ijpbs 2020; doi 10.22376/ijpbs.2020.11.1.b84-89

International Journal of Pharma and Bio Sciences

Original Research Article

Medicinal plants for better Natural therapy

Antibacterial Activity Of *Hemidesmus indicus* (L) Root Used By Tribes Of Kundam, Block District Jabalpur, Madhya Pradesh, (India).

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Abstract: The present study was aimed to examine the antibacterial activity of *Hemidesmus indicus* (L.). Root extract of this plant was screened against five human pathogenic bacteria in an *in vitro* condition. Phytochemical analysis of the root extracts indicated the presence of alkaloids, glycosides, carbohydrates, steroids, polyphenols, saponins and terpenoids. The antibacterial activity of the chloroform and aqueous extracts of root were tested by using well diffusion method and the zone of inhibition produced by extract in a dose of 50, 100 and 150 mg/ml, respectively against selected bacterial strains the overall results were compared with the standard drug ciprofloxacin (25µg/ml). Chloroform extract showed significant activity against all the tested bacteria. The biological efficiency was observed more prononced in chloroform extract in comparison to the aqueous extract against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Vibrio cholera* with zones of growth inhibition 16.33 mm, 12.66 mm, 12.00 mm, 9.00 mm and 3.00 mm, respectively. The present study mainly focuses to explore the antibacterial properties of the root extracts of plant that are generally used as traditional medicines. The overall results showed remarkable antibacterial efficiency of the extract and can be used as potent drug against some dreadful infectious diseases caused by the test organisms.

Keywords: *Hemidesmus indicus*, Antibacterial activity, photochemical analysis, Kundam, Tribes.

Article History	Date of Receiving	09 December 2019	Date of Revision	13 January 2020
	Date of Acceptance	14 January 2020	Date of Publishing	20 January 2020

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Funding This work is supported by University Grant Commission, India (RGNF) Grant no. F1-17.1/2014-15-ST_JAM_83141.

CItation Shoket Ali, Shikha Bansal and Ravi Prakash Mishra , Antibacterial Activity Of Hemidesmus indicus (L) Root Used By Tribes Of Kundam, Block District Jabalpur, Madhya Pradesh, (India)..(2020).Int J Pharm Sci.11(1), b84-89 http://dx.doi.org/10.22376/ijpbs.2020.11.1.b84-89

Int J Pharma Bio Sci., Volume11., No 1 (Jan) 2020, pp b84-89

1. INTRODUCTION

Hemidesmus indicus (L) is known as Indian sarsaparilla commonly called as anantmool in Sanskrit[1]. The plant belongs to the family Asclepiadaceae and is famous in Ayurvedic system of medicine[2,3]. It is a perennial climber and grows widely in upper Gangetic plains and in Central to South India. The plant being one of the traditionally used medicinal plants produces a variety of compounds of known therapeutic properties[4]. Root of *H. indicus* is used against various diseases such as urinary tract infections, skin infections, diarrhoea[5], eye infection, fever[6], bronchitis, rheumatism[7], gastric disorders[8] and blood infections[9]. It is a common medicinal plant widely used in the Indian system of medicine[10] and is also referred as an official drug in Indian pharmacopoeia[11] and in British pharmacopoeia[12]. Antibacterial activity of chloroform and methanol extracts of *H. indicus* root has been already demonstrated using various methods against different enterobacterial strains[13] possessing good scientific temper. Several biological activities like hepatoprotective, antioxidant, antithrombotic anti-ulcerogenic[14], anti-inflammatory[15] immunomodulatory and antidiabetic[16]. There are several reports on antimicrobial activity of different herbal extracts[17, 18]. According to World Health Organization, more than 80% of the world's population depends on traditional medicine for their primary healthcare needs[19]. In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infectious diseases. They are naturally synthesized in all parts of the plant body containing bioactive components[20]. In fact, there is a lack of information on the distribution of the biological[21] activity in different plant parts essentially related to the difference in the distribution of active compounds[22] which are more frequent in some plant parts than in others [23]. The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics makes it urgent to search for new infection fighting strategies[24]. The indigenous community of Jabalpur district of Madhya Pradesh has been using many plant species as traditional medicines since ages. Although there has been scarcity regarding their *in vivo* and *in vitro* efficiency. In view of the applications discussed above, this study was intended to explore the antibacterial activity profile of chloroform versus aqueous root extracts of *Hemidesmus indicus* against some common human pathogens bacteria.

2. METHODOLOGY

2.1 Collection and extraction of plant material

The plant sample was collected from Manera, village of district Jabalpur, Madhya Pradesh. The collection was done in Nov, 2016. To confirm and authenticate the identity of plant taxonomically, the samples were examined at State Forest Research Institute (SFRI) under voucher no. 7461. After collection, the roots were sun dried and powdered using mortar and pestle. The extraction of collected plant material was done according to the standard method reported by Kinanbakht and Jahaninani[24].The selected shade dried (for 7 to 14 days) plant part i.e. roots were grounded to fine powder. The powdered plant material was kept in soxhlet extractor for successive extraction with different solvents like chloroform, and aqueous water. For extraction, 25 g each of the powdered parts was taken with 250 ml of different solvents. The obtained liquid extracts were subjected to rotary evaporator 50 ^{0}C. The crude extract was collected in air tight bottle and stored at 4^{0}C for further use.

2.2 Procurement and maintenance of microbial culture

The five species of bacteria, *viz., Escherichia coli* (MTCC 77), *Bacillus subtilis* (MTCC 10110), *Staphylococcus aureus*(MTCC 96), *Pseudomonas aeruginosa* (MTCC 1688) and *Vibrio cholera* (MTCC 3904) were obtained from Institute of Microbial Technology (IMTECH) Chandigarh.The bacterial procured cultures were sub-cultured in nutrient agar medium (beef extract - 3 g, NaCl - 5 g, peptone - 5 g, agar - 15 g, distilled water - 1000 ml) and incubated at 37°C. The stock cultures of bacteria were maintained on nutrient agar slant at 4°C.

2.3 Determination of antibacterial activity

Antibacterial activity was determined using well diffusion method [25]. Petri plates were prepared with 20 ml of sterile Muller Hinton agar media (HiMedia). Wells (6 mm diameter) were punched in the Muller Hinton agar and filled with plant extracts. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10min for compound diffusion. The tests were conducted at three different concentrations 50mg/ml, 100mg/ml and 150mg/ml of the crude extracts. Ciprofloxacin (25µl) was used as a positive control. The plates were incubated for 18-24 hours at 37±1^{0}C. Zone of inhibition was recorded in millimeters using transparent (HiMedia) antibiotic zone. Experiments were carried out in triplicates. Activity index for each extract was calculated using following formula.[26]

2.4 Phytochemical screening

The qualitative phytochemical screening of root extract of *Hemidesmus indicus*(was employed by standard protocols like Raman [27] and Harborne[28] illustrate the presence of alkaloids, glycosides, saponins, carbohydrates, proteins, amino acids, flavonoids, steroids, tannins using the crude extract of chloroform and aqueous water.

2.4.1 Test for alkaloids

Crude extract was warmed with 2% H_2SO_4 for two minutes. It is filtered and a few drops of reagents were added and indicated the presence of alkaloids[29].

2.4.1.1 Mayer's reagent

A creamy- white colored precipitation gives positive result for alkaloids[29].

2.4.1.2 Wagner's reagent

A reddish-brown precipitation gives positive for alkaloids[29].

2.4.2 Test for steroids and terpenoid

2.4.2.1 Salkowski test

The extract was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) is carefully added to form a layer. A reddish brown coloration of the interface is formed to show positive result of the presence of steroids, terpenoids and triterpenoids respectively[30].

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2.4.3 Test for saponins

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins [30].

2.4.4 Test for phenols and tannins

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins[31,29].

2.4.5 Test for flavonoids

A small quantity of the extracts is heated with 10 ml of ethyl acetate in boiling water for 3 minutes. The mixture is filtered differently and the filtrates are used for the test[31].

2.4.5.1 Aluminum chloride test

The filtrates were shaken with 1 ml of 1% aluminum chloride solution and observed for light yellow color. It indicated the presence of flavonoid and diluted NaOH and HCl was added. A yellow solution that turns colorless indicated positive test [31].

2.4.5.2 Benedict's test for carbohydrate

Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrates[29].

2.4.6 Test for glycosides

2.4.6.1 Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars[29].

2.4.7 Test for proteins

2.4.7.1 Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein[30].

3. STATISTICAL ANALYSIS

The data obtained was analysed using Statistical Package for Social Sciences (SPSS) for mean ± Standard deviation (SD).

4. RESULTS AND DISCUSSION

The preliminary phytochemical screening of Chloroform extract showed positive results for steroids and terpenoids. The aqueous extract contains maximum number of phytoconstituents along with alkaloids, terpenoids, flavonoids, phenols, glycosides, saponins (Table 1). The zone of inhibition of chloroform and aqueous extracts of *Hemidesmus indicus* root on gram positive and negative bacteria at different concentrations, by using agar well diffusion method, was determined to access their antibacterial effect. The chloroform and aqueous extracts of the root of *Hemidesmus indicus* exhibited moderate to significant antibacterial activity against five tested bacterial organisms as compared to the standard ciprofloxacin (Table 2). The highest zones of growth inhibition were exhibited by chloroform extract against all the microorganisms compared to be aqueous extract. The chloroform extract produced the highest mean zone diameter of 16.33±1.24 mm at a dose of 150 mg/ml on *E.coli* and lowest zone of growth inhibition was observed on *Vibrio cholerae*, which gave a zone of inhibition measuring 1.33±0.47 mm at a dose of 50 mg/ml. Numerous studies demonstrated that the extracts of plant species possessed activity with regard to antimicrobial properties[32-36]. The study revealed that the chloroform extract of the crude drug was very much effective against *E. coli, S. aureus and P. aeruginosa* and moderately effective at *B. subtilis*and *Vibrio cholera*. The aqueous extract of the crude drug was moderately effective against all these bacteria. The findings from this work may add to overall value of the medicinal potential of *Hemidesmus indicus* root. (Fig 1, Fig 2) Further phytochemical studies are required to determine the purified bioactive compounds responsible for the antibacterial activities of these species, which could serve as useful sources for new antimicrobial agents. The observed activity have confirmed the tribal use of the plant in the treatment of infectious diseases.

Table 1: Qualitative Phytochemical screening of roots extract of *Hemidesmusindicus*(L.)

Phytoconstituents	Test	Solvent System	
		CR	AR
Alkaloids	Mayor's test Hager's test	-	+
Terpenoids	$CHCl_3$ + conc. $H2SO_4$	-	+
Steroids	Liebermann-Burchard test (acetic anhydride + Con. $H2SO_4$)	+	-
Phenols	$FeCl_3$ solution	+	+
Glycosides	Sodium nitroprusside solution Con. $H2SO_4$	-	+
Saponins	Foam test	-	+

+= Presence, -= Absent, CR=Chloroform root extract and AR= aqueous root extract.

Table 2. Antibacterial activity of Chloroform extracts of *Hemidesmus indicus*root against some human pathogenic bacteria.

Zone of inhibition in (mm)				
Bacter	**Chloroform extract**			**Ciprofloxacin**
	50mg/ml	**100mg/ml**	**150mg/ml**	**25mg/ml**
Escherichia coli	11.00±0.81	14.00±0.81	16.33±1.24	26.33±1.24
Staphylococcus aureus	9.00±0.81	11.33±1.24	12.66±1.24	28.66±1.24
Pseudomonas aeruginosa	7.00±0.81	9.00±0.81	12.00±0.81	31.00±0.81
Bacillus subtilis	6.33±1.24	7.00±0.81	9.00±0.81	24.66±0.47
Vibrio cholera	1.33±0.47	2.00±0.81	3.00±0.81	22.66±1.24

Values were expressed in mean ± Standard deviation.

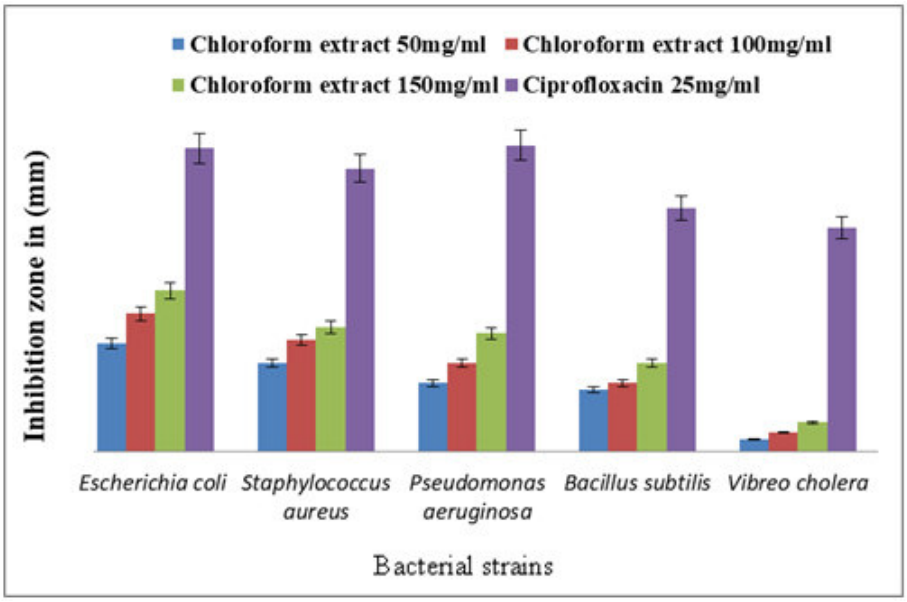

Fig 1. Antibacterial activity of chloroform root extracts of *Hemidesmus indicus* against some human pathogenic bacteria

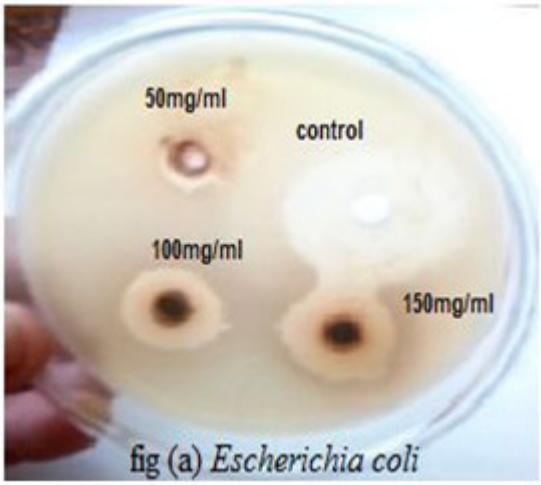

Fig (a).Escherichia coli

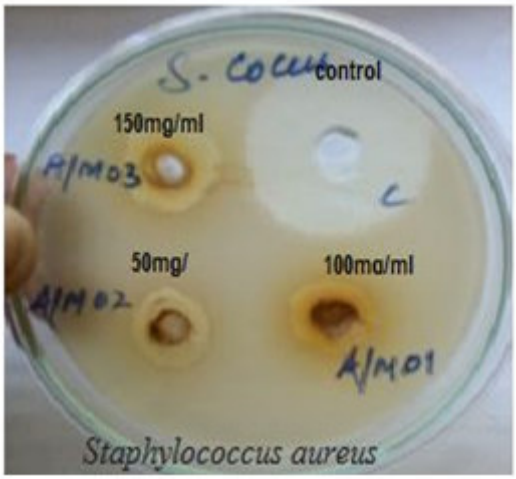

Fig (b). Staphylococcus aureus

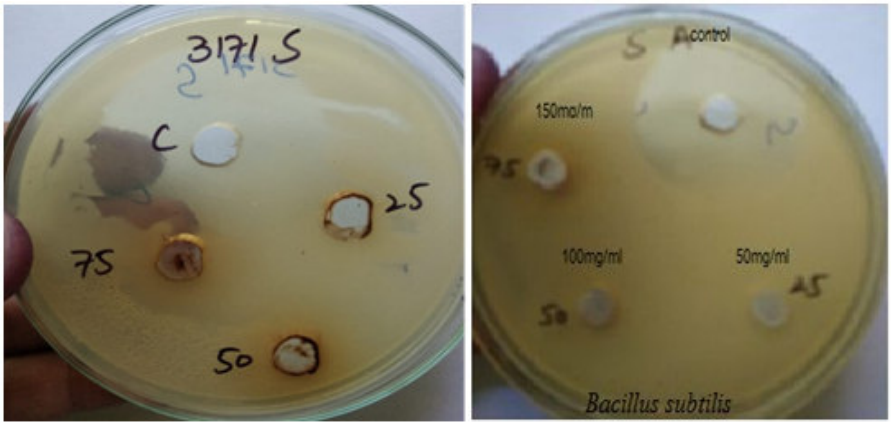

Fig (c).Pseudomonas aeruginosa Fig (d). Bacillus subtili

Fig 2. Antibacterial activity of Chloroform root extract of *Hemidesmus indicus*.

5. CONCLUSION

From the above study, it was concluded that the chloroform root extract of *Hemidesmus indicus* showed higher efficacy towards antibacterial activity against Gram-positive and Gram-negative bacteria. Herbal medicine represents one of the traditional medicines used all over the world. The occurrence of phytochemicals may be responsible for their therapeutic effects. Thus, the present study reflects importance of medicinal plant used locally by the tribes in remote areas, and leads the scope for the development of innovative drugs in the modern world.

6. FUNDING ACKNOWLEDGEMENT

Author is thankful to University Grant Commission for providing (RGNF) Rajiv Gandhi National fellowship for financial assistance to carry out this research work. (Ref. no. F1-17.1/2014-15-ST-JAM-83141). We the authors are also thankful to the Head of Department Post Graduate Studies and Research in Biological Science, Rani Durgavati University, Jabalpur, for providing the necessary laboratory facilities.

7. AUTHORS CONTRIBUTION STATEMENT

Mr. Shoket Ali devised and performed this work. Dr. Shikha Bansal and Dr. Ravi Prakash Mishra actively monitored the study as joint supervisors.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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