Preliminary phytochemical studies and antibacterial activity of leaf of *Holarrhena antidysentrica* (Roth) Wall. ex A.DC. and *Wrightia tomentosa* Roem. et Schulta

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Abstract- *Herbal medicines are significant and reliable sources for treating various infectious and non infectious diseases. The infectious diseases create health problem, mainly in developing countries. Microorganism has developed resistance to many antibiotics and this created major problem in the treatment of infectious disease. Holarrhena antidysenterica (Roth) wall. ex A.DC. and Wrightia tomentosa Roem. et Schulta is a very significant herbal drug in Unani system of medicine and Ayurvedic system of medicine to treat various infectious diseases. In the present work done on the preliminary phytochemical studies and antibacterial activity of leaf of Holarrhena antidysentrica (HAL) and Wrightia tomentosa (WTL) in aqueous, methanol and chloroform extracts. The leaf of H. antidysentrica showed the presence of alkaloids, carbohydrate, proteins, resins and steroid but W. tomentosa leaf revealed the presence of carbohydrate, resins, flavonoids and glycoside in all extracts. The microbial limit test were carried out against Staphylococcus aureus, Salmonella sp, Pseudomonas aeruginosa, Escherichia coli, total bacterial count (TBC) and Yeast and mould (Y & M). Antibacterial activity of methanol, ethanol and acetone extracts of both plants were tested against gram positive (Staphylococcus aureus) and gram negative (Escherichia coli, Pseudomonas aeruginosa and Salmonella spp.) bacteria by agar well diffusion method along with gentamycin run parallel as a control. These parameters are helpful in identification and quality control of drugs and also used to discover natural products that may serve as lead for the development of new biomedical application.*

Keywords: *Phytoconstituents, Antibacterial, Gentamycin, Holarrhena antidysenterica , Wrightia tomentosa.*

Introduction

When human civilization begins plants and its product are used as a source of food, fibre and medicine. The use of plant and its products for a long time that begins with folk medicine and after many years incorporated into traditional and allopathic medicine system to treat chronic as well as infectious diseases (Ramesh and Subramani, 2018; Dubey et al., 2011). Many plants species reported to have pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, triterpenes which are used to treat the causing by pathogens (Kamali et al., 2010; Zahan et al., 2013; Lalitha et al., 2010). With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs (Preethi et al., 2010). Antibiotics are one of the most important therapeutic discoveries which are effectiveness against various bacterial and fungus infections. About one third of the infectious diseases are treated by allopathic products (Akhtari et al., 2014). Due to this, emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases. Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistant inhibitors from plants (Kaneria et al., 2009). *Holarrhena antidysenterica* (Roth) wall. ex A.DC. and *Wrightia tomentosa* Roem. et Schulta is a very significant herbal drug in Unani system of medicine and Ayurvedic system of medicine. Both the plants belong to the family Apocynaceae. *H. antidysenterica* is a medium sized deciduous laticiferous shrub or small tree, 7-9 meter tall, distributed in central and peninsular India but mainly in the Chitrakoot forests of Satna district, Talawada forest of Guna district and Holipur forest of Sihor districts of Madhya Pradesh, Saharanpur, Travancore, Assam, and Uttar Pradesh While *W. tomentosa* is small tree upto 10 – 15 meter in height found throughout India but mainly in the Holipur forest of Sihor districts of Madhya Pradesh, Pondicherry and Orissa in dry and moist deciduous forests. The tree is distributed in other South Asian countries also in Malesia, Sri Lanka, Bangladesh, and Myanmar (Gupta, 2008; Sharma, 2009; Nishteswar and Hemadri, 2010). Kutaj bark is used in the treatment of diarrhoea, and dysentery which is caused by most dangerous enterobacteria *E-coli* and also effective in treatment of multi-drug resistance infection of Salmonella, which is an important cause of severe enteric diseases worldwide. Conessine or Kutaj is effective against the mycobacterium tuberculi and hence, it can be used treating pulmonary tuberculosis (Sudhakarrao et al., 2013; Srivastava, and Saxena , 2015).

W. tomentosa is widely used to treat stomach ache, tooth ache, fever, hemorrhage, arthritis and snake bite. The different extracts of leaf of W. tomentosa possess antibacterial activity (Srinivas et al., 2013; Chopra et al., 1956). However, this work is designed to preliminary phytochemical studies and antibacterial activity of leaf of *Holarrhena antidysentrica* (Roth) Wall. ex. A. DC. and *Wrightia tomentosa* Roem. et Schulta.

Material and Method

Collection and sample preparation of plant materials:

The leaves of *Holarrhena antidysentrica* (Roth) wall. ex A.DC. was collected from the sati Anusuiya' forest of Chitrakoot of Satna district and *Wrightia tomentosa* Roem. et Schulta was collected from Holipur forest of Sihor district of Madhya Pradesh, India. The leaves were identified by Taxonomist Dr. R.L.S. Sikarwar, J.R.D. Tata Foundation of research in Ayurveda and Yoga Sciences, Arogyadham, Deendayal Research Institute, Chitrakoot, Satna (M.P.). The leaves were washed under water to eliminate dust and other foreign particles. The materials were dried in Tray dryer at 35^{o}C and grinded with electric grinder. The powders were sieved (No. 43) and stored in air tight closed containers separately for protection from moisture and microbes growth for further uses.

Preparation of leaf extracts for phytochemical analysis:

Take 2.0g powder samples in 250 ml iodine flasks and add 100 ml solvents such as water, methanol and chloroform. The flasks were kept in rotatory flask shaker for 6 hours and leave for 18 hours for maceration period. After this, filter the filtrate by using Whatmann filter paper no. 1 and this extract were subjected to the qualitative phytochemical analysis (Anonymous, 2010; Gaddaguti et al, 2015; Thimmaiah, 2003; Sadasivam and Manickan, 1996).

Test for microbial limits:

Following tests were carry out as per standard methods (Anonymous, 2008; Tiwari et al., 2014)] to determine the microbial load in leaf of *H. antidysentrica* and *W. tomentosa* curna, a formulated compound drug powder of pharmaceutical substances

Enumeration of *Staphylococcus aureus* /gm

Enumeration of *Salmonella sp./gm*

Enumeration of *Pseudomonas aeruginosa/gm*

Determination of *E.coli*

Determination of total bacterial count (TBC)

Determination of Yeast and Mould (Y & M).

Preparation of leaf extracts for antibacterial activity:

Leaf extract prepared for determination of antibacterial activity by certain modification in given method (Vinoth et al., 2012).

Ethanol Extract: Ethanolic extracts of leaf both plants were prepared (Himal, 2008).

Acetone Extract: Acetone extracts of leaf both plants were prepared by certain modification (Puri, 1999).

Methanol Extract: Methanolic extract of leaf both plants were prepared (Jouad et al., 2001).

Procedure of antibacterial activity:

1. Preparation of Inoculums: Inoculums of *Salmonella spp*., *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were prepared from contaminated water by culturing them in Nutrient broth (Himedia grade). 10ml of inoculums was diluted with 10ml of sterilized distil water just before inoculation.

2. Preparation of Media:

100 ml of Xyline-Lysine deoxycholate agar for *Salmonella spp*., *Staphylococcus aureus* broth with agar for *Staphylococcus aureus*, Cetrimide agar for *Pseudomonas aeruginosa*, Eosin methylene blue agar for *E. coli*, Soyabean-casein digest agar for Total bacterial count and Potato dextrose agar for Yeast & Mould media (Himedia grade) were prepared following the API protocol or dehydrated culture media were used and sterilized by heating in an autoclave at 121^{o}C for 30 minutes.

Determination of antibacterial activity: The antibacterial activity the leaf extracts were determined by using agar well diffusion method. 1ml of diluted inoculums containing microorganism were poured in a sterilized Petridis. Then about 15ml of autoclaved liquefied specified media were poured and mixed well. Than the plate were allowed to solidify in laminar air flow for about 2 hours. After that wells were bored on the solidified agar plates with the help of sterile cork borer. 40 µl of drug extract was poured into the well and disk of Gentamycin antibiotics were kept on the agar surface also run parallel in the same plate (positive control). Whereas negative control containing neat solvents only. Than all the plates were allowed to stand at room temperature for 1 hours so that the drug diffuse in the agar. Than all the plates were incubated at 37^{0}C for 24 hour and the antibacterial activity were assessed by measuring the diameter of the zone of inhibition.

Result

Preliminary phytochemical analysis of aqueous, methanol and chloroform extracts of leaf of *H. antidysentrica* and *W. tomentosa* were carried out. The phytochemical results of both drugs are given in (Table 1).

The microbial profile of the *H. antidysentrica* and *W. tomentosa* leaf sample were developed four specific pathogens viz. *Salmonella Spp., Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and Total bacterial count (TBC) and Yeast and Moulds (Y & M). There results were given in (Figure 1 & Figure 2)

Antibacterial activity of methanol, ethanol and acetone extracts of leaf of *H. antidysentrica* and *W. tomentosa* against gram positive (*Staphylococcus aureus)* and gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa* and *Salmonella spp.)* were carried out by agar well diffusion method along with gentamycin as a standard run parallel. The zones of inhibition of three extracts of both samples were given in (Table 3 & Table 4 and Figure 3).

Discussion

The experimental finding of the preliminary phytochemical analysis and the results of antibacterial activity were showed in the respective tables. The preliminary phytochemical tests performed were of identification type and from the phytochemical investigations in *H. antidysentrica* leaf was observed that alkaloids, carbohydrate, proteins, resins, steroid were present showed the presence of alkaloids, carbohydrate, proteins, resins and steroid in all extracts but starch is absent in all of them. Whereas glycoside and tannins were presence in aqueous and methanol extracts but absent in chloroform extract. Flavonoids were observed in aqueous and chloroform extracts but absent in methanol extract. Along with saponin present only in aqueous extract but absent in both of the extracts. The highest solubility of phytoconstituents was found in aqueous or methanol solvents but lowest in chloroform. These findings are similar with a study conducted by Nahak *et al*. (2014), the phytochemicals analysis revealed the presence of flavonoids, amino acids, proteins, anthraquinone glycosides and phenolic compounds present in the leaf and callus extracts. Ganapathy *et al*. (2009), who observed the various phytoconstituents such as alkaloids, flavonoids, sterols, tannins and quinine in different extracts of petroleum ether, chloroform and ethanol of leaf, bark and inflorescence of *H. antidysentrica*. But in present study chloroform extract showed the presence of alkaloids, sterols but absent of flavonoids, tannins respectively. Shwetha *et al*. (2011), found the presence of alkaloids, flavonoids, tannins, phenolics in ethanol, chloroform and petroleum ether extracts of *H. antidysentrica* leaves but our study of chloroform extract showed the presence of alkaloids only but other were absent.

Where in case of *W. tomentosa* leaf revealed the presence of carbohydrate, resins, flavonoids, glycoside in all extracts but starch and proteins were absent in all of them. Steroid and tannins were presence in methanol and chloroform extracts but absent in aqueous extract. Saponin presence in aqueous and chloroform extracts but absent in methanol extract. Along with alkaloids is presence only in aqueous extract but absent in both the extracts. The highest solubility of phytoconstituents was found in chloroform solvent extract and lowest in aqueous and methanol solvent extracts. Similar results were reported by Khyade and Vaikos (2014) on the root, bark and leaf of *W. arborea* showed the presence of alkaloids, flavonoides, phlobatannin in bark and leaves; phenolics, reducing sugars, saponins, tannins were found in root, bark and leaf of plant while leucoanthocyanins, iridiods, steroids and terpenoid revealed in the leaves only. The methanolic extracts of leaves of *W. coccinea, W. tinctoria, W. mollissima* and *W. tomentosa* and revealed the presence of quinines, anthocyanins, flavonoids, cardiac glycoside, aurones, calcones and catchins in all four species but saponin, tannin, anthraquinone and alkaloids were absent in all four species of *Wrightia*. Whereas lucoanthocyanin and indole were also presence in *W. tomentosa* (Sharma et al. (2017). But current study showed the presence of alkaloids, carbohydrate, flavonoids, glycoside, tannin, resin, steroid but protein, starch, saponin were absent.

The microbial profile of the *H. antidysentrica* and *W. tomentosa* leaf sample was found satisfactory. Total bacterial count (average 369 cfu/g and 231 cfu/g), Yeast and Moulds (average 21 cfu/g and 46 cfu/g) counts were recorded less than the standard of WHO and pathogenic bacteria, i.e. *Salmonella, Pseudomonas, Staphylococcus* and *E.coli* were found to be absent.
Antibacterial activity of methanol, ethanol and acetone extracts leaf of *H. antidysentrica* and *W. tomentosa* were carried out. The acetone extract of *H. antidysentrica* leaf showed maximum zone of inhibition against *Escherichia coli* (24mm) followed by *Salmonella spp.* (23mm), *Pseudomonas aeruginosa* (22mm), *Staphylococcus aureus* (19mm) and compare with reference standard gentamycin. Whereas the ethanolic extract showed maximum zone of inhibition against *Escherichia coli* (26mm) followed by *Salmonella spp*.(23mm), *Pseudomonas aeruginosa* (23mm) and *Staphylococcus aureus* (13mm) compare with reference standard gentamycin. The methanolic extract revealed the maximum zone of inhibition (29mm) against *Escherichia coli* followed by *Salmonella spp*.(25mm), *Pseudomonas aeruginosa* (20mm) and *Staphylococcus aureus* (17mm) compare with reference standard gentamycin.

Where in case of *W. tomentosa* leaf the antibacterial activity of acetone extract of *W. tomentosa* leaf showed maximum zone of inhibition against *Pseudomonas aeruginosa* (18mm) followed by *Salmonella spp.* (13mm) *Staphylococcus aureus* (12mm), and *Escherichia coli* (11mm) compare with reference standard gentamycin. The ethanolic extract showed maximum zone of inhibition against *Pseudomonas aeruginosa* (20mm) followed by *Escherichia coli* (16mm), *Salmonella spp*.(15mm) and *Staphylococcus aureus* (9mm) compare with reference standard gentamycin. The methanolic extract revealed the maximum zone of inhibition against *Escherichia coli* (26mm) followed by *Salmonella spp*.(21mm), *Pseudomonas aeruginosa* (19mm), and *Staphylococcus aureus* (16mm) compare with reference standard gentamycin. Similar results were found in previous study conducted by Penchala on antibacterial potential of chloroform, petroleum ether, ethyl acetate, methanol and aqueous leaf extracts of *W. tomentosa* was tested against three gram positive and three gram negative bacterial pathogens viz., *Bacillus cereus, Staphylococcus aureus, Bacillus subtilis and E. coli, Klebsiella pneumonia, Salmonella typhimurium,* respectively. The methanolic extract showed slightly difference in zone of inhibition against *S. aureus* but in case of *E. coli* showed large difference between zones of inhibition along with also revealed the presence alkaloids, ellagic acid, iridoids, lignans, methylene dioxy compounds, steroids, tannins and triterpenoids in methanolic extract.

Conclusion

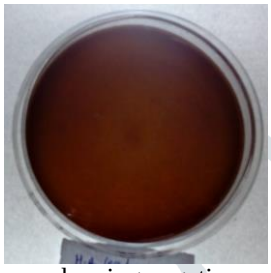
The maximum number of phytochemicals was found in leaf of *H. antidysenterica* compare to leaf of *W. tomentosa* in aqueous and methanolic extracts but chloroform extract vice versa. Due to the presence of secondary metabolites in drugs which directly linked to medicinal properties of plants such as therapeutic and pharmacological action. However, the methanolic extracts of leaf of both plants showed more antimicrobial activity than ethanol and acetone extracts. It is expected that using natural products as therapeutic agents will probably not elicit resistance in microorganisms. The experimental findings of the current study to control the pathogenic bacterial strains can be further explored in order to discover new drug molecules to combat human diseases. The plant can be further studied for the isolation, identification and quantification of important active phytochemical constituents for the development of new specific antibiotic.

Table -1 Showed preliminary phytochremical screening of aqueous, methanol and chloroform extract of *H. antidysentrica* leaf and *W. tomentosa* leaf

Name of phytoconbstitue nts	*Holarrhena antidysentrica* leaf			*Wrightia tomentosa* leaf		
	Aqueous extract	Methanol extract	Chlorof orm extract	Aqueous extract	Methan ol extract	Chlorofo rm extract
Alkaloid	Present	Present	Present	Present	Present	Absent
Carbohydrate	Present	Present	Present	Present	Present	Present
Protein	Present	Present	Present	Absent	Absent	Absent
Resins	Present	Present	Present	Present	Present	Present
Saponin	Present	Absent	Absent	Present	Absent	Present
Starch	Absent	Absent	Absent	Absent	Absent	Absent
Flavonoid	Absent	Present	Absent	Present	Present	Present
Steroid	Present	Present	Present	Absent	Present	Present
Glycoside	Present	Present	Absent	Present	Present	Present
Tannins	Present	Present	Absent	Absent	Present	Present

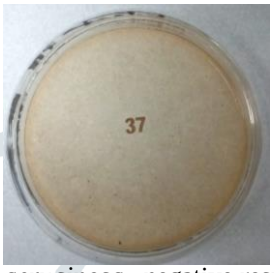
Table -2 Showed zone of inhibition of a acetone, ethanol and methanol extract of *H. antidysentrica* leaf and *W. tomentosa* leaf in mm

Name of pathogns	*Holarrhena antidysentrica* leaf			*Wrightia tomentosa* leaf			Control
	Acetone extract	ethanol extract	Methanol extract	Acetone extract	ethanol extract	Methanol extract	Gentamycin
E. coli	24	26	29	11	16	26	11
P. aeruginosa	22	23	20	18	20	19	12
S. aureus	19	13	17	12	9	16	21
S. spp	23	23	25	15	15	21	10

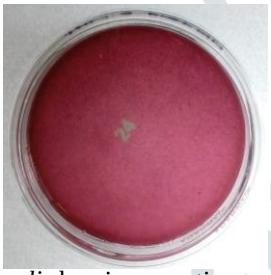


S. spp. showing negative result

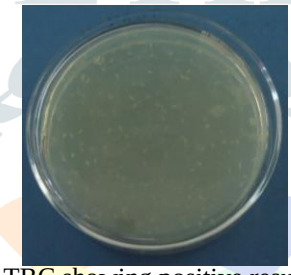
S. aureus showing negative result



P. aeruginosa negative result



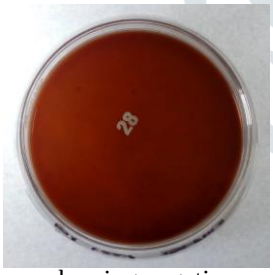
E. coli showing negative result



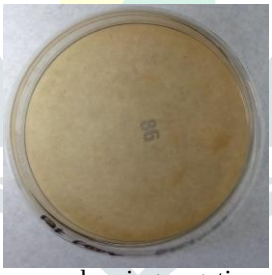
TBC showing positive result

Yeast & Mould showing positive result

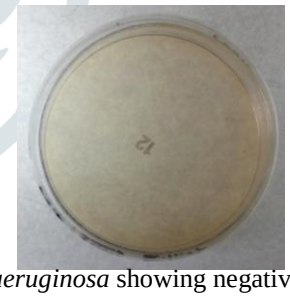
Figure 1: Showed microbial limit test of *H. antidysentrica* leaf against four specific pathogens, TBC and Y & M



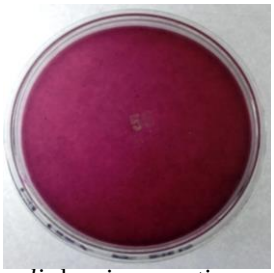
S. spp. showing negative result



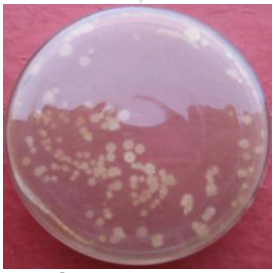
S. aureus showing negative result



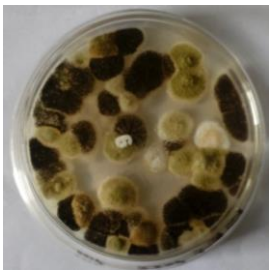
P. aeruginosa showing negative result



E. coli showing negative result

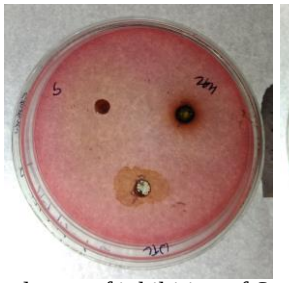


TBC showing positive result

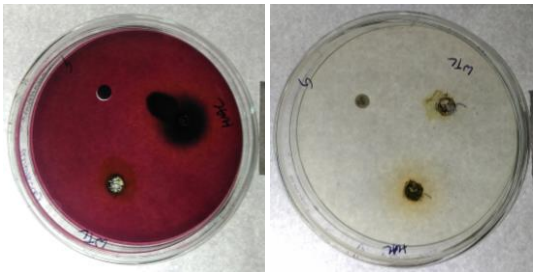


Yeast & Mould showing positive result

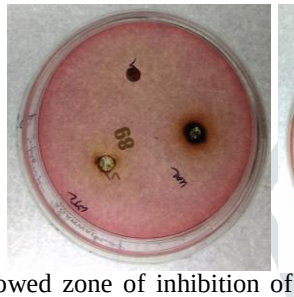
Figure 2: Showed microbial limit test of *W. tomentosa* leaf against four specific pathogens; TBC and Y & M.



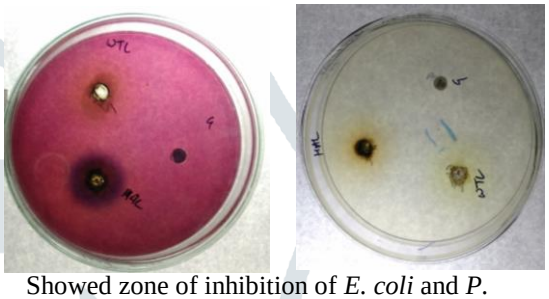
Showed zone of inhibition of *S. aureus* and *S. spp* against acetone extract of HAL & WTL with reference Gentamycin



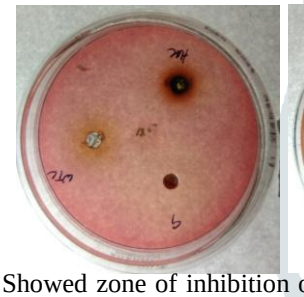
Showed zone of inhibition of *E. coli* and *P. aeruginosa* against acetone extract of HAL & WTL with reference Gentamycin



Showed zone of inhibition of *S. aureus* and *S. spp.* against ethanol extract of HAL & WTL with reference Gentamycin



Showed zone of inhibition of *E. coli* and *P. aeruginosa* against ethanol extract of HAL & WTL with reference Gentamycin



Showed zone of inhibition of *S. aureus* and *S. spp* against methanol extract of HAL & WTL with reference Gentamycin

Showed zone of inhibition of *E. coli* and *P. aeruginosa* against methanol extract of HAL & WTL with reference Gentamycin

Figure 3: Showed zone of inhibition of acetone, ethanol and methanol extract of *H. antidysentrica* leaf and *W. tomentosa* leaf against specific pathogens.

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