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Biochemical and Antibacterial Studies of *Hemidesmus Indicus* Root Extracts against selected Multi Drug Resistance Human Pathogens

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ABSTRACT

The present study undertaken to evaluate in vitro biochemical analysis and antibacterial activity of Methanol,Ethanol,Chloroform, Pet. Ether and Aqueous extracts of *Hemidesmus indicus* root extracts against selected multidrug Resistance Human Pathogens. The selected bacterial strains were *Escherichia coli*,(ATCC 25922), *Pseudomonas aeruginosa*(ATCC 27853), *Klebsiella pneumoniae (ATCC-13883) Staphylococcus aureus* (ATCC 25923) ,*Salmonella typhi*,(MTCC-734).,*Micrococcus luteus* (ATCC-9341), *Vibrio cholerae* (MTCC-756).The result proved to be most effectivity of Petrolium Ether extract against *S. typhi* and *S.aureus* Multidrug Resistance Human Pathogens.(21mm and 20mm resp).by Well Diffusion Method. Methanol and Ethanol extracts showed satisfactory Zone of inhibition against V. *cholerae* and *P.aeruginosa.* (14mm).The method used to test antibacterial activity of root extracts were Well Diffusion and Disc Diffusion Method. The result obtained from present investigation was proved for the presence of various biochemicals such as Alkaloid, Flavonoid, Phenol and Cardiac glycosides. The Phenolic compounds which were obtained in root extracts might be responsible for potential antibacterial activity.

Key Words :- *Hemidesmus indicus* root extracts, Multi Drug Resistance Human Pathogens, Streptomycin, Tetracycline,Well Diffusion and Disc Diffusion.

Introduction:-

Botanical Description :- It is thin slender, twinning perennial herb showed laticiferous naturewhen plucked its leaves or stem part. It is also prostrate or semi-erect herb. (Vijaya kumara K and Nishteswar K. 2012; Rajan *et al.*,2011).

Plate No.1. Roots and Twig of Hemidesmus indicus plant.

H. indicus are spindle shaped and woody with pleasant aroma. The stem is also slender, terete and shows bulgy at the nodes. The leaves are petiolated, opposite, elliptic-oblong to linear-lanceolate. Stem of *H. indicus* is flexible and bluish green in colour. *H. indicus* known as Anantmuli (in Marathi) belongs to (Fam. Asclepiadaceae). *Hemidesmus indicus* (Linn.) R. Br. commonly known as Indian sarsaparilla or Anantmool found all over India, from upper region of plains of Ganga to Assam, throughout Central, Western and Southern India upto an elevation of 600 m. (The Ayurvedic Pharmacopoeia of India.,1989 ; Anonymous, The Wealth of India .,2005).

Biomolecules of *H. indicus* :-

The biomolecules which are present in roots of *H.indicus* were repoted to possess 2-hydroxy-4-methoxy benzaldehyde, rutin, Lupeol Octacosanoate, hexatriacontane hemidesmine, hemidine, hemidesine. (Ayyangar M, and Ignacimuthu S.,2005).

Medicinal property:-

The roots are used as antipyretic, anticancerous, antidiarrhoeal, diuretic, diaphorectic, leucorrhoea and also used in urinary disorders.It was proved as it also act against certain skin diseases like leprosy, leucoderma. Methanolic extract of *H. indicus* roots showed remarkable anti-cancerious activity against MCF and breast cancer cell lines. Root extracts tested on hepatoma HepG2 cell lines showed good results against this cell lines. It is also used against snake poison as drug in snake bite, It act as a blood purifier. The roots of *H. indicus* also have potential to treat certain diseases such as Syphilis, Elephanthiasis,Kidney and Urinary Disorders.(Vijaya kumara K and Nishteswar K.,2012). The present study was performed to analyze Antibacterial activity of root extracts of *H. indicus* against MDR human pathogens.

Method and Material :-

Biochemical analysis was performed by using Practical Pharmacognosy book by Khandelwal.

Hemidesmus indicus plant roots were collected from cultivated own garden during the summer. The identification and authentification was performed by Prof and Head of Dept. of Botany, Dr. Dhabe A.S. Dr. BAM University Aurangabad. (Accession No-44444) Extraction of plant materials Prior to extraction, plant parts materials were cleaned 2-3 times with running water and once with sterilized distilled water then surface sterilized with 1%mercuric chloride. The materials were dried under shade at room temperature (25-35^{o}C). The air-dried plant materials roots again dried properly in Trey Dryer to avoid fungal infection.The roots were grounded into coarse powder form through electric blender. Two hundred grams of each air-dried plant material were soaked in 80% Methanol ,80% Ethanol, 60-80% Chloroform and and 80% Petrolium Ether used as organic solvents. The Soxhlet assembly was used for organic solvent extractions. The same procedure used for maceration with few changes. 200 gm of root sample mixed with 500ml of distilled water and kept it undisrupted for about 24 hours. It filtered with Whattman filter paper -1.

The extract's solutions were evaporated to dryness under reduced pressure at temperature of 45°C using a vacuum pump with the rotary evaporator. The paste obtained after rotary evaporation contained some water content evaporated for further drying on Hot Water Bath at 60 hour. The thick pastes obtained for each solvent extracts were stored in sterile vials at 4°C until further use.

Test microorganisms and microbial culture:- Test microorganisms and microbial culture of seven bacterial strains which are Multidrug resistance against antibiotics were collected on agar slant from Clinical Microbiology Department, Ghati Hospital Aurangabad. The selected strains were *Escherchia coli, Klebsiella pneumoniae,Pseudomonas aeruginosa,Staphylococcus aureus, Micrococcus luteus,Vibrio cholerae and Salmonella typhi*.Growth media used for testing the antibacterial assay was Nutrient agar media, which is best growth media for all kinds of bacteria.

Preparation Of Nutrient Agar :-The Media was composed of Beef extract 3.0g, Agar 20.0g and Peptone 5.0g. NaCl salt 3.0g, in half liter of distilled water. After complete dissolution, the final volume of the media was raised to 1000ml by adding more distilled water. The media was boiled using a hot plate. The PH was adjusted to 7.0 at 25 ºC, using 0.1M NaOH and 0.1M HCl. The needed media and all glassware were sterilized through autoclaving at 15psi at 121 for 20 minutes.

Antibacterial Activity :-The 25ml of nutrient agar was plated in Petri dishes and allowed to solidify for about 60 minutes. The antibacterial activity tested by using two methods .

Disc Diffusion Method ;- Antibacterial activity of tested plants parts was carried out by the disc diffusion method. First, the different extracts of plant parts tested were dissolved in DMSO at a concentration of 100 mg/mL and filtered through 0.45 µm sterile filter membranes. Then, 100 µL of bacterial inoculums containing 108 CFU/mL were spread over plates containing Nutrient agar, and discs (6 mm in diameter) impregnated with 10 µL of the extracts solutions (1 mg/disc) were placed on the surface of the media. Two control discs were used containing DMSO and Tetracyclin (10 µg/ disc) (for Disc diffusion) as negative and positive controls, respectively. The plates were incubated for 24 h at 37 °C, and the experiments were performed in duplicate. The diameters of inhibition zones were measured and antibacterial activity was considered for diameters of inhibition zone greater than 12mm to 18 mm. (Ahameethunisa AR and Hoper W. (2010); Mackie TJ and McCartney JE 1989).

Well Diffusion Method :- Antimicrobial assay of extracts of root plants was performed by agar well diffusion method on Nutrient Agar plates. The test organisms were inoculated in Nutrient Agar and incubated overnight at 37∘ C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5 × 108 CFU/ml. NA plate was lawn cultured with standardized microbial culture broth. Plant extracts of 50 mg/ml concentration were prepared in Dimethyl Sulfoxide (DMSO). Six wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well was filled with 50 ml extracts from different extracts of root. The positive control used for positive control was Streptomycin and negative control (DMSO). It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37∘ C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm.

Biochemical Analysis :- Qualitative Biochemical analysis was performed for detection of phytochemicals present in *H. indicus* roots .The result obtained were described as given.

Plate No.2. Rotary vacuum Evaporator and Soxhlet Apparatus

Result and Discussion:-

Table No-1. Biochemical Activity of Hemidesmus indicus extracts root

Chemical composition	**ME**	**EE**	**PE**	**CE**	**AQ**
Test for Carbohydrates.					
a. Molischs Test.	--	--	--	--	--
b. Fehlings Test.	--	--	--	--	--
c. Benedicts test	--	--	--	--	--
d. Barfoed test	--	--	--	--	--
e. pentose sugar.	--	--	--	--	--
f. hexose sugar.	--	--	--	--	--
Test for mucilaes	--	--	--	--	--
Test for Proteins.					
a.Biuret test.	++	++	++	++	++
b. Millons test	++	++	++	++	++
Test for Alkaloids					
a.Dragendorffs test	++	+++	++	++	++
b. Mayers Test	++	++	+++	++	++
c. Hagers reagent	++	++	++	++	++
d. Wagners test	+++	++	++	++	+++
Test for Flavonoids					
a.Shinoda Test	++	++	++	++	++
b. Sulphuric Acid	+++	++	++	++	++
c. Lead Acetate Test	++	++	++	++	++
d.Zinc powder Test	++	+++	++	++	++
Test for phenols					
a.5%$FeCl_3$ Sol^n	++	++	++	++	++
b. Lead acetate sol^n	++	++	++	++	++
c. Gelatin Sol^n	++	++	++	++	++
d.dil. Iodine	++	++	++	++	++
e. dil. HNO_3	++	++	++	++	++

Test for Saponin					
Foam test	++	++	--	--	++
Test for Amino acids					
a.Ninhydrin test	++	++	++	++	++
Test for Tryosine	--	--	--	--	--
Test for Tryptophan	--	--	--	--	--
Test for Terpenoids	++	++	++	++	++
Test for Tannins	--	--	--	--	--
Test for Steroids	--	--	--	--	--
Test for cardiac glycosides	++	++	--	++	++

Carbohydrates,Mucillage,Tannin,Tryosine,Tannin and steroids were absent But there was presence of alkaloids,cardiac glycosides,proteins,flavonoids, terpenoids and phenolic compounds.

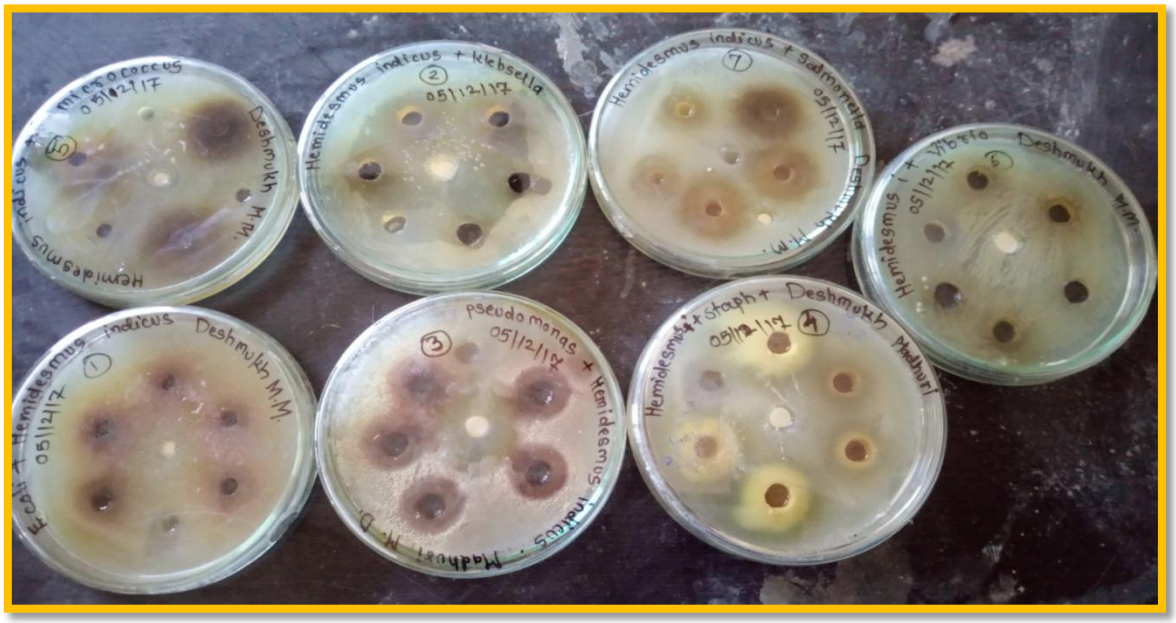
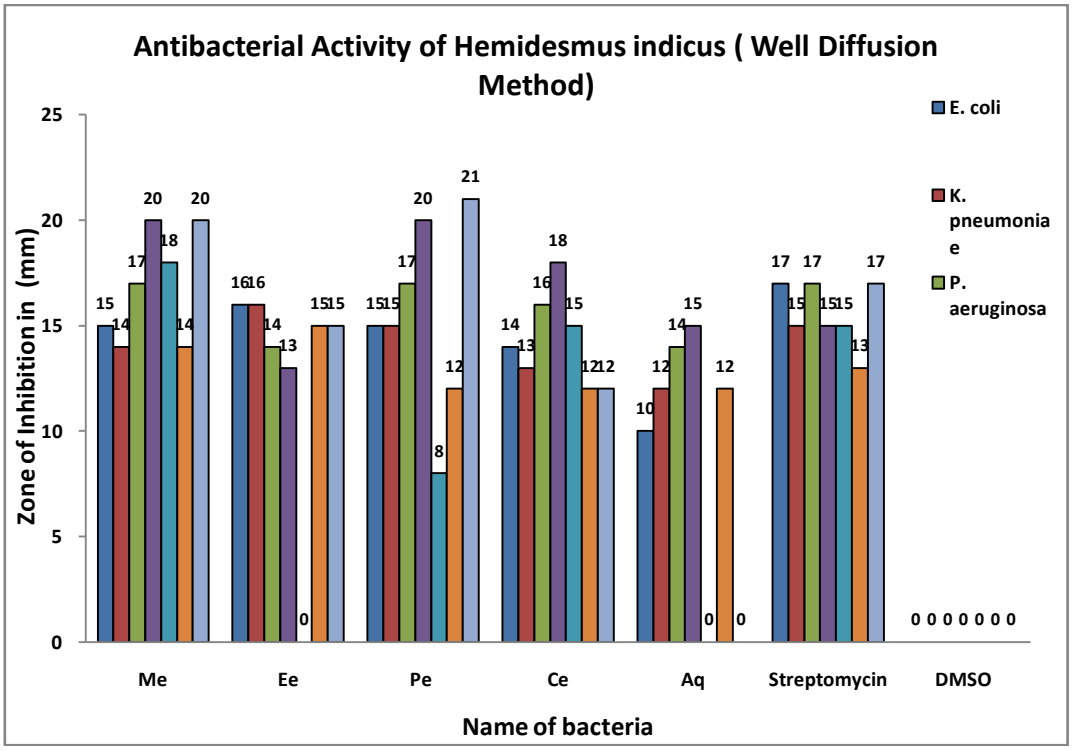


Plate No. 3.Antibacterial Activity Of *H. indicus* extracts against (MDR) human pathogens by using Well Diffusion Method.

Sr. No.	**Name of bacteria**	**Zone of Inhibition in (mm)**						
		Me	**Ee**	**Pe**	**Ce**	**Aq**	**Streptomycin**	**DMSO**
1	*E. coli*	15	**16**	15	14	10	17	Nil
2	*K. pneumoniae*	14	**16**	15	13	12	15	Nil
3	*P. aeruginosa*	**17**	14	**17**	16	14	17	Nil
4	*S. aureus*	**20**	13	**20**	18	15	15	Nil
5	*M. luteus*	**18**	nil	08	15	nil	15	Nil
6	*V. cholerae*	14	**15**	12	12	12	13	Nil
7	*S. typhi*	20	15	**21**	12	nil	17	Nil

The potent effect of Methanol extract was showed on *S.aureus* and *S.typhi* (20mm) with highest ZOI amonag all tested bacterial strains.The least effect showed on *V. cholerae* and *K.pneumoniae* (14mm) with small inhibition zone. Ethanol extract of *H. indicus* proved effective against E.coli and *K.pneumoniae* (16mm) but did not showed any activity against *M. luteus* strain.*Staphylococcus aureus* is most susceptible to Petrolium Ether extract.(20mm) inhibition Zone. It was followed by *P.aeruginosa* (17mm),but less or almost nill activity against *M.luteus.(*8mm). The chloroform extract showed highest inhibitory potential against *S.aureus* but less against *V. cholerae* and *S. typhi* bacteria.Aqueous extract also showed activity against S.aureus (15mm) but it was not active against *M.luteus* and *S.typhi.* (Mahon. C and Manuselis. G.,1995 Gulluce M, 2007)

Graph No-1. Graphical Representation of Antibacterial Activity (Well Diffusion)



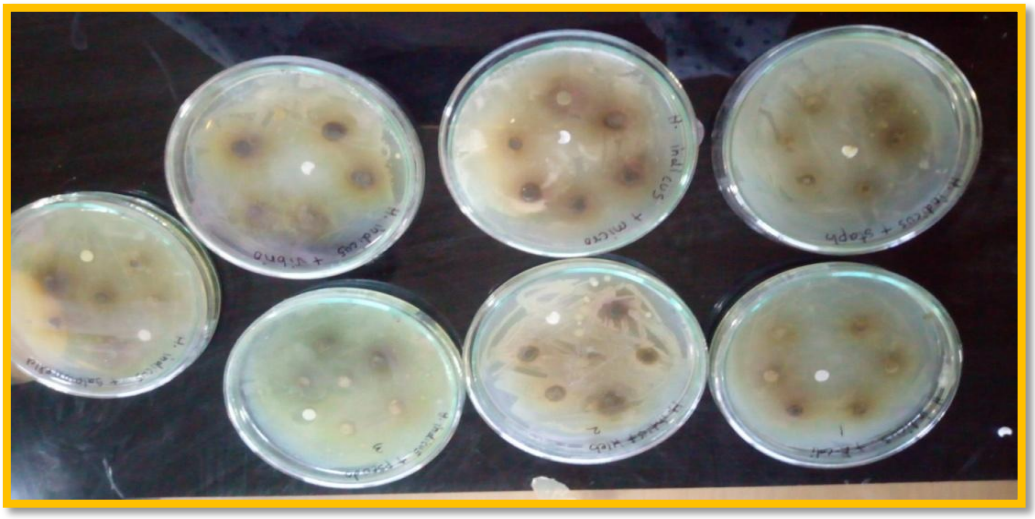


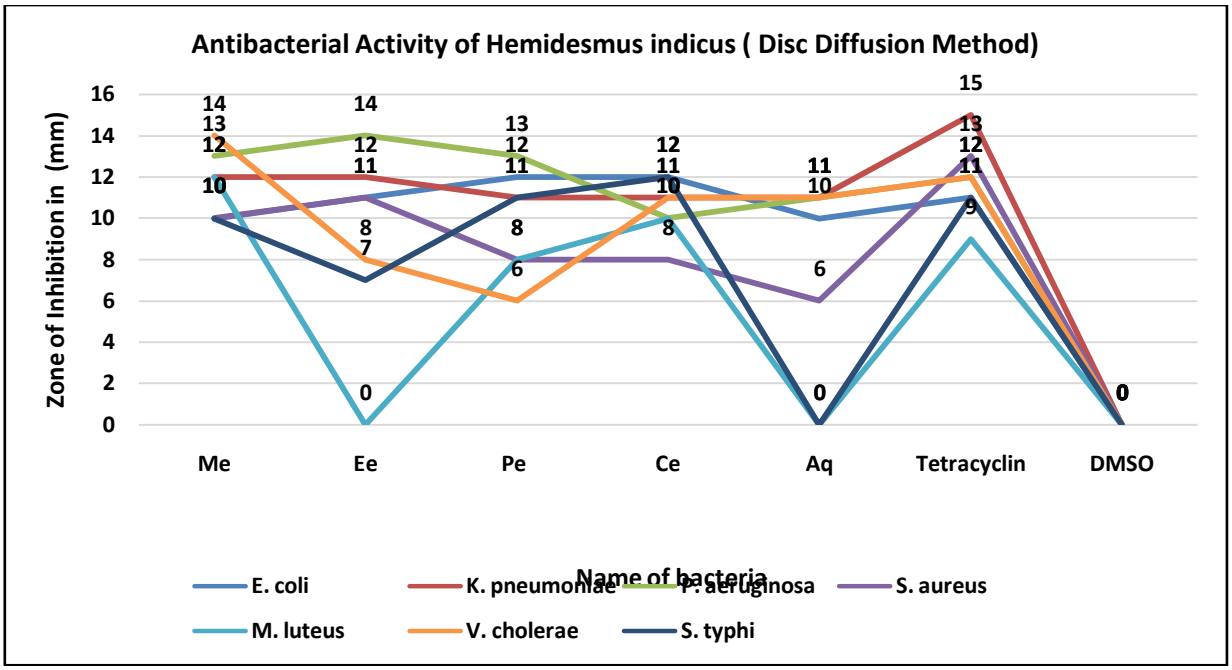
Plate No. 4.Antibacterial Activity Of *H. indicus* extracts against (MDR) human pathogens by using Disc Diffusion Method

Table No. – 3. Antibacterial Activity of Hemidesmus indicus (Disc Diffusion Method).

Sr. No.	Name of bacteria	Zone of Inhibition in (mm)						
		Me	Ee	Pe	Ce	Aq	Tetracyclin	DMSO
1	*E. coli*	10	11	12	12	10	11	Nil
2	*K. pneumoniae*	**12**	**12**	11	11	11	15	Nil
3	*P. aeruginosa*	13	**14**	13	10	11	12	Nil
4	*S. aureus*	10	**11**	08	08	06	13	Nil
5	*M. luteus*	**12**	nil	08	10	nil	09	Nil
6	*V. cholerae*	**14**	08	06	11	11	12	Nil
7	*S. typhi*	10	07	11	**12**	nil	11	Nil

In Disc Diffusion Methanol extract showed highest Zone of inhibition against *V.cholerae* and least on *E. coli* and *S.typhi.*(10mm). *P.aeruginosa* was more susceptible to ethanol extract (14mm) ZOI. But Ethanol extract does not showed any activity against *M.luteus* strain.Pet.Ether extract was most active against *P.aeruginosa* (13mm) and least on *V.cholerae.* Chloroform extract showed moderate activity against *E.coli* and *S. typhi.*(12mm) and very less against *S.aureus* strain of MDR pathogen.*M.luteus* and *S.typhi* strains were resistance to aqueous extract of *H. indicus* root.It does not showed considerable activity against selected pathogens.

Graph No.2. Graphical Representation of Antibacterial Activity (Disc Diffusion)



Conclusion:-

In this study, *H. indicus* root extracts were assessed for their antibacterial activity showed satisfactory results for their antibacterial activities. The results indicated that Methanolic extracts of *H. indicus* have more potential antibacterial effects on bacterial strains tested, This was confirmed by determination of diameters of inhibition zones.It indicated that these plants have potentially antibacterial properties and could be used in the development of novel antibacterial agents.Other investigations are necessary to be done on a wide range of bacterial and viral infection to assess the spectrum of such plant parts extracts. Moreover, other parts of the examined plants are also needed to be assessed for their antibacterial activity. Further analysis on isolation and purification,characterization and chemical structure determination of active compounds from these extracts are necessary for their utilization for treatment of infections diseases caused by pathogenic and often multi-drug resistant bacteria.

Conflict Of Interest :- Nill.

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