International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 29-10-2021, Accepted: 15-11-2021, Published: 30-11-2021
Volume 6, Issue 6, 2021, Page No. 1567-1570

Studies on antibacterial activities of two ethnomedicinally important plants

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Abstract
All over the world, specifically in rural areas of developing countries, people use traditional medicines for curing numerous diseases. Medicinal plants are widely used in the field of ethnomedicine for various ailments. Plant derived medicinal products are being used in ethnomedicinal system from times unknown and have been recorded in many pharmacopoeia as antimicrobial agents. In the present study two ethnomedicinally important plants i.e. *Lawsonia inermis* and *Adhatoda vasica* were evaluated for their antimicrobial potential against some pathogenic bacteria *viz*. *E. coli*, *Enterobacter aerogenes*, *Salmonella typhi* and *Agrobacterium tumefaciens*. Different plant parts were examined using Water, Ethanol, Chloroform, Acetone and Petroleum ether as solvents. The antibacterial activity was determined by using Disc Diffusion method. Ciprofloxacin was used as positive control whereas DMSO and water as negative controls. Significant antibacterial activity was observed in most of the plant extracts evaluated. In case of *Lawsonia inermis* the highest antibacterial potential was exhibited by Petroleum ether extracts of leaves against *E. coli* whereas acetone extract of flowers of *Adhatoda vasica* were found highly effective against *E. aerogenes.* The phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, saponins, phenols, steroids and flavonoids. The findings of present study indicate that the plant extracts evaluated can prove to be potential antimicrobial agents against disease causing pathogenic bacteria.

Keywords: antibacterial, *Lawsonia*, *Adhatoda*, disc diffusion, *Enterobacter aerogenes*

Introduction

Globally, the indigenous people have been using diverse medicinal plants as traditional medicines for combating infectious diseases. Resistance to antibiotics is a worldwide serious problem and is challenging the medical sector in all the countries as evidenced by spread of drug resistant pathogens. This has led to search for new antimicrobial substances like plants which are source of bioactive compounds with therapeutic capacities [1]. Indian culture is enriched by herbal preparations as plants are used not only to combat diseases but also used in several traditional ceremonies. Indian people are using traditional system of health care as India is repository of medicinal plants evidenced by various ethno-botanical studies done in search of new plant resources as source of food and drugs [2]. Plants play significant role in producing healthcare pharmaceutical drugs and also serve as resource for developing biomedicines in many communities of the world. Plants with medicinal properties are usually found more frequently in specific plant families and humans make direct use of only a small proportion of medicinal plant species. Ethnomedicinal studies in various regions of the country can prove to be an initiating point for the development of novel drugs but due to modern life styles people are getting far from this traditional treasure, which is popular in rural areas due to high cost of allopathic medicines and lethal after effects [3].

Lawsonia inermis L. locally referred to as henna belongs to the Lythraceae family and it has the appearance of a small shrub like tree with spine-tipped branch-lets. Leaves are opposite, sub-sessile, elliptically shaped and broadly lancelate [4]. The plant grows in a semi-arid and can withstand prolonged drought and poor soil. In Middle East, leaves of this plant in powdered form are used as cosmetic product and also remedy for boils, wounds, and fungal infections [5]. The plant (Henna) is traditionally used to colour fingertips, nails and toes as a weekly application and this practice is observed to reverse the damage caused by finger nail fungi [6]. Henna powder is used to dye hair which protects from UV rays which damages hair and also helps to get rid of dandruff [7].

Adhatoda vasica locally popular as *Adusa* belongs to acanthaceae family and present throughout the tropical regions of Southeast Asia and has several medicinal properties and is efficacious in the treatment of inflammatory diseases [8]. Plant derived non-toxic products are becoming people's first choice for health care, due to benefits of vasaka derived medicines [9]. The plant is medicinally very significant as the complete plant is used due to its therapeutic effects and is a main constituent of cough syrups along with tulsi and ginger [10]. Leaves and Flower's decoction are used on fresh wounds, inflammatory swellings and rheumatic joints. Vasicine is most important ingredient for the removal of sputum from the body[11]. Experimental studies have proved that this plant is hypotensive, bronchodilator, expectorant, hypoglycemic, antibiotic, antitubercular and is also used in uterine problems [12]. Natural products are a significant source of synthetic and traditional herbal medicine and are still the primary health care system and as a part of culture religious plants have been explored for their antibacterial activity. Owing to significant medicinal properties of both the above mentioned ethnomedicinally important plants, the present investigation is an attempt to study the antimicrobial potential of *Lawsonia inermis* and *Adhatoda vasica* against some pathogenic bacteria.

Materials and Methods

Plants of *Lawsonia inermis* were collected from Sojat city of Pali district in Rajasthan. Sojat city is world famous for production of *Mehandi* or *Henna*. Fresh plants of *Adhatoda vasica* were collected from residential gardens and farm houses, at different localities, in Jodhpur. Their identity was confirmed from the literature available in Department of Botany, J.N.V, University, Jodhpur. Different plant parts viz. root, leaves and stem of *Lawsonia inermis* and; root, leaves and flowers of *Adhatoda vasica* were thoroughly washed and then dried under shade at 28± 2^0C for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were Petroleum ether, Chloroform, Acetone, Ethanol and Aqueous; and the extracts were dried. The dried extracts were stored in a refrigerator at 4^0C. Finally, concentration of 5 mg per disc was loaded on each disc.

Antimicrobial Susceptibility Test

All the plant part extracts were screened against *E. coli*, *Salmonella typhi*, *Enterobacter aerogenes* and *Agrobacterium tumefaciens* pathogenic bacterial strain. The disc diffusion method was used to test the antimicrobial activity of the plant extracts [13]. 20ml of sterilized nutrient agar medium for pathogens were poured into each sterile petri dish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) loaded with 5 mg/ disc of dry extract were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37^0C for 24h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Phytochemical Analysis

Plant extracts were subjected to standard phytochemical analyses to find the presence of alkaloids, glycosides, tannins, saponins, phenols, steroids and flavonoids [14].

Results and Discussion

With increasing antibiotic resistance in pathogenic bacteria, prevalence of infectious diseases have become worldwide problem and as a result globally researchers are exploring interest in developing anti-bacterial components [15]. All over the world scientist are evaluating antimicrobial potential of different plant parts for combating various bacterial pathogens causing human and plant diseases [16, 17]. In the present investigation different plant parts of *Lawsonia inermis* and *Adhatoda vasica* were evaluated for their antibacterial potential. In case of *Lawsonia inermis* roots showed significant activity against *E. aerogenes* and *E.coli* in chloroform and Petroleum ether extracts respectively. Chloroform extract of root was also effective against *A. tumefaciens*. All the extracts of leaves exhibited great antimicrobial potential and the highest antibacterial action was shown by petroleum ether extracts of leaves against *E. coli* followed by chloroform extract against *A. tumefaciens*. Bissa (2018) also studied antimicrobial potential of leaves of some religious plants against pathogenic bacteria [18]. The stem extracts in acetone, chloroform and petroleum ether solvents were found effective against all the tested bacteria. Similarly Yusuf (2016) reported leaves extracts possessed antibacterial activity against Gram negative and Gram-positive bacterial strains and ethanol extract showed the highest antibacterial effects followed by ethyl acetate and n-hexane extracts [19]. Habbal *et al* (2005) also evaluated dried leaves extract of *Lawsonia inerms* and showed high effects against all the tested bacterial strains as well as against *Candida albicans* [20]. Similarly Kannahi and Vinotha (2013) showed that maximum antibacterial activity was possessed by methanol and ethanolic extracts against the tested bacteria and fungi [21]. The preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, saponins, phenols, steroids and flavonoids in all the plant parts tested. Similarly Semwal *et al* (2014) reviewed the ethnobotanical, phytochemical and pharmacological aspects of *Lawsonia inermis* and observed Phenolic compounds, flavonoids and naphthoquinones in henna extracts [22]. Al-Snafi (2019) reported the presence of carbohydrates, phenolic, flavonoids, saponins, proteins, alkaloids, terpenoids and quinones, from all parts of *Lawsonia inermis* [23].

The antibacterial potential explored in the plants encourages to search and develop new classes of antibiotic compounds to control the diseases caused by pathogenic bacteria. In case of *Adhatoda vasica* petroleum ether extract of roots was found to be highly effective against *E. aerogenes* and *E. coli*. The petroleum ether extract of leaves was found effective against *E. coli* whereas chloroform extract was able to inhibit *E. aerogenes*. Leaves extract in three solvents i.e. acetone, chloroform and petroleum ether exhibited antimicrobial potential against *A. tumefaciens.* All the flower extracts of *Adhatoda vasica* were found effective against tested pathogens. The highest activity was noticed in acetone extract against *E. aerogenes* and *E. coli*. Petroleum ether extract of flowers were observed to inhibit all the experimental pathogenic bacteria. The crude phytochemical analysis revealed the presence of alkaloids, glycosides, tannins, saponins, phenols, steroids and flavonoids in ethanolic extracts of flowers. Similarly antifungal and antibacterial activity of *Adhatoda vasica* was evaluated against *Candida albicans*, *Aspergillus flavus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes* by Pa and Mathew (2012) [24]. Shahzad *et al* (2020) also reported that chloroform and ethanol extract of plant exhibited the antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus* and *E. coli* [25]. The chemical composition and antimicrobial activity of essential oil collected from *Adhatoda vasica* leaves was done by Sarker *et al* (2011) [26]. Dhankar *et al* (2011) proposed that this plant can be used as an important source of natural medicines due to the presence of various phytochemicals in the plant [27]. Similarly Suthar *et al* (2009) estimated the phytoconstituent vasicine and vasicinone in *Adhatoda vasica* [28]. Medicinal plants have been proved to exhibit potential therapeutic effects and are widely used as a significant alternative to control infectious diseases. The present study reveals that both the ethnomedicinally important plants tested *viz. Lawsonia*

inermis and *Adhatoda vasica* exhibited significant antibacterial activity against the tested bacterial strains and can be used as a potential source to extract antimicrobial drugs.

Table 1: Antibacterial activity of Plant part extracts of *Lawsonia inermis*

Plant part	Plant Extract	Zone of Inhibition (mm)			
		E.coli	***Enterobacter aerogenes***	***Salmonella typhi***	***Agrobacterium tumefaciens***
Root	Aqueous	-	7±1.00	5±1.00	-
	Alcoholic	8.67±0.58	7.67±1.53	6±1.00	-
	Acetone	11±1.00	9±1.00	9.67±1.53	11±2.00
	Chloroform	8±2.00	14.33±1.15	8.67±1.08	14±1.65
	Petroleum ether	12.33±1.52	12.33±0.58	9±1.65	-
Leaves	Aqueous	5.67±1.08	7±1.00	6.33±1.15	7.33±1.53
	Alcoholic	8.33±1.15	9.33±2.08	12±2.00	10.67±1.06
	Acetone	7±1.00	10±2.00	11.67±1.53	11±2.00
	Chloroform	11±1.65	11±1.00	10.67±1.06	14.67±1.08
	Petroleum ether	15.67±1.53	12.33±1.53	13±1.00	13±2.00
Stem	Aqueous	6.67±1.06	9±1.65	8.33±1.51	5.67±1.08
	Alcoholic	9±1.65	7±2.00	6.67±1.08	9±2.00
	Acetone	12.67±1.08	11.67±1.53	13.67±1.08	10±1.00
	Chloroform	11.67±0.58	12.67±1.08	11.33±1.52	7.67±1.53
	Petroleum ether	11.33±1.52	7.67±1.52	11.67±1.08	10.33±1.53

Table 2: Antibacterial activity of Plant part extracts of *Adhatoda vasica*

Plant part	Plant Extract	Zone of Inhibition (mm)			
		E.coli	***Enterobacter aerogenes***	***Salmonella typhi***	***Agrobacterium tumefaciens***
Root	Aqueous	-	5.67±1.08	5.33±1.53	-
	Alcoholic	9.67±1.08	7.67±1.53	9±1.00	-
	Acetone	10.67±1.53	8±1.00	9.33±1.08	7.67±1.08
	Chloroform	12±1.00	13±1.65	11±1.00	7±1.00
	Petroleum ether	13.33±1.53	14±2.00	9.67±1.53	7.67±1.51
Leaves	Aqueous	8±2.00	5±1.00	-	4.67±1.53
	Alcoholic	8.33±1.08	7.33±1.53	6.67±1.15	9±1.15
	Acetone	8.67±1.53	9.67±1.06	7.33±0.58	10.33±1.53
	Chloroform	6±1	12.33±1.06	9±1.00	10.67±1.08
	Petroleum ether	12.33±1.53	11±2.00	9.67±1.53	11±1.00
Flower	Aqueous	6±1.00	8.67±1.08	7±1.00	5±1.00
	Alcoholic	9.33±1.08	10.33±1.52	8±1.00	9.33±1.53
	Acetone	13.67±1.06	15.67±1.53	9±2.00	7.33±1.53
	Chloroform	11.67±1.08	9.33±1.06	9.33±1.08	12±1.00
	Petroleum ether	11.33±1.52	7.33±1.52	10.67±1.15	10±1.00

Conclusion

From ancient times plant derived therapeutic agents are ample source of antibacterial drugs for treatment of diseases. During recent times also the awareness about importance of locally used medicinal plants is growing due to their safe, efficient use and ready availability. The present investigation suggest that both the plants i.e. *lawsonia inermis* and *Adhatoda vasica* possess high antimicrobial activities and can be further explored for obtaining antimicrobial drugs. The knowledge gained in this research work might open novel areas leading to discovery of new drugs against common infectious agents.

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