

# 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,

# *Content*

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# *Introduction*

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- This has been the rationale for the development of new antimicrobial drugs and the search for novel molecules has been extended to herbal drugs that offer better protection.
- Plants and other natural substances have been used as the rich source of medicine.

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- ❖ All ancient civilizations have documented medicinal uses of plant in their own ethnobotanical texts.
- ❖ Most of the remedies were taken from plants and proved to be useful.
- ❖ Twenty known flavonoids were isolated from *Artemisia vulgaris* a plant used as an emmenagogue in traditional medicine
- ❖ However, the literature review revealed that *Artemisia vulgaris* has not been studied for the antimicrobial activity.
- ❖ Hence, in the present study, the leaves of *Artemisia vulgaris* have been selected for phytochemical investigation, *in-vitro* antibacterial and antifungal activity.

# Objective of Study

- ❖ Exploring the traditional medicines with proper chemical and pharmacological profiles.
- ❖ To conduct systematic Pharmacognostic investigation of leaves of *Artemisia vulgaris* Linn.
  - ❖ Collection and authentication
  - ❖ Organoleptic evaluation
  - ❖ Physicochemical evaluation
- ❖ Phytochemical investigation of leaves of *Artemisia vulgaris* Linn
  - ❖ Extraction
  - ❖ Preliminary Phytochemical investigation

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Antibacterial activity of *Artemisia vulgaris* Linn leaves extract on following Bacteria selected for the study.

- *Bacillus subtilis* (+)
- *Bacillus cerius* (+)
- *Staphylococcus aureus* (+)
- *Salmonella typhi* (+)
- *Pseudomonas aerogenosa* (-)
- *Escherichia coli* (-)
- *Klebsiella pneumoniae* (-)
- *Vibrio cholerae* (-)
- *Proteus mirabilis* (-)
- *Serratia marsupium* (-).

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Antifungal activity of *Artemisia vulgaris* Linn leaves extract on following fungal species selected for the study

Aspergillus fumigatus

Candida albicans

Rhizopus japonicun

Candida tropicallis.

# *Introduction To Plant*

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❁ *Artemisia vulgaris* is an aromatic perennial shrub belonging to family Asteraceae.

❁ Synonyms:

❁ *Artemisia nilagirica* (C.B. Clarke) Pamp. ,

❁ *Artemisia vulgaris* auct., non Linn

❁ **Vernacular Names**

❁ **Sanskrit:**Damanakah, Nagadamani

❁ **English:**Indian wormwood, Fleabane

❁ **Hindi:**Nagadouna, Dauna

❁ **Marathi:**Gathona, Nagdona

❁ **Kannada:**Urruvalu, Urigattige

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## Occurrence and distribution:-

Throughout India in, Himalaya, sikkim, khasia hills, western ghats kokan to south wards.

## Description:-

*Artemisia vulgaris* is a tall aromatic perennial shrub, often pubescent or villous. The leaves are 5 to 10 cm long and the margins are often rolled back. The upper surface is usually dark green and glabrous, occasionally pubescent, and the lower surface is tomentose.

## Chemical Constituents:

*Artemisia vulgaris* contains Artemisia alcohol borneol, camphene, camphor, 1,8-cineole, p-cymene,  $\beta$ -eudesmol,  $\alpha$ -gurjunene,  $\alpha$ -pinene, terpene-4-ol

## Traditional Medicinal Uses of Plant Parts

Plant is used in asthma and nervous and spasmodic Emmenagogue, anthelmintic, and stomachic also used as febrifuge antilithic. It is used in China for female complaints as well as for ulcer .



# Review of Literature

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## Hernandez H and et.al

Aqueous extract of *Artemisia vulgaris* showed a 89.8% growth **inhibition of the *Plasmodium falciparum*** in *In-vitro* culture

## Abdual Ghani AS and et.al

The effect of aqueous extract of leaves and stems of *Artemisia vulgaris* were studied on picrotoxin induced seizures in mice. *A. vulgaris* **delayed the onset of seizures** and decreased the mortality rate

## Gilani AH and et.al

The effect of a crude extract of the aerial parts of *Artemisia vulgaris* was investigated against D-galactosamine and lipopolysaccharide induce hepatitis in mice. Pretreatment of mice with different doses of extract (150-600mg/kg) significantly reduced the toxin induced and showing **hepatoprotective activity**

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## **Uniyal GC and et.al**

The **essential oil** of aerial parts of plant constituents were camphor, beta-eudesmol, 1,8-cineole, borneol, Artemisia alcohol, camphene, alpha-gurjunene, p-cymene, terpinene-4-ol and  $\alpha$ -pinene

## **Marco JA and et.al**

The aerial parts of *Artemisia vulgaris* yielded two new eudesmane acids and a known eudesmane dialcohol

## **Worner M et.al.**

54 **volatile constituents** was fractionated from solid liquid extraction with pentane dichloromethane.

## **Dung NX et.al.**

**Essential oil** of the leaves of *Artemisia vulgaris* **fourty six** components have been identified of which the major ones were found to be  $\beta$ -caryophyllene (24.1%) and  $\beta$ -cubebene(12%).

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## Ahmad R et.al

Essential oil from *Artemisia vulgaris* plant reported have shown **insect repellent, nematocidal** and insect attractant activities has been enumerated.

## Milhau G et.al

Essential oil of *Artemisia vulgaris* were showing In-vitro **antimalarial activity** on *Plasmodium falciparum*.

## Lee SJ

Twenty known flavonoids were isolated from *Artemisia vulgaris* a plant used as an **emmenagogue** in traditional medicine.

## Herrera CL and et.al

Using modified screening method of Hall et.al for antifertility activity in female mice extracts and/or juice of *Artemisia vulgaris* gave promising results 50% or **more reduction in fertility**

# Collection Of Plant Material

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- 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.

## *Macroscopic Character of Artemisia vulgaris Linn. Leaf.*

<b>Colour</b>	Upper surface dark green and lower surface is silvery green
<b>Odour</b>	Aromatic
<b>Taste</b>	Bitter
<b>Shape</b>	Lanceolate, acuminate, entire margined or slightly serrated.
<b>Texture</b>	Upper surface is glabrous, pubescent, Lower surface is tomentose.

# *Pharmacognostic Investigations*

Sl. No.	Physico-chemical parameter	Leaves
1.	Foreign matter	Nil
2.	Ash Values	
	• Total ash	7 % w/w
	• Acid insoluble ash	3.25 % w/w
	• Water-soluble ash	2.12 % w/w
3.	Extractive values	
	• Alcohol soluble extractive	7.2 % w/w
	• Water soluble extractive	9.6 % w/w
4.	Loss on drying (at 105 <sup>0</sup> C)	9 % w/w
5.	Fluorescence	No fluorescence

# Extraction

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- ❖ The air-dried leaves of *Artemisia vulgaris* Linn. were reduced to fine powder (40 size mesh)
- ❖ 100 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether, chloroform, ethyl acetate and ethanol.
- ❖ Another batch of powdered drug was macerated with chloroform-water I.P.
- ❖ After the effective extraction, solvent were concentrated using rotary flash evaporator

Sr. No.	Extracts	Nature of Extract	Colour of extract	Weight (g)	%Yield w/w
1.	Pet-ether (40-60°C)	Solid sticky	Green	2.2	2.2
2.	Chloroform	Semi Solid	Light green	2.5	2.5
3.	Ethyl acetate	Semisolid viscous	Yellowish green	1.8	1.8
4.	Ethanolic	Semisolid viscous	Yellowish brown	15.5	15.5
5.	Chloroform-Water I.P.	Solid	Reddish brown	11	11

# *Preliminary Qualitative Tests of Various Extracts of Artemisia vulgaris.*

-  The Petroleum ether extract shown the presence of steroid, fats and oil.
-  The Chloroform extract shown the presence of steroid, alkaloid, fats and oil.
-  The ethyl acetate extract shown the presence of flavonoids, glycosides, tannin, phenolics.
-  The Ethanolic extract shown the presence of glycoside, flavonoids, saponin, alkaloid, tannin, phenolic substances.
-  The Aqueous extract shown the presence of glycosides, flavonoids, proteins, saponins, tannins.

# *Antibacterial Activity By Cup-plate Method*

-  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.

# *Antifungal Activity*

-  In the present study antifungal extract is diffused from the cup through an agar layer in a petri dish 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.

## **Standard used**

-  Benzyl penicillin for gram +ve bacteria
-  Streptomycin for gram-ve bacteria
-  Griseofulvin for fungal species

 DiMethyl Formamide (DMF) as control

## **Preparation of sample solution**

-  Different concentration of extracts equivalent to 50  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150  $\mu\text{g}$ , 200  $\mu\text{g}$  and 300  $\mu\text{g}$ /0.1ml 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  $\mu\text{g}$ , 100  $\mu\text{g}$ , 150  $\mu\text{g}$ , 200  $\mu\text{g}$  and 300  $\mu\text{g}$ /0.1ml of benzyl penicillin and streptomycin for antibacterial and Griseofulvin were prepared for antifungal activity.

# *Preparation of inoculum for antibacterial and antifungal activity*

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- 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  $37\pm 1^{\circ}\text{C}$  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 Nutrient agar medium (Himedia labs)

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## **Preparation of cultural medium**

- 27 gm of nutrient agar (Himedia) readymade medium was dissolved in freshly prepared distilled water (in 1000 ml) by gentle heating.

## **Preparation of agar plate**

- The sterilized medium was cooled at 40°C 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 Petri dishes so as to obtain 6 mm thickness of medium. The medium in the plate was allowed to solidify at room temperature.

# *Experimental procedure for antibacterial and antifungal activity*

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- ❖ The sterile borer was used to prepare 4 cups of 8 mm diameter in the medium of each Petri dish.
- ❖ 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  $37\pm 1^{\circ}\text{C}$  for 24 hrs.
- ❖ The presence of definite zones around the cup of any size indicated antibacterial and antifungal 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.

# *Antibacterial activity shown by extracts*

- For the gram +ve organisms like *Bacillus subtilis*, *Bacillus cereus*, *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 pneumoniae*,
- 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.

# *Antifungal activity shown by extracts*

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- ❁ For fungal organisms like *Candida albicans*, *Rhizopus japonicum* the MIC was 150  $\mu\text{g}/0.1\text{ml}$  with aqueous and petroleum ether extract,
- ❁ For *Aspergillus fumigatus* the ethyl acetate and aqueous extracts has MIC 100  $\mu\text{g}/0.1\text{ml}$ ,
- ❁ For *Candida tropicallis* the ethyl acetate and petroleum ether extracts has MIC at 150  $\mu\text{g}/0.1\text{ml}$ .

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- ❖ The present investigation reveals that the *Artemisia vulgaris* aqueous, chloroform and ethyl acetate extracts shows significant antibacterial and antifungal activity whereas petroleum ether extract showed significant antifungal activity when compared with standard.
- ❖ However, further experiment are required to establish and elaborate the molecular mechanisms(s) of its Anti-ulcer activity.
- ❖ Hence, to put into a nutshell, more significant antibacterial activity and *antifungal* activity of aqueous, chloroform and ethanolic extract may be due to the presence of flavonoids may be due to the combine effect of glycoside, saponin, alkaloid, tannin and flavonoid. However, this claims demands further study of isolation of individual components and observing their effect in the protection against various bacterial and fungal organism

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

Extracts	Bacillus Subtilis					Bacillus Cerius					Staphylococcus aureus					Pseudo aerogenosa				
	50	100	150	200	300	50	100	150	200	300	50	100	150	200	300	50	100	150	200	300
<b>Conc. (µgm/0.1mL)</b>																				
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***

Extracts	Salmonella typhi					E. Coli					Klebsiella Pneumnae					Vibrio-cholerae				
	50	100	150	200	300	50	100	150	200	300	50	100	150	200	300	50	100	150	200	300
<b>Conc. (µgm/0.1mL)</b>																				
<b>Ethanolic</b>	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
<b>Ethyl acetate</b>	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*
<b>Chloroform</b>	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*
<b>Pet ether</b>	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
<b>Aqueous</b>	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*
<b>DMF</b>	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
<b>STD</b>	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 ulgaris***

	<b>Proteus mirabilis</b>					<b>Serratia marsupium</b>				
	<b>50</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>	<b>50</b>	<b>100</b>	<b>150</b>	<b>200</b>	<b>300</b>
<b>Ethanolic</b>	9.3	10.3	11.5	12.7	13.1	9.5	10.2	10.7	11.8	12.7
<b>Ethyl acetate</b>	13.8	15.9	17.9	20.1	22.7*	14.4*	16.6	18.7	20.3	23.4
<b>Chloroform</b>	14.8	16.7	19.1	21.5	24.1*	14.6*	16.6	19.2	20.8	23.1
<b>Pet ether</b>	10.1	10.5	11.4	12.1	12.6	9.5	10.8	13.1	14	14.9
<b>Aqueous</b>	14.9	16.8	18.9	20.9	24*	14.5*	16.8	19.1	21	22.9
<b>DMF</b>	R	R	R	8.5	8.7	R	R	R	8.5	8.7
<b>STD</b>	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***

Extracts	Aspergillus fumigatus					Candida albicans					Rhizopus japonicum					Candida Tropicallis				
	50	100	150	200	300	50	100	150	200	300	50	100	150	200	300	50	100	150	200	300
Conc. (µgm/0.1mL)																				
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



THANK YOU