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ANTIMICROBIAL ACTIVITY OF *Adhatoda Vasica* Nees

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ABSTRACT

Adathoda vasica Nees well known in the indigenous systems of medicine for its beneficial effects, effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicobials . They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials. The *in vitro* antimicrobial activity of crude ethanol and benzene extracts of various Plant Parts of *Adhatoda vasica*(Nees) was evaluated by using agar well diffusion method. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 4 to 20 mm. Maximum inhibition zone (20mm.) was observed in roots against bacillus and minimum inhibition in stem, leaves (4mm.) against many strains such as Streptomyces, pseudomonas, *A.niger*. The Crude extracts of *Adhatoda vasica* Nees have showed its ability to inhibit the growth of various bacteria and fungi.

KEYWORDS: Antimicrobial activity, *Adhatoda vasica* Nees, Antibacterial, Antifungal.

INTRODUCTION

Adhatoda vasica Nees. belongs to the medicinal family Acanthaceae, is an evergreen shrub, distributed from the Punjab in the North and Bengal and Assam in the South-East to the Ceylon, Malaya and Singapore in the South.[1] It is well known in Ayurveda by its Sanskrit name Vasaka and commonly known as Adusa. First botanically described as *Justicia adhatoda* by Linnaeus (*Species Plantarum*, 1753), redefined as *Adhatoda vasica* by Nees (1831) the name by which it is generally known today. The leaves of Adusa have been in use in Indian systems of medicine for last more than 2000 years. The plant is appreciated for containing bronchodilator alkaloids, mainly vasicine. The shrub is the source of the drug-

vasaka, well known in the indigenous systems of medicine for its beneficial effects, predominantly in bronchitis. All parts of the plant are used in herbal medicine and particularly the leaves are endorsed with insecticidal and parasiticidal properties. The root is valuable in strangury, leucorrhoea, bronchitis, asthma, bilious vomiting, sore eyes, fever and gonorrhoea. It is a valuable as an antiseptic, antiperiodic and anathematic.[2] The leaves, flowers, fruits and roots are extensively used for treating cold, cough, whooping cough and chronic bronchitis and asthma as sedative expectorant, antispasmodic and as anthelmintic.[3]

Plant-based antimicrobials represent a vast untapped source for medicines with enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects of synthetic antimicobials. [4]They may act as lead compounds for the pharmaceutical industry or as the base for the development of new antimicrobials.[5.6]

A review of literature reveals that a significant contribution has been made on antimicrobial potential of family Acanthaceae.[7-21] Antimicrobial activity of leaf extracts of *Adhatoda vasica* Nees alone as well as in combination of extract of different plants has been studied by various workers. Crude leaf extract of the plant exhibited significant inhibition of bean mosaic virus,[22] chloroform and ethanol extracts of leaves of the plant showed *in vitro* antifungal activity against systemic fungal pathogens,[23] aqueous and organic solvent extract of *Adhatoda vasica, Allium sativum, Azadirachta indica, Embelica officinalis, Euphorbia pilulifera, Ocimum sanctum, Solanum trilobatum* and *Withania somnifera* showed bactericidal activity against *Mycobacterium tuberculosis in vitro.*[24-29]

The effectiveness of a particular antimicrobial agent results in the production of growth-inhibition zones that appear as clear areas surrounding the disc from which the agent diffused. The diameter of the zones can be measured and the results of such an experiment are represented as zone of inhibition in mm.[30]

Botanical Description

Adhatoda vasica (Nees)is commonly known as Adusa and Malabar Nut (English name).

Kingdom Plantae

Division Angiosperms

Class Dicotyledenae

Sub class Gamopetalae

Series Bi-carpellatae

Order Personales

Family Acanthaceae

Genus Adhatoda

Species Vasica

Other Common Names

Malabar Nut, Adulsa, Arusha, Vasaka, Justicia, adhatoda, Adulra, Bakasa, Adusoge, Addasardmu, Lion's muzzle, Stallon's tooth.

Plant description

Adhatoda vasica Nees .belongs to the medicinal family Acanthaceae. It is an evergreen shrub of 1-3 feet in height with many long opposite branches. Stem herbaceous above and woody below. The leaves are opposite, exstipulate, broad,, lanceolate, sharp and pointed . Flower spikes or panicles, small irregular zygomorphic, bisexual, and hypogynous. K4-5, C5, imbricate, A, didynamous, epipetalous, G (2), two celled. Style simple, stigma two of unequal size.[31]The flower has large white Petals, streaked with purple on the lower tip. It has capsular four seeded fruits. Its trade name Vasaka is based on Sanskrit name.[26] Inflorescence is axillary spicate cyme, densely flowered, peduncles short, bracts broadly ovate, foliaceous. The leaves, flowers, fruit and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma, as sedative, expectorant and antispasmodic.[32]

The plant has been recommended by ayurvedic physicians for the management of various types of respiratory disorders. In the present investigation benzene and ethanol extract of various plant parts of *Adhatoda vasica* (Nees) Has been studied for their antimicrobial efficiency.

MATERIALS AND METHODS

Plant Material Various plant parts of *Adhatoda Vasica* (leaves, stem and roots) were collected from the fields at Jaipur and authenticated from the herbarium of University of Rajasthan. Plant parts were Separated, cleaned and oven dried at 35ºC for 30 min and then at 25^0C till constant weight was achieved and powdered.

Antimicrobial Activity

Ethanolic and Benzene extract was used for determination of antimicrobial activity. Four bacterial and four fungal strains were selected for the antimicrobial screening.

Microorganisms Used

Clinical laboratory isolates of bacteria viz *Streptococcus viridians*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter cloacae* and fungi viz *Trichoderma viridae* and *Aspergillus niger* were procured from the Microbiology Laboratory, SMS Medical College, Jaipur.

Preparation of Extract

The ethanol and benzene extract was obtained separately by macerating 10g of dried powder of different plant parts in 95% ethanol, benzene respectively, kept on a rotary shaker for 24 h, separately. Each of the extract was filtered, centrifuged at 5000rpm for 15 min, dried under reduced pressure and stored at 4 ºC in airtight bottles.

Determination of Antibacterial Assay

In vitro antibacterial activity of the crude ethanol and benzene extract was studied against bacterial strains by the agar well diffusion method.[33] Mueller Hinton Agar No.2 (Hi Media, India) was used as the bacteriological medium. The extracts were diluted in 100% dimethy lsulphoxide at the concentrations of 5 mg mL^{-1}. The Mueller Hinton agar was melted and cooled to 48-50 ºC and a standardized inoculum (1.5×108 CFU mL^{-1}, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile petri dishes to give a solid plate. Wells were prepared in the seeded agar plates. The test compound (100 µl) was introduced in the well (6 mm). The plates were incubated overnight at 37^{o}C. The antimicrobial spectrum of the extract was determined for the bacterial species in terms of zone sizes around each well. The diameters of zone of inhibition produced by the agent were compared with those produced by the commercial control antibiotic ciprofloxacin. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter was measured with antibiotic zone reader to nearest mm. The experiment was performed in triplicate to minimize the error and the mean values are presented.

Determination of Antifungal Assay

Antifungal activity of the experimental plant was investigated by agar well diffusion method.[34] The fungi were subcultured on potato Dextrose Agar (PDA) medium and respectively incubated at 37 °C for 24 h and 25 °C for 2 - 5 days. Suspensions of fungal spores were prepared in sterile PBS (phosphate buffered saline) and adjusted to a concentration of 106 cells mL^{-1}. Dipping a sterile swab into the fungal suspension was rolled

on the surface of the agar medium. The plates were dried at room temperature for 15 min. Wells of 6 mm in diameter and about 7 mm apart were punctured in the culture media using sterile glass tube. 0.1 mL of several dilutions of fresh extracts was administered to fullness for each well. Plates were incubated at 37 °C. After incubation of 24 h, bioactivities were determined by measuring the diameter of inhibition zone (mm). The diameters of produced inhibition zone were compared with those of standard ketokenozol used as standard antifungal agent. All the experiments were performed in triplicate and mean values were taken.

RESULTS AND DISCUSSION

The increasing microbial resistance for the use of antibiotics has made it necessary to carry out research to evaluate plants as source of potential chemotherapeutic and antimicrobial agent along with their ethnomedicinal use.[35]

The antimicrobial activity (MIC) of *Adhatoda vasica* against clinical pathogen was assessed by disc diffusion method, solvents like methanol, ethanol, acetone, Chloroform, diethyl ether and water were used for the preparation of plant extracts in various concentrations.[19] The solvents showed higher activity in the order of diethyl ether > methanol > ethanol > acetone > Chloroform> water. The plant extract of *Adhatoda vasica* showed higher activity for different clinical pathogens in the order *of Klebsiella pneumoniae>Staphylococcus aureus> Proteus valgaris> Pseudomonas aeroginosa> Streptococcus Pyogens*.

Leaf extracts of *J. adhatoda* showed the higher activity for different clinical pathogens in the order of *P. aeruginosa>B. cereus >E. coli >K. pneumoniae>S. aureus*. Among the tested bacterial pathogens, *B. cereus* exhibited maximum susceptibility (18.33±0.94 mm) toward ethyl acetate extract. The maximum activity index (2.67±0.42) and fold area increase (6.31±2.24) were also observed against *B. cereus* for ethyl acetate extract.[36]

Seven compounds from the stems extract of *Adhatoda vasica* viz., *β*-sitosterol, daucosterol palmitate, monopalmitin, vanillin, vanillic acid, vasicinolone and vasicinone.[37] The petroleum ether fraction, daucosterol palmitate, monopalmitin, vanillin and vanillic acid showed strong antibacterial activity towards *E. coli* and *S. aureus*. Whereas daucosterolpalmitate and vanillic acid showed pronounced antifungal activity against *C. albicans*. Antiquorum-sensing assay showed that *β*-sitosterol, vanillin and vanillic acid were the most active compounds. While, petroleum ether and methylene chloride fractions,

vasicinolone and daucosterol palmitate showed moderate antiquorum-sensing activity. Petroleum ether fraction showed a remarkable inhibition of Ach-induced contraction at 200 and 250 µg/mL (89.5 and 95.2%, respectively) for antispasmodic activity. Methylene chloride fraction showed remarkable inhibition (97.6%) at 150 µg/mL. Vasicinone and vasicinolone showed significant inhibition at 150 µg/mL (89.9% and 84.8%, respectively).

The methanolic leaf extracts of *J.adhatoda,* where methanol as negative control and antibiotic as positive control against *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Penicillium digitatum was used.*[38] Methanolic and leaf extract were most effective against *Fusarium oxysporum* (20 mm and 22 mm), followed by *Penicillium digitatum* (18 mm and 21 mm), (15 mm and 16mm) and *Aspergillus flavus* (13 mm and 14 mm) respectively. With decreasing concentration of the extract the antifungal activity also decreased. Leaves exhibited better antifungal activity.

In the present investigation initial screenings of the experimental plant for possible antimicrobial activities was done using crude ethanol and benzene extracts. Nearly all of the identified components from plants that are active against microorganisms are saturated organic compounds and most often obtained through ethanol or Benzene extractive. In the present study *Adhatoda vasica* showed antimicrobial potent activity against bacterial strains as compared to fungal strains.

The Antimicrobial activity of ethanolic and benzene extract of different plant parts of *Adhatoda vasica* were tested against 5 bacterial strains (*Bacillus, E. coli, S. aureus, Streptomyces, A. niger, Fusarium, Penicillium*). The Inhibition zone (IZ) was measured by antibiotic zone reader (Table 1 To 4). Individually against Bacillus maximum IZ (20mm) was observed in ethanolic extract of root. *S. Aureus* showed no activity. Ethanolic extracts of root showed activity for *E.coli*. In Streptomyces maximum Inhibition zone was in ethanol roots extract and minimum in benzene extracts of leaves and stem. Pseudomonas shows activity in Benzene extract of stem only. Among the Fungal strain against *T.ressi* maximum IZ was observed in ethanol extract of stem and root and minimum in leaves. In *A. niger* benzene extract of root show maximum IZ (8mm) and stem and root did not show activity. Against Fusarium only stem show activity in ethanol extract and all other parts did not show activity. In case of Penicillium ethanol stem extract shows maximum activity and leaves (of both extract) shows minimum activity in both ethanol as well as benzene extract.

Table 1: Antimicrobial activities of ethanol extract of *Adhatoda vasica.*(Nees)

Test Organisms	Plant parts and inhibition zones of growth inhibition(mm.)			Standard
	Leaves	Stem	Roots	C/K
Bacteria				
Baccillus	NA	NA	20	20
E. Coli	-	-	12	20
S. aureus	NA	NA	NA	20
Streptomyces	NA	4	12	20
Psuedonomas	NA	NA	-	20

Table 2: Antifungal activities of ethanol extract of *Adhatoda vasica.*(Nees)

Test Organisms	Plant parts and inhibition zones of growth inhibition(mm.)			Standard
	Leaves	Stem	Roots	C/K
Fungi				
T.reesei	4	12	12	22
A.niger	NA	4	6	22
Fusarium	NA	4	NA	22
Penicillium	4	12	8	22

Table 3: Antimicrobial activities of benzene extract of *Adhatoda vasica.*(Nees)

Test Organisms	Plant parts and inhibition zones of growth inhibition(mm.)			Standard
	Leaves	Stem	Roots	C/K
Bacteria				
Baccillus	NA	NA	12	20
E. Coli	-	-	NA	20
S. aureus	NA	NA	NA	20
Streptomyces	NA	4	NA	20
Psuedonomas	NA	4	-	20

Table 4: Antifungal activities of benzene extract of *Adhatoda vasica.*(Nees)

Test Organisms	Plant parts and inhibition zones of growth inhibition(mm.)			Standard
	Leaves	Stem	Roots	C/K
Fungi				
T.reesei	4	10	NA	22
A.niger	NA	NA	8	22
Fusarium	NA	NA	NA	22
Penicillium	4	10	11	22

IZ=Inhibition Zone **NA**=No Activity

Plate-1

Antimicrobial activity of Ethanolic and Benzene extract of different plant parts(Leaves and Stems) of *Adhatoda vasica* (Nees)

A-Activity against *Bacillus subtilis.*

B-Activity against *S. aureus.*

C-Activity against *Streptomyces griseus.*

D-Activity against Pseudomonas.

E-Activity against *T. reesei.*

F-Activity against *A. niger.*

G-Activity against Fusarium.

H-Activity against Penicillium.

Abbreviations

S-Standard.

L-Leaves.

St-Stem.

Ben-Benzene.

Eth-Ethanol.

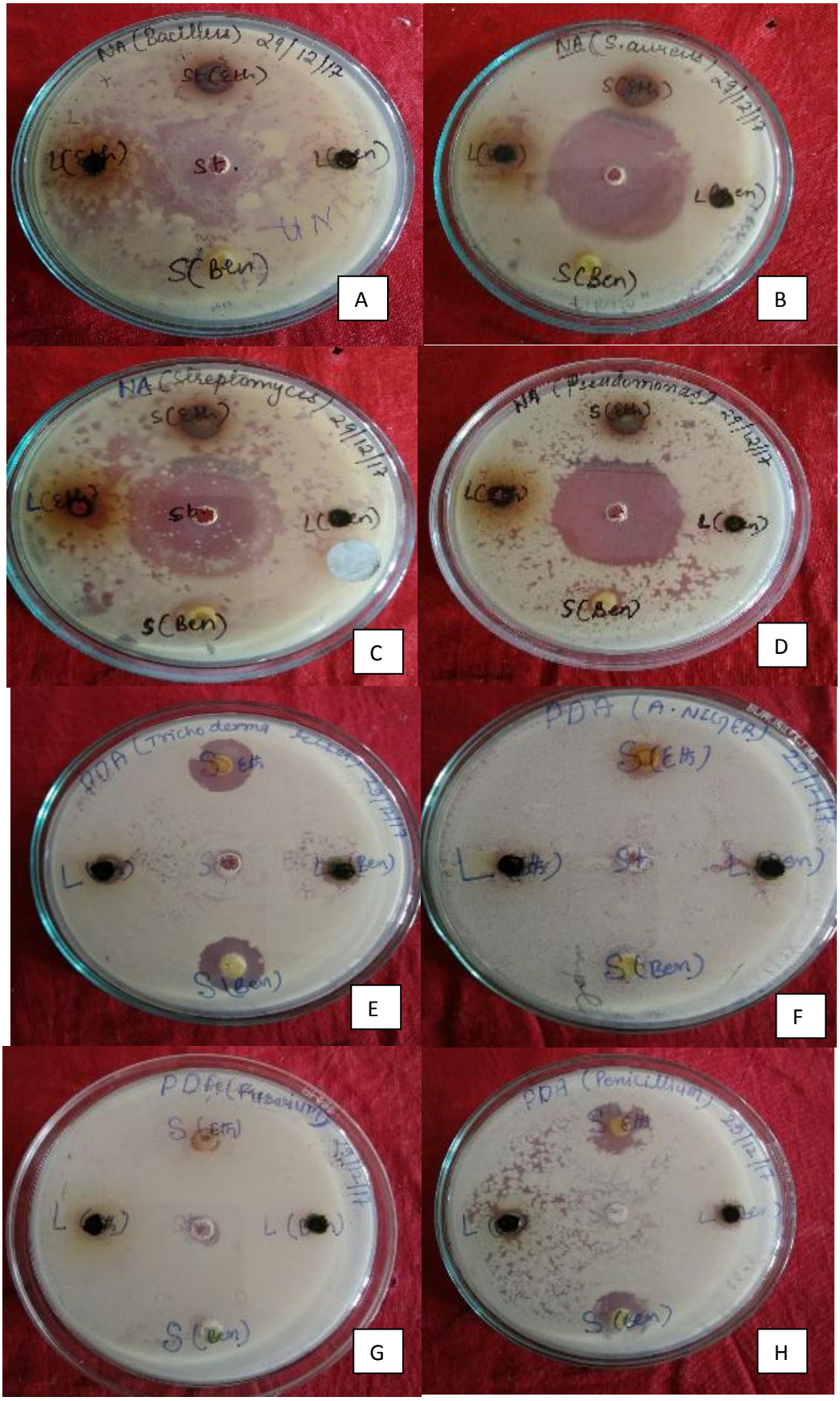

Plate No. 1: (STEM& Leaf)

Plate-2

Antimicrobial activity of ethanolic and benzene extract of different plant parts(Roots) of *Adhatoda vasica* (Nees)

A-Activity against *Bacillus subtilis.*

B-Activity against *S. aureus.*

C-Activity against *Streptomyces griseus.*

D-Activity against *E.coli*.

E-Activity against *T. reesei.*

F-Activity against *A. niger*.

G-Activity against Fusarium.

H-Activity against Penicillium.

Abbreviations

S-Standard.

Ben-Benzene.

Eth-Ethanol.

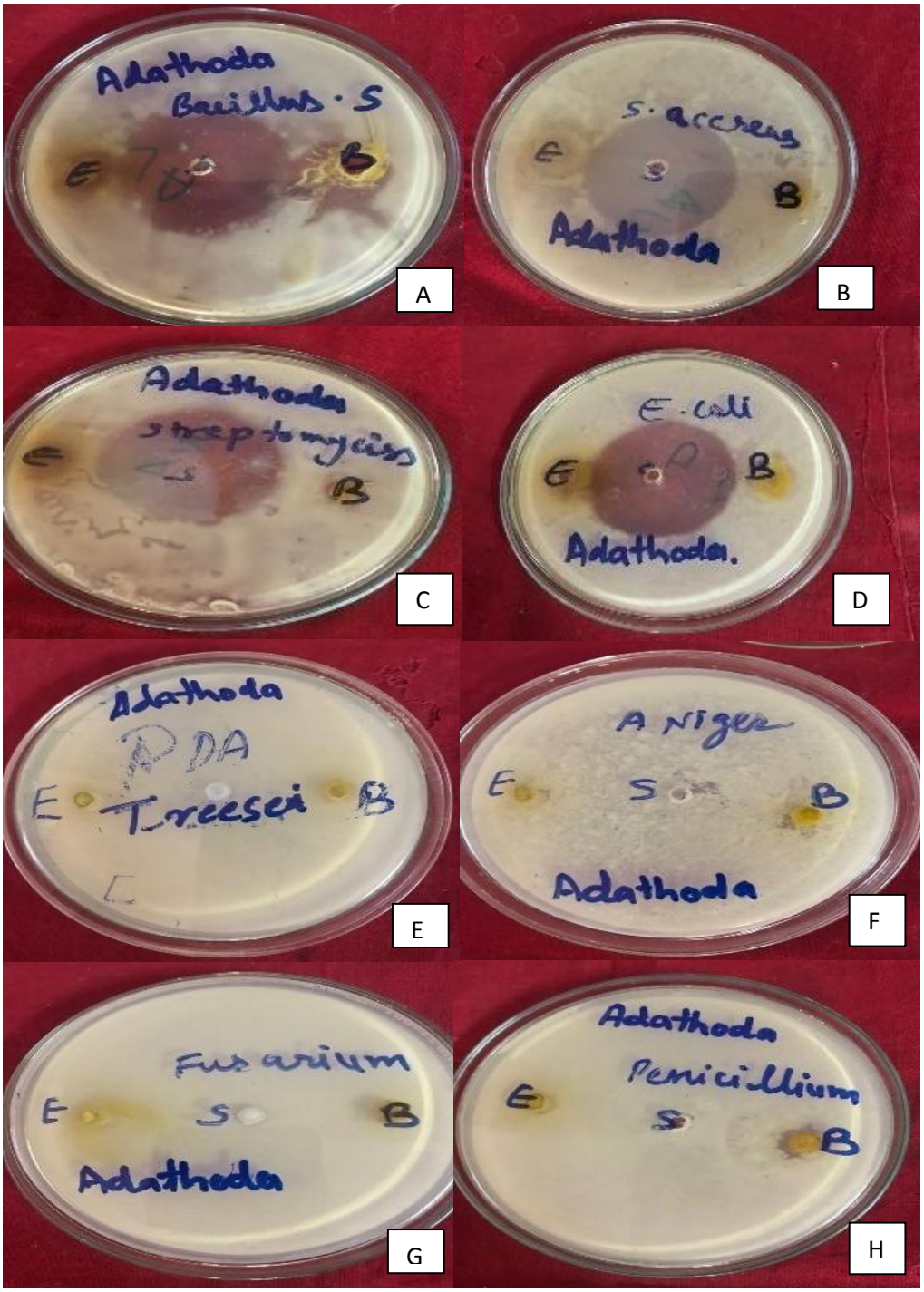

PLATE NO. 2 (ROOT)

This is in agreement with previous researchers that *Adhatoda vasica*(Nees) has potent antimicrobial activity and in future can be used as an antibacterial and antifungal agent for a number of pathogens, proper research is needed to find out the active principle for antimicrobial potential of this plant.[19-39]

CONCLUSION

Adhatoda vasica Nees by virtue of high activity can produce antibiotics have more promising therapeutic value over the known antibiotics like ciprofloxain and ketokenozol . The present investigation can be of economical and commercial interest to both pharmaceuticals companies and research institutes in the production of antimicrobial drugs. There have been no side effects or toxicity reports from many years on this plant therefore for further research there is a lot of scope from this plant.

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