal activity (Table 1., 2, 3and Table 4).

In antibacterial activity both gram +ve and gram –ve organisms were used. For the gram +ve organisms like *Bacillus subtilis*, *Bacillus cerius, Staphylococcus aureus* the chloroform and ethyl acetate extracts showed significant anti bacterial activity at 50 µg/0.1ml, when compared with standard. For *Salmonella typhi,* the aqueous and ethyl acetate extracts showed minimum inhibitory concentration (MIC) at 100 µg/0.1ml.

For the gram –ve organisms like *Pseudomonas aerogenosa*, the aqueous and ethyl acetate extracts showed significant antibacterial activity at 50 µg/0.1ml, for *Escherichia coli,* the aqueous and chloroform has MIC at 50 µg/0.1ml, for *Klebsiella pneumonae,* the ethyl acetate and chloroform extracts has MIC at 300 µg/0.1ml, for *Vibrio cholerae,* the aqueous and chloroform has MIC at 300 µg/0.1ml, for *Proteus mirabilis,* the aqueous and chloroform has MIC at 300 µg/0.1ml,while for *Serratia marsupium* it was 50 µg/0.1ml, when compared with standard.

For fungal organisms like *Candida albicans, Rhizopus japonicum* the MIC was 150 µg/0.1ml with aqueous and petroleum ether extract, for *Aspergillus fumigatus* the ethyl acetate and aqueous extracts has MIC 100 µg/0.1ml, for *Candida tropicallis* the ethyl acetate and petroleum ether extracts has MIC at 150 µg/0.1ml.

The present investigation reveals that the aqueous, chloroform and ethyl acetate extracts shows significant antibacterial and antifungal activity whereas petroleum ether extract showed significant antifungal activity when compared with standard.

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References

1) **Arya Vaidya sala** *Indian medcinal plants*. orient Longman publisher, Chennai (1994) 202-5.
2) **Ambasta SP**. *The useful plants of India*. CSIR Publication, Delhi, (1994) 55-6.
3) **Agrwal VS**. *Drug plants of India*. Kalyani publishers, Ludhiana (1997) 1: 200.
4) **Laxmi US, Rao JT.** Antimicrobial properties of the essential oils of *Artemisia pallens* and *Artemisia vulgaris.* Perfumerie und Kosmetik.1991;72:8:510-11.
5) **Lee SJ, Chung HY, Maier CGA, Wood AR, Dixon RA, Mabry TJ**. Estrogenic flavonoids from *Artemisia vulgaris* L. *J of Agri & food chem*. (1998) 46: 8: 3325-29.
6) **Harbone JB**. *Pytochemical Methods of Analysis*. Chapman and Hall Ltd, London: (1984)1-7.
7) **Leibovich SJ and Ross R**. *Am.J.Pathol.* (1975) 78: 71.
8) **Indian Pharmacopoeia [editorial],** The Controller of Publication Publisher, Delhi (1996) Vol.2: Appendix-9: 100.

Table No. 1: **Antibacterial Activity of *Artemisia vulgaris***

Antibacterial Activity of *Artemisia vulgaris*																				
Extracts	**Bacillus Subtilis**					**Bacillus Cerius**					**Staphylococcus aureus**					**Pseudo aerogenosa**				
Conc. (µgm/0.1mL)	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	9.5	10.5	11.5	12.4	13.6	10.4	11.3	11.9	12.3	12.8	9.6	10.8	11.5	12.3	12.5	10.3	11.5	12.6	13.1	13.4
Ethyl acetate	15.8*	17.5	19.6	21.5	23.8	13.8*	15.8	18.2	19.1	20.5	16.8*	18.5	20.6	22.4	24.1	14.5*	17.1	19.5	21.5	23.1
Chloroform	14.8*	16.3	18.1	19.8	22.5	14.4*	16.5	18.6	21.3	23.3	15.8*	17.2	18.8	20.5	22.3	14.3*	15.4	17.5	20.3	22.3
Pet ether	10.8	11.3	12.2	12.8	13.9	10.5	11.5	12.3	12.9	13.5	9.6	10.8	12.5	13.1	13.5	10.1	10.7	11.7	12.5	13.2
Aqueous	14.2*	15.2	17.1	18.5	21.5	14.8*	16.3	18.9	21.3	23.1	14.5*	16.3	17.6	19.8	22.5	15.5*	17.5	20.1	21.6	23.5
DMF	R	R	R	8.7	8.8	R	R	R	8.8	8.9	R	R	R	8.8	8.9	R	R	R	8.5	8.7
STD	17.2	21.1	22.7	15.2	28.3	17.3	21.3	22.8	25.3	29.3	16.8	20.6	23.8	26.3	28.3	16.5	19.6	22.7	25.8	27.6

Diameter of cup (8mm)
Average of 3 readings
Readings are in millimeter (mm)
R- Resistant

Table No. 2: Antibacterial Activity of *Artemisia vulgaris*

Antibacterial Activity of *Artemisia vulgaris*																				
Extracts	**Salmonella typhi**					**E. Coli**					**Klebsiella Pneumnae**					**Vibrio-cholerae**				
Conc. (µgm/0.1mL)	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	9.1	10.1	10.7	11.5	12.5	9.3	10.1	10.7	11.5	12.5	9.5	10.2	10.9	11.6	12.4	9.9	10.7	11.6	12.5	13.1
Ethyl acetate	14.6	16.5*	19.2	20.6	22.6	15.2*	16.6	18.6	20.8	22.6	14.7	16.7	19.6	22.7	24.8*	14.1	16.1	18.3	20.1	22.7*
Chloroform	15.5	17.6*	19.6	21.6	22.5	15.4*	17.5	19.5	21.5	23.6	12.8	15.8	18.6	20.7	23.1*	14.4	16.8	19.6	22.1	23.8*
Pet ether	9.9	10.6	11.5	12.4	13.2	10.1	10.6	11.2	11.6	12.1	9.5	10.8	11.2	11.8	12.5	10.1	10.8	11.9	12.4	13.1
Aqueous	15.2	16.2*	17.6	20.8	23.6	14.8*	16.4	18.6	20.6	23.4	14.5	16.4	18.1	19.5	21.8*	15.4	17.9	19.8	22.4	24.6*
DMF	R	R	R	8.7	8.8	R	R	R	8.7	9	R	R	R	8.7	8.9	R	R	R	8.7	8.9
STD	17.5	18.8	21.5	23.8	25.9	16.5	18.8	20.8	23.6	25.8	18.3	20.5	22.5	24.1	25.5	17.8	19.6	21.5	23.7	25.5

Diameter of cup 8mm)
Average of 3 readings
Readings are in millimeter mm)

R- Resistant

Table No. 3: Antibacterial Activity of *Artemisia vulgaris*

Antibacterial Activity of *Artemisia ulgaris*										
Extract	**Proteus mirabilis**					**Serratia marsupium**				
Conc. (µgm/0.1mL)	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	9.3	10.3	11.5	12.7	13.1	9.5	10.2	10.7	11.8	12.7
Ethyl acetate	13.8	15.9	17.9	20.1	22.7*	14.4*	16.6	18.7	20.3	23.4
Chloroform	14.8	16.7	19.1	21.5	24.1*	14.6*	16.6	19.2	20.8	23.1
Pet ether	10.1	10.5	11.4	12.1	12.6	9.5	10.8	13.1	14	14.9
Aqueous	14.9	16.8	18.9	20.9	24*	14.5*	16.8	19.1	21	22.9
DMF	R	R	R	8.5	8.7	R	R	R	8.5	8.7
STD	19.5	21.5	23.1	23.5	25.5	18.4	20.6	22.7	24.6	28.5

Diameter of cup (8mm)
Average of 3 readings
Readings are in millimeter (mm)
R- Resistant

Table No. 4: Antifungal Activity of *Artemisia vulgaris*

Antifungal Activity of *Artemisia vulgaris*																				
Extracts	**Aspergillus fumigatus**					**Candida albicans**					**Rhizopus japonicum**					**Candida Tropicallis**				
Conc. (µgm/0.1mL)	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**	**50**	**100**	**150**	**200**	**300**
Ethanolic	R	10.5	12.7*	14.9	16.4	R	10.5	12.7*	15.5	17.7	R	10.8	12.6*	15.9	17.4	R	R	10.5	12.9	15.4
Ethyl acetate	R	10.6	12.4*	14.6	18.5	R	10.6	13.5*	15.9	19.8	R	10.8	13.6*	15.6	20.4	R	R	13.7	15.8	19.4
Chloroform	R	R	10.5	11.6	12.5	R	R	R	10.7	11.8	R	R	10.7	11.6	12.5	R	R	10.7	11.8	13.5
Pet ether	R	10.4	13.7*	15.4	20.5	R	10.5	13.6*	15.5	20.5	R	11.4	14.6*	16.5	20.4	R	R	13.7	15.4	19.4
Aqueous	R	10.6	12.6*	16.7	20.6	R	10.8	14.5*	16.7	20.6	R	10.4	15.5*	18.5	21.4	R	11.4	13.5	15.5	20.4
DMF	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
STD	R	10.5	12.8	14.5	20.5	R	11.3	13.7	15.7	21.5	R	11.7	14.5	16.8	22.5	R	10.2	12.4	14.7	20.7

Diameter of cup (8mm)
Average of 3 readings
Readings are in millimeter (mm)
R- Resistant