Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 13 (2), 520-526

Evaluation of antibacterial activity of selected plant species from the State of Punjab, India

Research Article

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Abstract

Objective: Evaluation of antibacterial potential of aqueous and ethanol extracts of *Solanum nigrum* L., *Eclipta alba* (L.) Hassk., *Achyranthes aspera* L., *Sida acuta* Burm.f., *Justicia adhatoda* L. (Syn. *Adhatoda vasica* Nees), *Boerhavia diffusa* L. and *Withania somnifera* (L.) Dunal against human pathogenic bacteria *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.* Materials and Methods: Powdered form of the whole plant of each species was extracted in aqueous and ethanol solvents. The antibacterial activity experiment was performed using agar well diffusion method. A dose of 50µl, 100µl and 150µl of each extract (aqueous and ethanol) were tested. Results: Only the ethanol extracts have shown activity against test organisms. The 50µl and 100µl doses of extracts were not much effective against all the bacteria. Thus 150µl volume was selected for further study. The ethanol extract of *Adhatoda vasica* has yielded maximum zone of inhibition 24±1.7mm and 22±2.1mm against *S. aureus* and *E. coli*, respectively. Type AA_3 of *A. aspera* and SN_3 of *S. nigrum* were the most effective against *E. faecalis* with inhibition zones 22±2.2mm and 21±2.2mm, respectively. Similarly, *B. diffusa* has shown maximum inhibition against *P. aeruginosa.* Conclusion: The present study suggests the use of ethanol extract since none of the aqueous extracts have shown activity against all the tested bacteria. The difference in activity of plant extracts may be due to variable chemical composition of the plant species. The plant species can be selected based on the bacterial species to be investigated. Further studies can be planned to understand the bioactive molecules responsible for antibacterial activity.

Key Words: Chemical compounds, Cytomorphotypes, Extracts, Herbal, Pathogens, Zone of inhibition.

Introduction

Plants are continuously being used throughout the world by various sections of the society to meet their day to day requirements including medicines. They have served as potential reservoir for new drugs. Mother Nature has provided a variety of plant genetic resources for this purpose. Medicinal plants contain bioactive compounds that have been used in traditional medicine for various ailments (1). Plants have been recognized as richest source of antimicrobial compounds as well (2). The demand for medicinal plants is continuously increasing because of their easily availability, cost-effectiveness, no or least side-effects and in some area, the only source of health care (3). A number of plants have been known for their biological (4; 5) and antimicrobial properties (6; 7; 8; 9). Despite of advancement in healthcare facilities including medicines, some infectious diseases are still a major threat to human health (10). Bacterial infections are one of the most serious concerns of global health in 21st century (11). The regular use of antibiotics has developed antibiotic resistance at an alarming rate (12). The antibiotic resistance of some bacteria has become a major global challenge (13). In addition to resistance, some adverse effects of antibiotics including allergies, hypersensitivity and immune-suppression have also been reported (14). Thus, there is a need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases (15).

Present study is concerned with the antibacterial activity of cytomorphotypes and morphotypes of selected medicinal plants such as *Solanum nigrum, Eclipta alba, Achyranthes aspera, Sida acuta, Adhatoda vasica, Boerhavia diffusa,* and *Withania somnifera* against four pathogenic bacteria. Emphasis has been given to the antibacterial properties of cytomorphotypes and morphotypes. This will help to draw a correlation between the cytomorphological variations of the plant species with respect to antibacterial activity against the selected bacteria.

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Materials and Methods

Plant Materials

The plant species *Solanum nigrum* L., *Eclipta alba* (L.) Hassk., *Achyranthes aspera* L., *Sida acuta* Burm.f., *Adhatoda vasica* Nees, *Boerhavia diffusa* L.

and *Withania somnifera* (L.) Dunal were collected from different sites of Punjab. The three morphotypes of *S. nigrum* differ in morphological as well as cytological traits and named as shiny bluish-black (diploid)- SN_1, orange-red (tetraploid)- SN_2 and purplish-black (hexaploid)- SN_3. The three morphotypes of *Eclipta alba i.e.* Prostrate, Semi-erect and Erect have also been reported and named as EA_1, EA_2 and EA_3, respectively. Four populations of *Achyranthes aspera* collected from different localities of Punjab differs in morphological features were named as AA_1, AA_2, AA_3 and AA_4. Two forms of *S. acuta* has also been established, and named as SA_1 and SA_2.

Test Organisms

Two Gram positive (*Enterococcus faecalis* MTCC 439 and *Staphylococcus aureus* ATCC 25923) and two Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853) bacteria were used for the evaluation of antibacterial activity. The extracts, both aqueous and ethanol of the selected plant species have been studied for their activity against different bacteria using agar well diffusion method.

Preparation of Extracts

Aqueous

Twenty (20) grams of each whole plant powder was added to 250ml conical flasks containing 100 ml of distilled water and mixed well by shaking it thoroughly. The flasks were then kept on an orbital shaker at room temperature for 24hrs. The mixtures were filtered through a muslin cloth followed by Whatman filter paper no.1. The final extracts were stored in vials at 4°C.

Ethanol

Ten (10) grams of whole plant powder of each sample was extracted in 130ml of ethanol. The extraction was carried out in Soxhlet apparatus at 50°C to 60°C. After the extraction is completed, the extract was allowed to evaporate at room temperature till it reaches 1/3rd of the original volume. Then it was stored at 4°C in a screw cap bottles.

Preparation of Medium

The liquid bacterial growth medium, *i.e.,* Muller Hinton Broth (MHB) was prepared by mixing 21gm of MHB powder in one liter of distilled water followed by autoclaving it at 121°C for 15 minutes. Solid bacterial growth medium, *i.e.*, Muller Hinton Agar (MHA) was prepared by adding bacteriological grade agar to MHB medium at final concentration of 2% w/v followed by autoclaving it at 121°C for 15 minutes. For preparing plates, MHA medium was poured on to sterile Petri plates under aseptic conditions and kept at room temperature for solidification.

Preparation of Inoculums

Streaked bacterial cultures from MHA plates were inoculated into the freshly prepared 5ml MHB medium in 50ml tube and incubated for 15 hours at 37°C, 150rpm. The optical density (OD) of 15 hour grown culture was adjusted to 0.1 at 600nm using fresh growth medium as diluents. The properly diluted culture further used to spread it on MHA plates for performing agar-well diffusion assay.

Agar Well Diffusion Method

Aliquots of 100µl from 0.1OD adjusted cultures were spread uniformly on MHA plates for the agar well diffusion assay. The wells were made in the plates using sterile 200µl pipette tips. Doses of (50µl, 100µl and 150µl) each plant extract were poured into the wells using micropipettes. Pure solvents (aqueous or ethanol) employed in the extraction process were used as a solvent (negative) control and 4µg of rifampicin (2µl of 2mg/ml stock) was used as positive control in each plate. The plates were incubated overnight at 37°C. After 16 hours of incubation, the plates were observed for the appearance of zones of inhibition (ZOI). The ZOI were measured in millimeters using Antibiotic zone scale (HiMedia).

Statistical Analysis

The assays were independently repeated three times with 150µl dose of extracts against each bacterium. The average values for ZOI were calculated and represented as mean± standard error of mean (SEM).

Results and Discussion

The extracts (50µl) of only two plant species *i.e. S. acuta* (SA_2 against *E. coli*) and *B. diffusa* (against *E. faecalis*) were found effective. The 100µl volume of extract was better than the 50µl but was not as effective as 150µl against all the bacteria. Therefore, to compare the antibacterial activity of different plant extracts, 150µl volume of the extract was selected for further experimentation. Only the ethanol extracts have shown antibacterial activity against tested bacteria. The zone of inhibition (ZOI) of pure solvent (ethanol) was ≤10mm. The zones of inhibition measuring 10mm or less has been considered the activity of the solvent and not recorded as the activity of the extract. However, the ZOI reported do not exclude the ZOI formed by ethanol alone. The antibacterial activity measurements of the extracts from different plants were independently repeated at least three times and the average ZOI were expressed with Standard Error of Mean (±SEM) in Table 1. The observations pertaining to antibacterial activity of the ethanol extracts of different plant species are discussed as follows:

Solanum nigrum

The 150µl extracts of all the three cytomorphotypes has shown activity against all the bacterial strains. The SN_1 type has formed maximum ZOI for *S. aureus* (14±2.9mm). Maximum ZOI has also been observed both for *S. aureus* and *E. faecalis* with SN_2 extract. The extract SN_3 appeared to be best amongst all the three as it gave the largest ZOI against all bacteria tested (Table 1; Figs. 1a-d). The whole plant ethanol and methanol extracts of stem and berries of *Solanum nigrum* had shown antibacterial activity (16).

The ethanol extracts recorded inhibitory zones against *P. aeruginosa* (19.1± 0.32mm) and *E. coli* (15.1±0.2mm). However, in present study, the ethanol whole plant extract of SN_3 has shown almost similar activity against *P. aeruginosa* (18±1.2mm) and *E. coli* (15±3.6mm). Their findings for antibacterial activity are comparable to the present study. Yogananth *et al.* (17) used ethanol, hexane and chloroform extracts of stem and leaf of *S. nigrum* against eight bacterial strains. A 100µl dose of chloroform extract of stem and leaf showed maximum activity against *V. cholerae*, *P. aeruginosa* and *E. faecalis*. The zone of inhibitions, 4.1mm and 2.7mm for stem and leaf, respectively were recorded against *E. faecalis.* In present study, all the three forms have shown activity against *E. faecalis* and a maximum zone of inhibition has also been observed only for this bacterium with SN_3 at 150µl. Thus observations made during the present study are similar to their report. Similarly in another study, ethanol root extract of S. nigrum was tested against *S. aureus, E. coli and P. aeruginosa* and maximum inhibition was against *S. aureus* (16mm) (18). However, the whole plant extract of *S. nigrum* (SN_3) has been found most effective against *S. aureus* with 18±1.5mm inhibitory zone in present study.

Eclipta alba

Three morphotypes of *Eclipta alba* (EA_1, EA_2 and EA_3) have been studied for their antibacterial activity. Ethanol extracts EA_1 and EA_2 (150µl each) had shown activity against *E. faecalis* with ZOI 15±1.5mm and 15±0.9mm, respectively. The EA_3 extract had shown comparable activity against all tested bacteria (Table 1; Figs. 2a-d). The extracts of three morphotypes were prepared in methanol, acetone and water. But none of the extract had shown activity against *P. aeruginosa* (19). The leaf extracts of erect type had shown maximum activity against all the bacteria under investigation except *B. subtilis*. However, during the present study, all extracts of *E. alba* were found active against *P. aeruginosa*. In another study, the aqueous extracts of *E. alba* leaves, stem and flower were tested against some pathogens including *E. coli, E. faecalis* and *S. aureus* (20). Only the aqueous stem extract had shown inhibition (10mm) against *E. faecalis*. But in present study, three morphotypes have activity against *E. faecalis*. In another study, ethanol whole plant extracts of *E. alba* had shown ZOI of 15mm, 13mm and 11mm against *S. aureus*, *E. coli* and *P. aeruginosa*, respectively (21). Similarly in present study, ethanol whole plant extracts of *E. alba* have shown comparable activity against these three bacteria.

Achyranthes aspera

All the extracts (AA_1, AA_2, AA_3 and AA_4) at 150µl volume exhibited zones of inhibition between 11±1.0mm to 22±2.2mm. The ZOI 16±3.5mm, 13±1.5mm, 17±4.4mm and 18±3.8mm had measured against *E. faecalis, S. aureus*, *E. coli* and *P. aeruginosa*, respectively with AA_1. Similarly, for the extract AA_2, inhibitory zones of 19±2.0mm, 15±2.0mm, 18±4.6mm and 21±2.7mm were observed against *E. faecalis, S. aureus*, *E. coli* and *P. aeruginosa*, respectively. The extract AA_3 was also found to be active with 22±2.2mm, 12±1.2mm, 15±3.6mm and 19±1.9mm of ZOI against *E. faecalis*, *S. aureus, E. coli* and *P. aeruginosa* correspondingly. The inhibitory zones of the extract AA_4 were 14±3.0mm, 18±3.7mm, 11±1.0mm and 18±0.6mm against *E. faecalis*, *S. aureus, E. coli* and *P. aeruginosa*, respectively (Table 1; Figs. 3a-h). The extracts of types AA_2 and AA_3 performed well against *P. aeruginosa* and *E. faecalis*, respectively.

Out of aqueous and ethanol leaf extracts of *Achyranthes aspera* only the ethanol extract was active against *B. subtilis*, *P. aeruginosa* and *S. aureus* (22). These findings have corroborated the present study where only the ethanol extracts have shown activity. In another study, aqueous, ethanol, methanol and chloroform leaf extracts of *A. aspera* were screened against *B. subtilis*, *E. coli, P. aeruginosa* and *S. aureus* through agar well diffusion method (23). A 100µl volume of ethanol extract had formed 31mm, 40mm and 24mm of ZOIs against *E. coli, P. aeruginosa* and *S. aureus*. During the present study, whole plant ethanol extract (AA_2) at 150µl has formed zones of inhibition 18±4.6mm and 21±2.7mm against *E. coli* and *P. aeruginosa,* respectively whereas, AA_4 against *S. aureus* (18±3.7mm). Thus, their findings are in a similar trend to the present study. Similarly, a leaf extract (80µl) of *A. aspera* had shown zone of inhibition (17mm) against *E. faecalis* (24). However, in present study, AA_3 has been best among all the extracts and formed 22±2.2mm ZOI against *E. faecalis* at 150µl. In general, the ethanol extracts from all four populations (AA_1 to AA_4) of *A. aspera* had displayed activity against *P. aeruginosa*.

Sida acuta

A 150µl ethanol extract of SA_1 and SA_2 has been active against all four bacteria. The extract SA_1 showed maximum inhibition (19±2.9mm) against *S. aureus* whereas SA_2 has formed maximum inhibitory zone against *E. faecalis* (18±2.0mm). (Table 1; Figs. 4a-d). According to Stanley *et al.*, ethanol leaf extracts of *Sida acuta* had zones of inhibition of 16mm, 14mm and 18mm against *E. coli, P. aeruginosa* and *S. aureus*, respectively (25). However, in present investigation, ethanol extract of SA_1 inhibited the growth of *S. aureus* with ZOI 19±2.9mm. Similarly, SA_2 showed activity against *E. coli* (15±0.0mm) and *P. aeruginosa* (17±3.2mm). Their study has supported the present findings. Ethanol extracts of 14 plant species including *Sida acuta* possess activity against *B. cereus*, *P. aeruginosa, S. aureus* and *E. faecalis* (26). Their observations have corroborated the present study.

Adhatoda vasica

The ethanol extract (150µl) of *Adhatoda vasica* has formed a maximum ZOI (24±1.7mm) against *S. aureus* followed by *E. coli* (22±2.1mm), *E. faecalis* (18±1.5mm) and minimum 15±1.2mm against *P. aeruginosa* (Table 1; Figs. 5a-d). Josephin and Selva tested the activity of *Adhatoda vasica* extracts prepared in methanol, ethanol, acetone, chloroform, diethyl either and water on *S. aureus, S. pyrogens, P. vulgaris, E. coli, P. aeruginosa* and *K.*

pneumoniae (27). Maximum activity was reported for *S. aureus* which is in consonance with the present study. Likewise, Pradhan *et al.* (28) studied the antibacterial activity of hexane, methanol and aqueous extracts of *A. vasica* and reported ZOI against *E. coli* (12±0.71mm), *E. faecalis* (13±0.56mm), *P. aeruginosa* (9±0.47mm) and *S. aureus* (11±0.69mm) with aqueous extract. However, during the present study, only the ethanol extract has shown 22±2.1mm, 18±1.5mm, 15±1.2mm and 24±1.7mm ZOI against *E. coli, E. faecalis, P. aeruginosa* and *S. aureus*, respectively. The variation in activity *i.e.* on higher side in the present study may be due to the use of different solvent.

Boerhavia diffusa

Boerhavia diffusa ethanol extract (150µl) has been effective against all the four bacteria under investigation. Maximum ZOI has been measured against *P. aeruginosa* (22±0.9mm) followed by *E. coli* (20±2.0mm), *E. faecalis* (18±2.8mm) and 17±1.8mm against *S. aureus* (Table 1; Figs. 6a-d). The ethanol extract of *B. diffusa* leaves had formed zone of inhibition 9mm against *E. coli* and 11mm each against *P. aeruginosa* and *S. aureus* (29). During present study, maximum zone of inhibition has been recorded against *P. aeruginosa*. The decoction of *B. diffusa* roots was studied for activity against different bacteria including *E. faecalis*, *S. aureus*, *P. aeruginosa* and *E. coli* (30). The zones of inhibition were 12mm, 17mm and 22mm for *E. coli*, *E. faecalis* and *P. aeruginosa*, respectively whereas, *S. aureus* was resistant. However, during present investigation, the whole plant ethanol extract has shown activity against all these four bacteria.

Withania somnifera

Withania somnifera ethanol extract (150µl) has measured the inhibition zones ranging from 12±2.0mm (*P. aeruginosa*) to 18±2.1mm (*E. coli*). The remaining two bacteria, *S. aureus* and *E. faecalis* have ZOI measuring 16±1.3mm and 14±1.3mm, respectively (Table 1; Figs. 7a-d). Gauniyal and Teotia reported 25mm ZOI against *E. faecalis* with ethanol root extract of *W. somnifera* (31). It shows that activity of root extract of this species is more than the whole plant extract of present study. However, amount and concentration of the extract has to be considered to reach at the conclusion. In another study, the antibacterial study of aqueous, methanol and ethanol extracts of stem, leaves, roots and fruits of *W. somnifera* were conducted against different bacteria (32). The ethanol leaf extract showed maximum activity against *S. aureus* (22.4mm) followed by *P. aeruginosa* (20.2mm) and *E. coli* (11.1mm).The antibacterial activity of different plant species have also been presented in graphics (Figs. 8-12). The variation in activity reported in several studies likely because of the use of diverse solvents, plant parts and doses.

Rifampicin (4µg) and Ethanol (50µl) was included as positive and negative controls, respectively. The positive control produced ZOI of 12±0.7, 29±0.6, 15±0.3 and 17±0.3 against *E. faecalis*, *S. aureus, E. coli* and *P. aeruginosa*, respectively. Similarly, the negative control has produced has ZOI ≤10mm. The data were represented as mean of three independent repeated values with Standard error of mean (±SEM).

Table 1: Antibacterial activity of different plant species

Plant species	Inhibition Zone ±SEM (in mm)			
	E. faecalis	***S. aureus***	***E. coli***	***P. aeruginosa***
Solanum nigrum				
SN_1	12±1.3	14±2.9	12±2.3	10±0.3
SN_2	16±2.0	16±2.6	11±1.3	13±1.3
SN_3	21±2.2	18±1.5	15±3.6	18±1.2
Eclipta alba				
EA_1	15±1.5	13±0.9	12±0.7	11±0.6
EA_2	15±0.9	13±0.6	11±1.3	13±1.2
EA_3	14±0.0	13±0.9	13±1.5	13±0.3
Achyranthes aspera				
AA_1	16±3.5	13±1.5	17±4.4	18±3.8
AA_2	19±2.0	15±2.0	18±4.6	21±2.7
AA_3	22±2.2	12±1.2	15±3.6	19±1.9
AA_4	14±3.0	18±3.7	11±1.0	18±0.6
Sida acuta				
SA_1	18±1.7	19±2.9	14±0.0	16±2.4
SA_2	18±2.0	17±1.9	15±0.0	17±3.2
Adhatoda vasica	18±1.5	24±1.7	22±2.1	15±1.2
Boerhavia diffusa	18±2.8	17±1.8	20±2.0	22±0.9
Withania somnifera	14±1.3	16±1.3	18±2.1	12±2.0

Conclusion

The results obtained from the present investigation suggest that the whole plant ethanol extracts of *Solanum nigrum*, *Eclipta alba, Achyranthes aspera, Sida acuta, Adhatoda vasica, Boerhavia diffusa* and *Withania somnifera* have activity against reference strains. *Adhatoda vasica* has shown maximum ZOI of 24±1.7mm and 22±2.1mm against *S. aureus* and *E. coli*, respectively. The type AA_3 of *A. aspera* (22±2.2mm) and SN_3 of *S. nigrum* (21±2.2mm) have shown best zones against *E. faecalis*. Similarly, *B. diffusa* and *A. aspera* (AA_2) has recorded maximum ZOI against *P. aeruginosa*. The plant species under investigation contain compounds with antibacterial properties. The activity in plant extracts may be due to the presence of antibacterial compounds which can be further studied for their therapeutic use as these plants are easily available and economically affordable. The use of additional or alternative plant species in medicinal preparations may reduce the pressure on the species which are already used for this purpose.

Acknowledgements

We are thankful to the Chairperson, Department of Botany, Panjab University, Chandigarh for providing necessary facilities during this research work. Authors are also grateful to the SERB (DST), New Delhi for providing financial assistance. We are also thankful to Dr. Samer Singh, Department of Microbial Biotechnology, Panjab University, Chandigarh for scientific discussion and help in the experimental work.

Fig. 8: Antibacterial activity of cytomorphotypes of *S. nigrum*

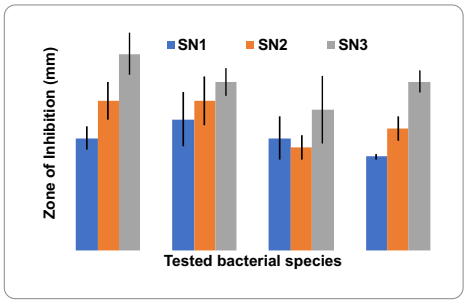


Fig. 9: Antibacterial activity of morphotypes of *E. alba*

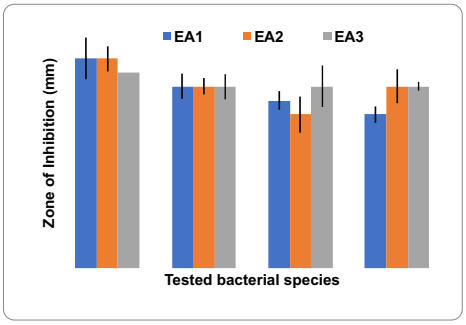


Fig. 10: Antibacterial activity of different populations of *A. aspera*

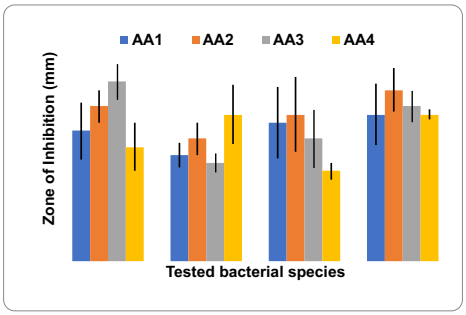


Fig. 11: Antibacterial activity of two populations of *S.acuta*

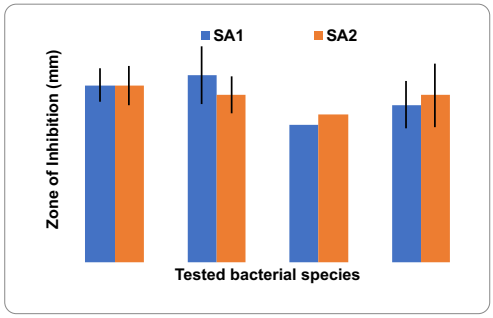
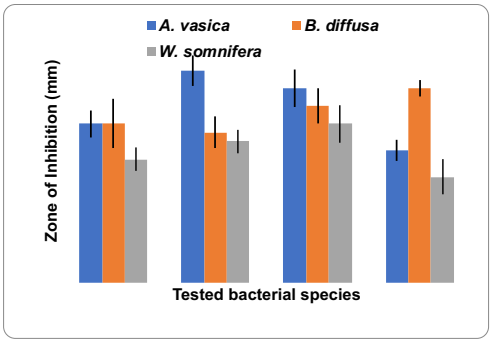
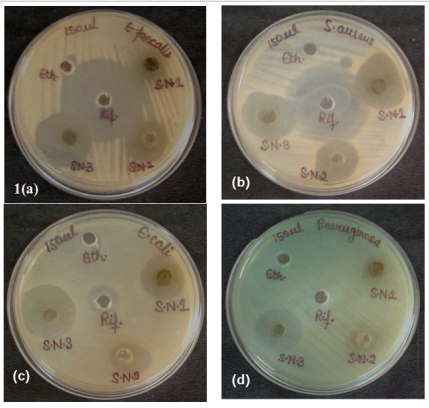


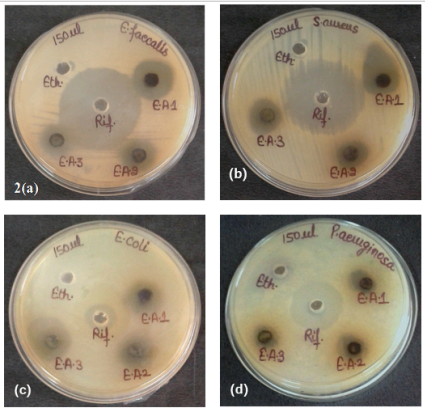
Fig. 12: Antibacterial activity of *A. vasica, B. diffusa* and *W. somnifera*



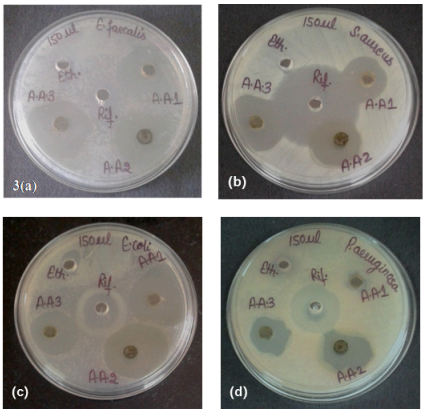
Figs. 1(a-d): Antibacterial activity of three cytomorphotypes of *Solanum nigrum*



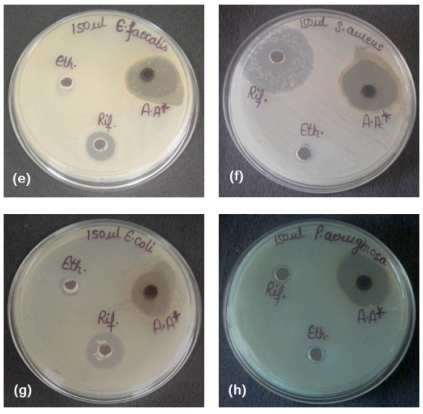
Figs. 2 (a-d): Antibacterial activity of three morphotypes of *Eclipta alba*



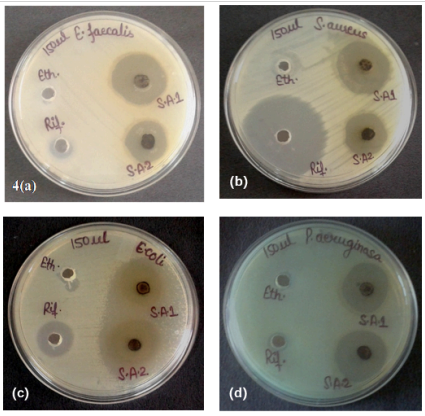
Figs. 3 (a-d): Antibacterial activity of *Achyranthes aspera* plant populations (AA_1 - AA_3)



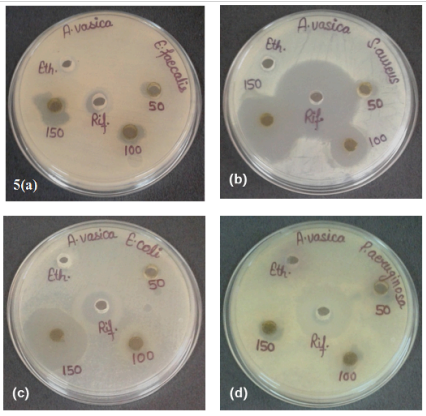
Figs. 3 (e-h): Antibacterial activity of *Achyranthes aspera* plant population (AA_4)



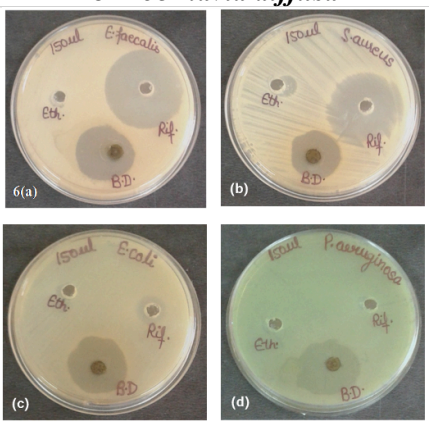
Figs. 4(a-d): Antibacterial activity of two populations of *Sida acuta*



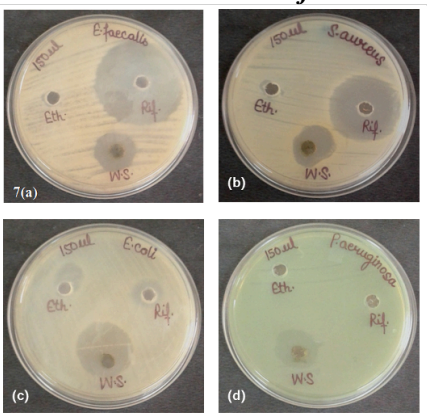
Figs. 5(a-d): Antibacterial activity of *Adhatoda vasica*



Figs. 6(a-d): Antibacterial activity of *Boerhavia diffusa*



Figs. 7(a-d): Antibacterial activity of *Withania somnifera*



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