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IN-VITRO STUDIES ON ANTIBACTERIAL ACTIVITY OF SOME MISTLETOE SPECIES.

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ABSTRACT
In vitro evaluation of antibacterial activity was carried out on leaf extracts of *D.falcata, D. falcata var pubescens, V. monoicum* and *V. orientale* and stem extracts of *D. falcata, D. falcata var pubescens, V. articulatum* and *V.orientale* in three different solvents viz., methanol, n-hexane and ethyl acetate against four bacterial strains viz., *Staphylococcus aureus, Bacillus subtilis, Escherichia coli* and *Pseudomonas aeruginosa* by using disk diffusion method. Among the test species, all the three solvent extracts of *V. monoicum* were shown to be effective against the four bacterial strains. Overall, ethyl acetate extracts of both leaf and stem of the tested species exhibited higher inhibitory activity compared to its methanol and n-hexane counterparts.

KEYWORDS: mistletoe, Antibacterial activity.

INTRODUCTION
Mistletoes are semi-parasitic evergreen shrubs prevail in tropical and subtropical climates worldwide and are widely distributed throughout India. Of about 1300 species of Mistletoes, majorly of them fall into two families Viscaceae and Loranthaceae in the order Santalales (Calder, 1983). Mistletoe, with distinct habit, have been reported to exist on more than 300 different host plants (Sampatkumar and Selvaraj, 1981) & (Shanavaskhan *et al*., 2012).

Mistletoes considered to be hemiparasites as they live on host plants and derive water and minerals by haustorial connections. However, they perform photosynthesis activity at some point of time in their life cycle (Barlow, 1987) (Richter & Popp, 1992, 1998). As there is no phloem connection with the host, organic substances of the host are only transported via xylem that includes amino acids, cyclohexols and thiols etc. Often the quality and quantity of various phytoconstituents of a hemiparasite influenced by its host plant. Many reports indicate that mistletoes have higher nutrient concentrations than their host (Lamont, 1983) (Karunaichami *et al.*, 1993).

Though various species of Mistletoe have been used historically as a medicinal herbs in the curative methods of high blood pressure, infertility, epilepsy, cancer and arthritis problems etc., however, exhaustive exploration of their ethanomedicinal values are comparatively very meager (Karola Maul *et al.*, 2018). (O'Neill *et al*., 2019**)**.

MATERIAL AND METHODS: In the present study, (one species and one variety of genus *Dendrophthoe*) and three species of genus *Viscum*) were collected from different forest regions (Srisailam & Talakona) of Andhra Pradesh. All the plant species selected for the present study are Hemi parasitic flowering plants belong to the families Loranthaceae and Santalaceae. The tested species are collected from different regions (Srisailam &Talakona) are duly authenticated by Botanical Survey of India (BSI), Deccan regional centre, Hyderabad. Herbarium specimens of each of the species have been maintained separately in the lab. The list of the species tested is presented in Table.1&Fig-I

Table 1: The list of tested plant species (semi-parasites).

S.NO	Scientific name*	Family	Location	Evaluated part of the plant	Host species
1	*Dendrophthoe falcata var pubescens* (Hook.f.) V.Chandras	Loranthaceae	Srisailam	Stem, Leaves	*Samanea saman*
2	*Dendrophthoe falcata* (L.f.) Ettingsh	Loranthaceae	Talakona	Stem, Leaves	*Azadirachta indica*
3	*Viscum articulatum* Burm.f.	Santalaceae	Talakona	Stem	*Dalberjia paniculata*
4	*Viscum monoicum* Roxb.ex DC.	Santalaceae	Srisailam	Leaves	*Ficus racemosa*
5	*Viscum orientale* Willd.	Santalaceae	Srisailam	Stem, Leaves	*Strychnos nux vomica*

*Authentication by BSI, Deccan regional center Hyderabad

Dendropthoe falcata

Dendropthoe falcata var pubescens

Viscum articulatum

Viscum monoicum

Viscum orientale

Fig. 1: Plant species and Variety.

Selection of micro-organisms

The organisms employed in this study were two gram-positive bacteria viz. *Bacillus subtilis* (MTCC 121), *Staphylococcus aureus* (MTCC 96)and two gram-negative bacteria *Pseudomonas aeruginosa* (MTCC424), *Escherichia coli* (MTCC 443).The bacterial cultures were obtained from the Microbial Type Culture Collection, Chandigarh, India.

Preparation of plant extracts

The leaves and stems were separated, and surface sterilized with 0.1% $HgCl_2$ for 5 minutes, washed thrice with sterilized distilled water for 5 minute each time. They were shade dried and powdered. Powders of the test material were dissolved in three different solvents viz methanol, ethyl acetate, and n-hexane for *in vitro* antibacterial studies.

Disc-diffusion method

A stock solution of extract was prepared with the dried powdered plant materials by hot extraction process by using a Soxhlet extraction device with respective solvents viz. methanol, ethyl acetate and n-hexane (1:1). The stock solution was then diluted with different concentrations. 0.2 ml of each dilution was impregnated into sterile, blank disks (Whatman N0.1 filter paper) 6 mm in diameter. All discs were thoroughly dried.

Agar plates with Mueller Hinton Agar (MHA) are seeded with the test bacterium strain and labelled. Leave culture Plate for 5-10 min at room temperature by closing the culture plate. Place the impregnated disc at the centre of plate using disc dispensers. Gently press antibiotic paper disc by forceps so that there is no gap left between the disc and bacterial culture. Incubate plates at 37^0C for 24 hours. After 24 hours, the inhibition diameter around each disc was measured and recorded. Each extract was tested in triplicate. Negative control was prepared with only methanol extract used for extraction.

RESULTS

Antibacterial activity of plant extracts

In the present study leaf extracts of *D.falcata, D. falcata var pubescens, V. monoicum* and *V. orientale* and stem extracts of *D. falcata, D. falcata var pubescens, V. articulatum* and *V.orientale* plant species were evaluated in three different solvents viz., methanol, n-hexane and ethyl acetate against two of gram-positive bacteria *Staphylococcus aureus* (MTCC– 96), *Bacillus subtilis* (MTCC – 121) and two of gram-negative bacteria *Escherichia coli* (MTCC – 443), *Pseudomonas aeruginosa* (MTCC – 424) using disk diffusion method.

Among the four test species, methanol leaf extracts of *V. monoicum* exerted inhibitory effect against the four bacterial strains. Methanol leaf extracts of *D.falcata var pubescens* exerted high zone of inhibition against *S.aureus,* while *V.orientale* showed inhibitory activity on both *E.coli* and *S.aureus*. Among four test species, methanol leaf extracts of *V. monoicum* was most effective against the bacterial strains over the other three species.

Among N-hexane leaf extracts *V.monoicum* has shown inhibitory activity against *B.subtilis and E.coli* whereas *V.orientale* was effective only on *S.aureus.* The inhibitory effect of n-hexane leaf extracts of both *D.falcata* and *D.falcata var pubescens* were negative on the four bacterial strains tested.

Ethyl acetate leaf extracts of *D.falcata* and *D.falcata var pubescens* exerted inhibition against all the four bacterial strains tested. Whereas *V. monoicum* and *V. orientale* were effective only against three of the four bacterial strains tested. *V. monoicum* tested negative against *P.aeruginosa* while *V. orientale* was ineffective against *B. Subtilis*.

Overall, compared to methanol leaf extracts, most of the ethyl acetate leaf extracts exhibited consistent inhibitory activity against three of the four test strains, while n-hexane extracts were observed to be least effective. Among the four test species, *V. monoicum* was shown to be effective against the four bacterial strains by the three solvent extracts.

Methanol stem extracts of *D.falcata* exerted inhibitory effect against all the four bacterial strains tested. *D.falcata var pubescence* induced inhibitory activity against three of the four bacterial strains tested.

V.articulatum was very effective against the two gram-positive and the gram-negative *P.aeruginosa*.

None of the N-hexane stem extracts have shown any inhibitory activity against the bacterial strains except for *V.orientale* which has shown inhibitory activity against *B.subtilis.*

Ethyl acetate stem extracts of *D.falcata*, *V.articulatum* & *V. orientale* exerted inhibitory effect on all the four tested bacterial strains, while *D.falcata var pubescens* exerted inhibitory effect on three of the four bacterial strains tested excepting against *S.aureus.*

Table 2: Antibancterial activity of different leaf extracts.

Bacterial type	Bacterial Species	*Dendrophthoe falcata var pubescens*			*Dendrophthoe falcata*			*Viscum monoicum*			*Viscum orientale*		
		ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA
Gram(+)	***Bacillus subtilis***	**	**	2.5	**	**	4	5	4	6	**	**	**
	Staphylococcus aureus	6	**	4	2	**	3	5	**	3	5	2	2
Gram (-)	***Pseudomonas aeruginosa***	**	**	2	**	**	2	4	**	**	**	**	2
	Escherichia coli	**	**	2	**	**	2	4	3	3	3	**	3

Key: ME =Methanol extract, NH=N-Hexane extract, EA=Ethyl acetate extract; **= No activity (+)=positive and (-) =negative

Overall, ethyl acetate extracts of both leaf and stem of the tested species exhibited higher inhibitory activity compared to its methanol and n-hexane counterparts. On the other hand methanol leaf extracts of *V.monoicum* and

methanol stem extracts of *D.falcata* were very efficacious against all the bacterial strains tested. Among the test species, all the three solvent extracts of *V. monoicum* were shown to be effective against the four bacterial strains.

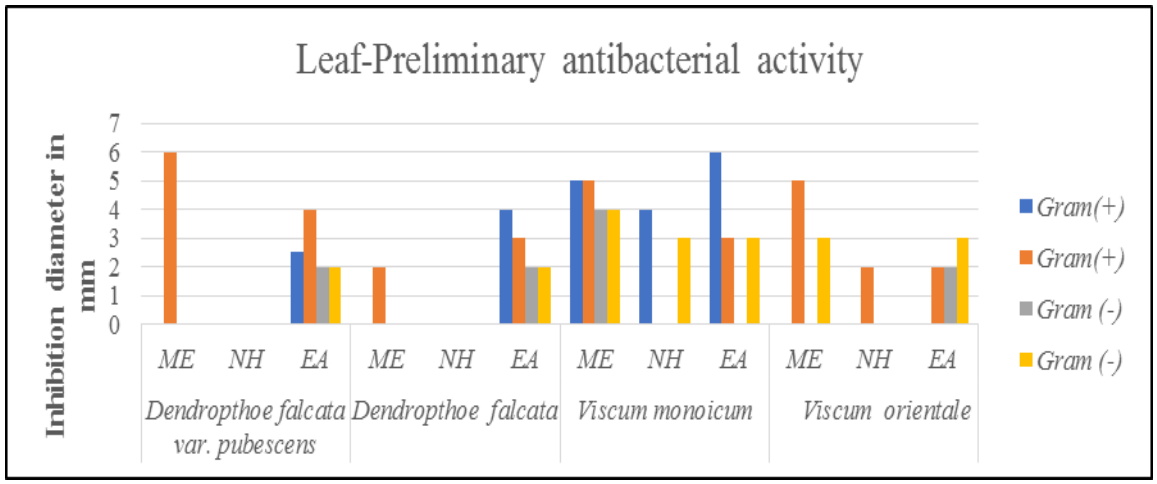


Fig. 2: Antibacterial activity of different leaf extracts.

Table 3: Antibacterial activity of different stem extracts.

Bacterial type	Bacterial Species	*Dendrophthoe falcata var pubescens*			*Dendrophthoe falcata*			*Viscum orientale*			*Viscum articulatum*		
		ME	NH	EA	ME	NH	EA	ME	NH	EA	ME	NH	EA
Gram(+)	***Bacillus subtilis***	**	**	2	5	**	3	**	3	4	3.5	**	4
	Staphylococcus aureus	5.5	**	**	4	**	4	**	**	2	4	**	2
Gram (-)	***Pseudomonas aeruginosa***	2	**	2	5	**	3	**	**	2	4	**	3
	Escherichia coli	2	**	2	5	**	4	**	**	4	**	**	3

Key: ME =Methanol extract, NH=N-Hexane extract, EA=Ethyl acetate extract; **= No activity (+)=positive and (-) =negative

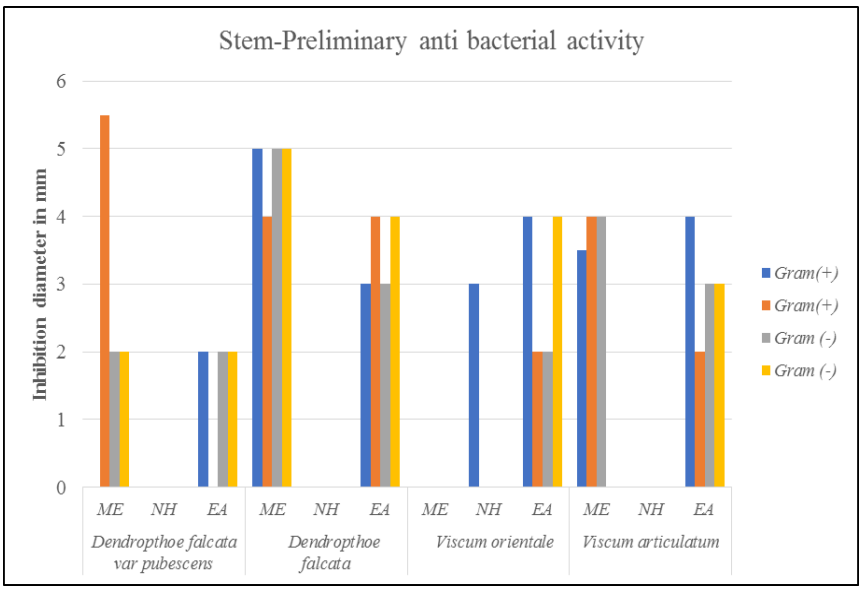
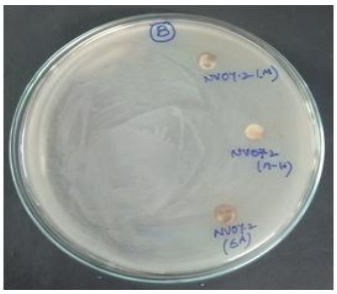


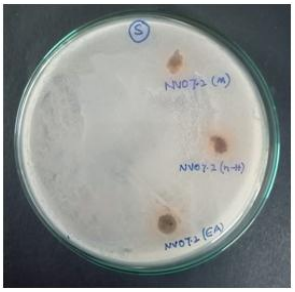
Fig. 2: Antibacterial activity of different stem extracts.

PLATE I

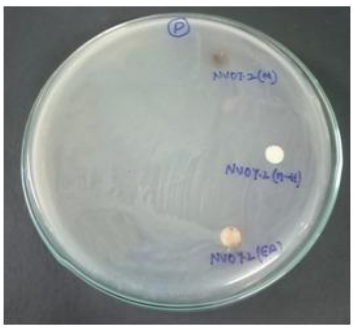
Antibacterial activity of different solvent extracts of *Dendrophthoe falcate.*



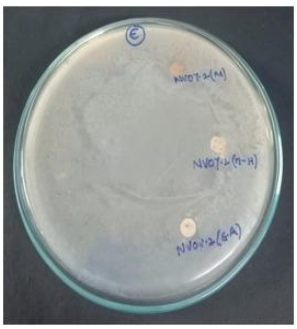
Bacillus subtilis



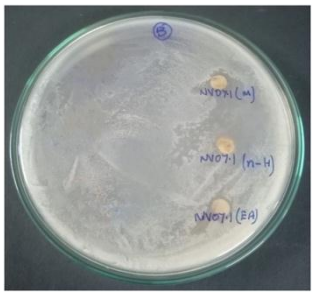
Staphylococcus aureus



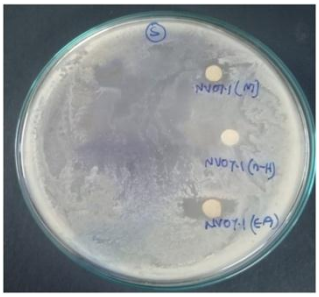
Pseudomonas aeruginosa



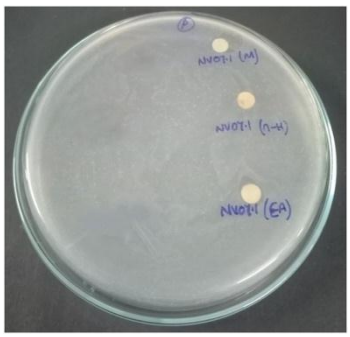
Escherichia coli



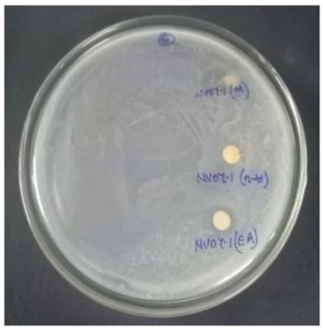
Bacillus subtilis



Staphylococcus aureus



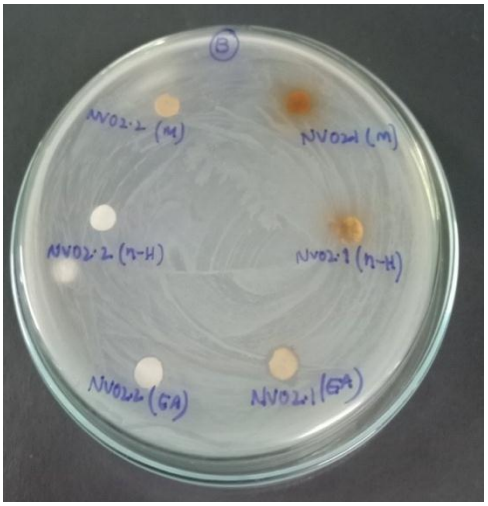
Pseudomonas aeruginosa



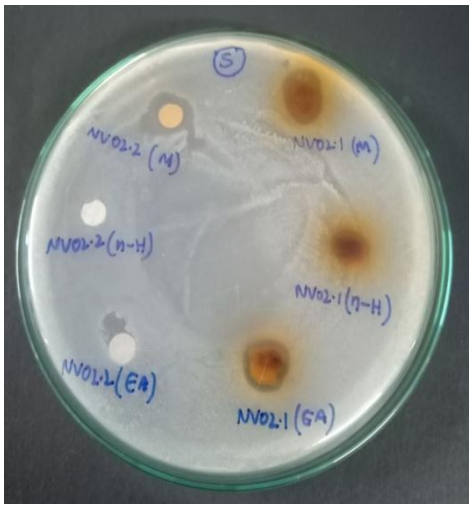
Escherichia coli

NV 07.1=leaf extract, NV 07.2=stem extract.

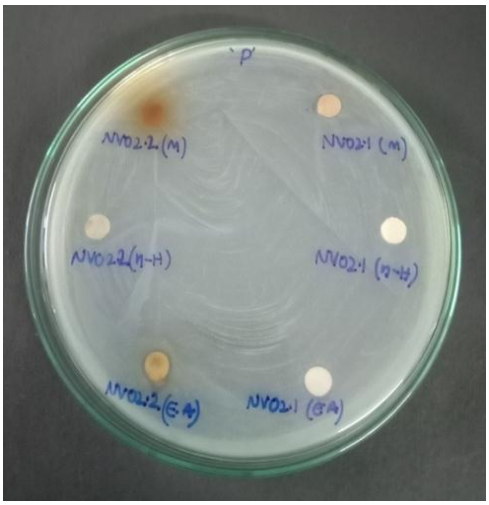
PLATE II
Antibacterial activity of different solvent extracts of *Dendrophthoe falcata var pubescens*



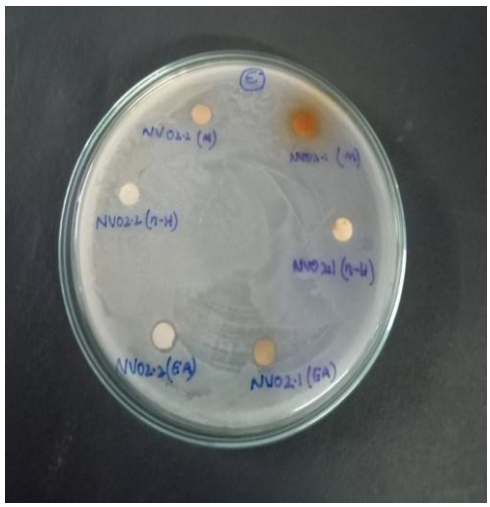
Bacillus subtilis



Staphylococcus aureus



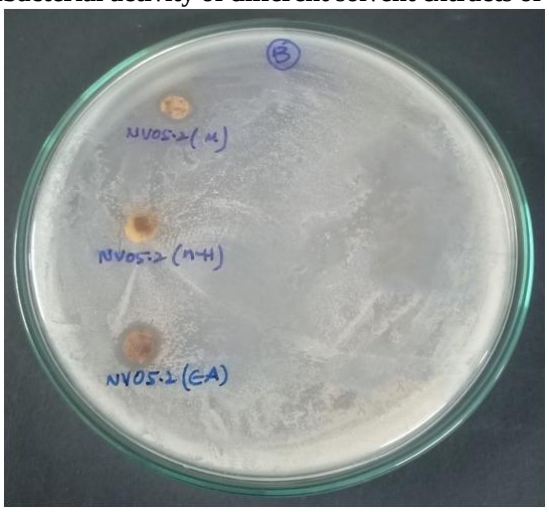
Pseudomonas aeruginosa



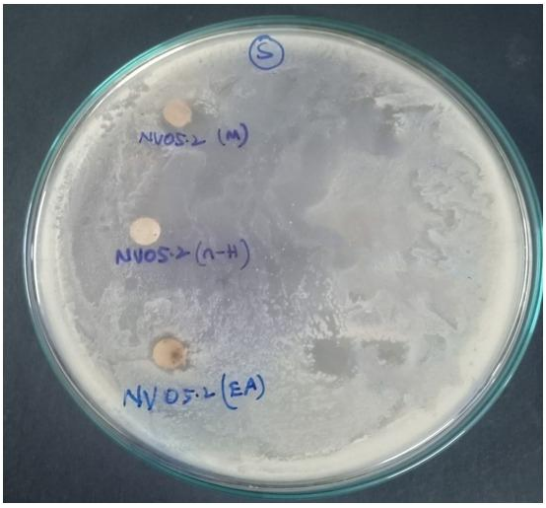
Escherichia coli

NV 02.1=leaf extract, NV02.2=stem extract.

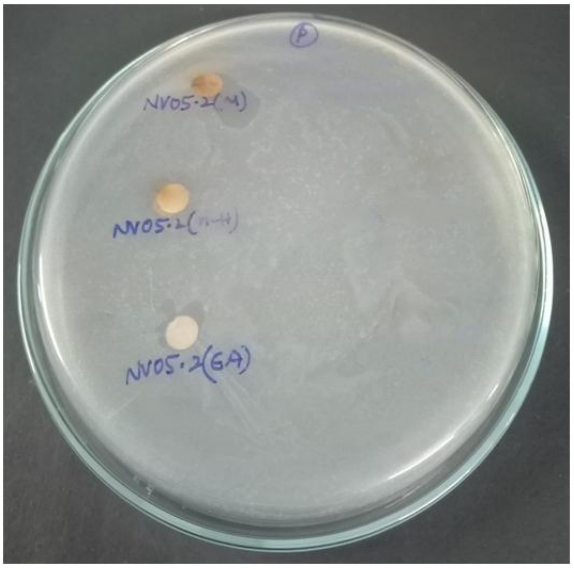
PLATE III
Antibacterial activity of different solvent extracts of *Viscum articulatum*



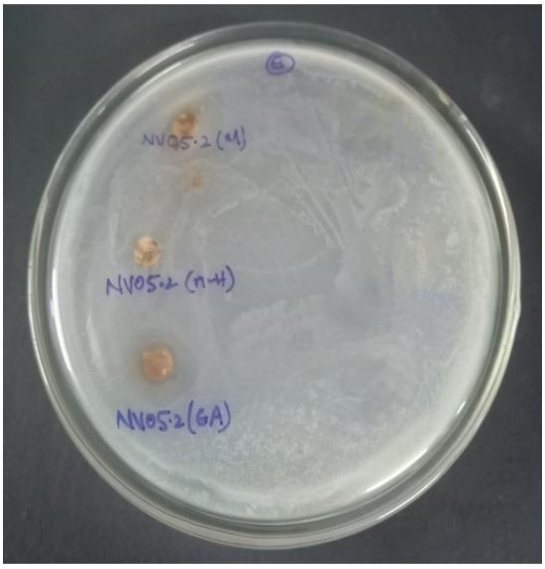
Bacillus subtilis



Staphylococcus aureus



Pseudomonas aeruginosa

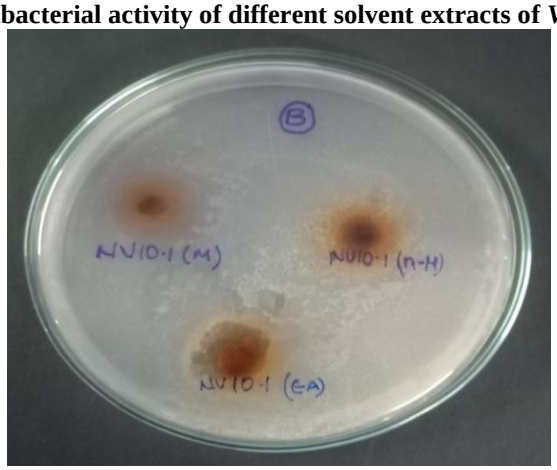


Escherichia coli

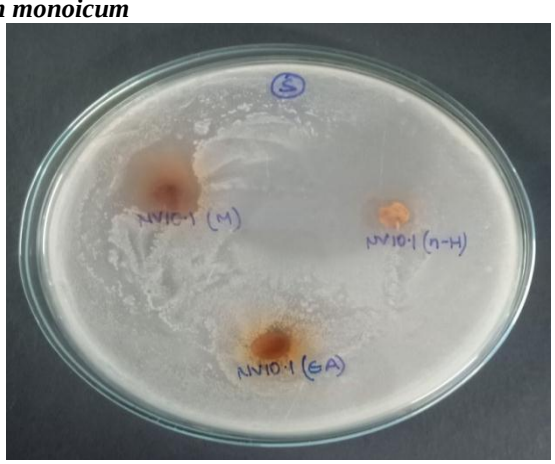
NV05.2=Stem extract

PLATE IV

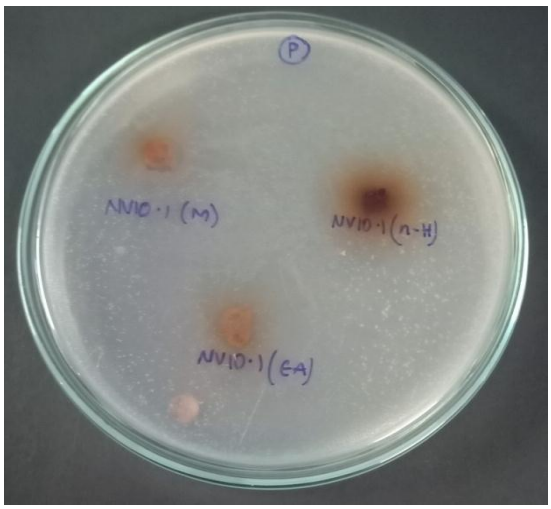
Antibacterial activity of different solvent extracts of *Viscum monoicum*



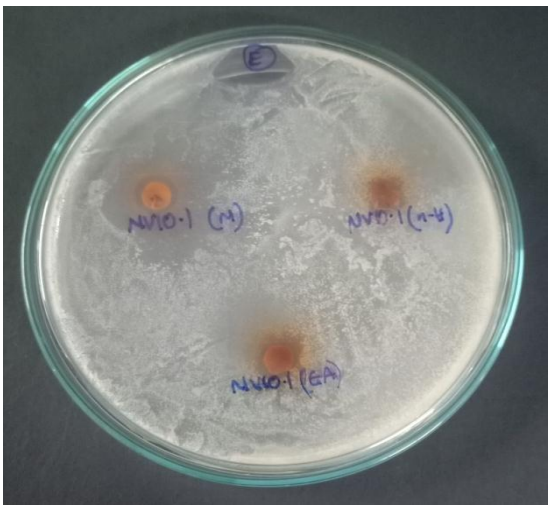
Bacillus subtilis



Staphylococcus aureus



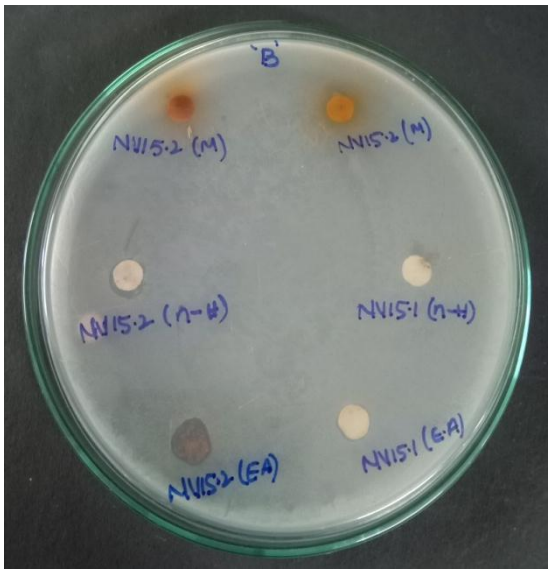
Pseudomonas aeruginosa



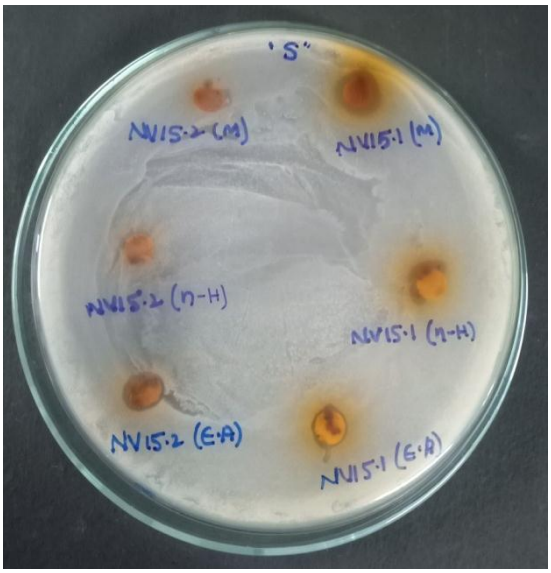
Escherichia coli

NV10.1=Leaf extract

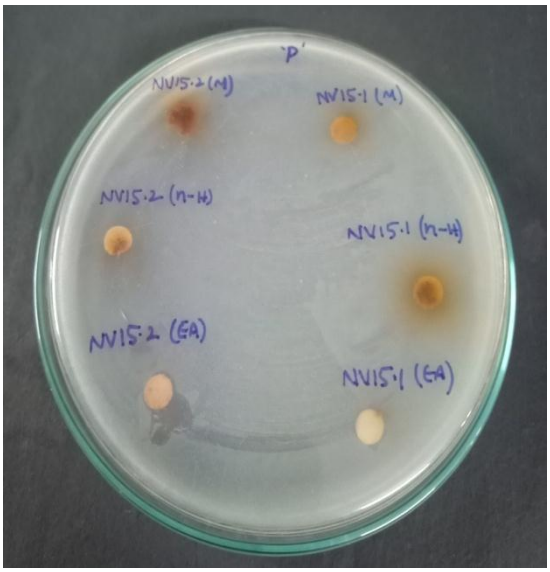
PLATE V
Antibacterial activity of different solvent extracts of *Viscum orientale*



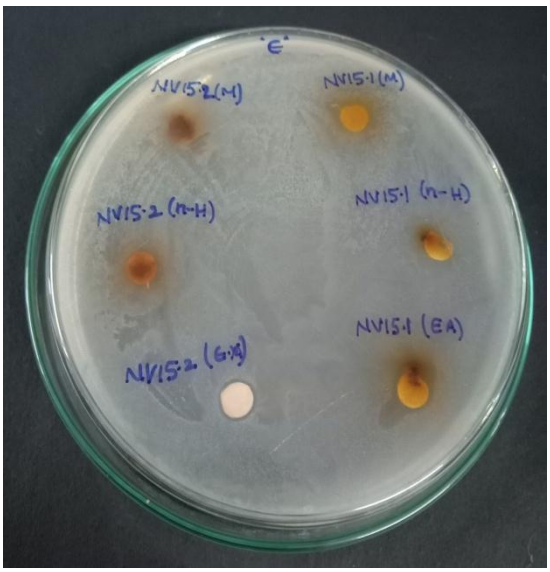
Bacillus subtilis



Staphylococcus aureus



Pseudomonas aeruginosa



Escherichia coli

NV 15.1=leaf extracts, NV 15.2= stem extracts.

DISCUSSION AND CONCLUSIONS

The ever enlarging world population and the need for better human healthcare, need not be reiterated. The increasing antibiotic resistance exhibited by pathogenic microbial infectious agents and failures of chemotherapeutics have lead to the screening of several medicinal plants for their natural and potential antimicrobial activity (Cowan, 1999 & Fahey, 2005). Overall, ethyl acetate extracts showed more inhibitive activity against tested bacteria compared to its methanol and n-hexane counterparts. Reduced ability of n- hexane to extract polar solutes could be the reason for low-performance of n-hexane extracts. (Siek, 1978; Thanh *et al.*, 2017).

All the test species exerted inhibition against the four bacterial strains tested. Among leaf extracts *V.monoicum* was most effective against the test bacterial strains. (Foyaj Ahmmed *et.al.*,2013).

Ethyl acetate leaf and stem extracts of both *D.falcata* and its variety *D.falcata var pubescens* exhibited similar inhibition activity against the test strains. (Pattanayak *et al.*, 2008) (Bhagat and Kondawara, 2021).

Ethyl acetate stem extracts of *V.articulatum* followed the suit in its bacteriostatic activity. The overall antibacterial efficacy of the mistletoes could be attributable to the presence of metabolites like terpenoids, phenols and flavonoids which are synthesized in response to plants

protective mechanism to microbial infections. (Cowan, 1999; Atun *et al.*, 2019).

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