WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 9, 462-469. Conference Article ISSN 2277–7105

EVALUATION OF PHYTOCHEMICAL CONSTITUENTS AND IN VITRO ANTI-BACTERIAL ACTIVITY OF THE BARK EXTRACTS OF ADENANTHERA PAVONINA L

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Article Received on
18 March 2018,
Revised on 08 April 2018,
Accepted on 28 April 2018
DOI: 10.20959/wjpr20189-12129

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ABSTRACT

Adenanthera pavonina L. (Fabaceae) commonly called as red sandal-wood is a deciduous tree and its bark is traditionally used for treatment of various disease conditions such as gonorrhoea, hematuria, ulcers etc. The objective of the present study was to investigate the hidden anti-bacterial potential of *Adenanthera pavonina* L. against some selected bacterial human pathogenic strains. The research work was conducted in the Microbiology Research Laboratory of Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha. Five bacterial strains i.e. *Escherichia coli* (MTCC-614), *Shigella flexneri* (MTCC-9543), *Staphylococcus aureus* (MTCC-1430), *Salmonella paratyphi* (MTCC-3220) and *Salmonella enteric typhi* (MTCC-733) were used in the study. The study revealed that methanolic extract of *A. pavonina* was most potent and exhibited the highest zone of inhibition against *Shigella flexneri* at 6 mg/ml which was quite comparable with reference antibiotic - Ciprofloxacin (0.5 mg/ml). The n-hexane extract was moderately effective against all the test bacteria. Phyto-chemical analysis showed that the bark of the plant contained various constituents like flavonoids, tannins, alkaloids and triterpenoid. The presence of these phyto-constituents indicated that the bark extracts of *Adenanthera pavonina* might be a source for a new drug against diseases like diarrhoea and dysentery and needs further investigation.

KEYWORDS: *Adenanthera pavonina*, anti-bacterial activity, phyto-chemical analysis, Ciprofloxacin.

I. INTRODUCTION

Plants have been a source of food, fibre and medicine since the beginning of the human civilization. The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine system.[1] Since antiquity, 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 therefore, should be utilized to combat the disease causing pathogens.[2,3,4] Application of plant-derived biocides in agriculture have been more popular during the past for their low health risk and feasibility. In recent years much attention has been given to non-chemical systems for seed treatment to protect them against many plant pathogens.[5] 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.[6] Antibiotics are undeniably one of the most important therapeutic discoveries of the twentieth century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products.[7] This is because of the emergence of resistant pathogens as a consequence of years of widespread indiscriminate use, incessant and misuse of antibiotics.[8,9] 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.[10,11] Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics might be active against drug resistant pathogens.[12]

Adenanthera pavonina is found scattered in evergreen to dry deciduous rainforests and also in savannah. The tree is native to China and India. *Adenanthera pavonina* L. (Family: Fabaceae) commonly known in Bangladesh as ‘Rakta-Kombol’, is an important medicinal plant native to tropical Asia, Western and Eastern Africa as well as in most islands of both the Pacific and Caribbean regions. The tree is generally erect, ranges in height from 6-15 m with diameter up to 45 cm (depending upon location) and young parts glabrous with spreading crown. Bark is dark brown to greyish in colour. Various parts of this plant have also been known to be used in traditional medicine for the treatment of asthma, boil, diarrhoea, gout, inflammations, rheumatism, tumour and ulcers, and as a tonic.[13] Several alkaloids have been isolated from this plant including pilocarpine, pilocarpidine and

physostigmine. The bark contains stigmasterol, glucoside and wood contains robinetin, chalcone, butein, ampelopsin and dihydrobinetin. Phytochemical investigations showed the presence of phytosterols, flavonoids, terpenoid, saponins, carbohydrates, tannins, glycosides, alkaloids and proteins. In India decoction of young leaves is used against rheumatism and gout. Pulverised wood mixed with water is taken orally for migraines and headaches and dysentery, diarrhoea and tonsillitis are treated with a bark and leaf decoction. Previous phytochemical investigation of the plant reported the presence of robinetin, chalcone**,** butin and flavanolampelopsin**,** stigmasterol glucosides**,** oleanolic acid, echinocystic acid, sapogenins and many other bioactive phyto-constituents.[14] Generally the optimal effectiveness is due to the combined action of different compounds originally present in the plant.[15] Diarrhoea and dysentery are the two dreaded diseases causing heavy toll in Odisha. The causative organisms of these diseases are *Escherichia coli*, *Shigellaflexneri*, *Staphylococcus aureus*, *Salmonella paratyphi* and *Salmonella enteric typhi*. As the plant is claimed for treatment in diarrhoea and dysentery, the authentication is needed for further investigation to find out compounds which are responsible for bacterial inhibition. The interest in the scientific investigation of *Adenanathera pavonina* was based on the claims of its effective use in the treatment of diarrhoea and dysentery by the rural communities of Odisha.

II. MATERIALS AND METHODS

2.1 Collection and identification of plant material

The plant *Adenanthera pavonina* was collected from the Chandaka reserve forest area near Bhubaneswar, Odisha. Identification of the voucher specimen was done by following available literatures (Saxena and Brahamam 1995)[16] The voucher specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar. The bark samples were collected in bulk amount, washed in running tap water, dried under shade and made to coarse powder form.

2.2 *Processing of plant material and preparation of extract*

The collected bark which was shade dried and ground to form course powder and had been successively extracted with the solvent n-hexane and methanol by Soxhlet apparatus[17] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.the dried extracts were weighed and their percentage in terms of dry weight of the plant were estimated by the following formula.

Percentage of extract yield = (weight of dried extract/ weight of dried plant material) x100

2.3 *Evaluation of the extracts for antibacterial activity*

The *in vitro* antibacterial screening was carried out against selected bacterial pathogens causing diarrhoea and dysentery in human. The bacterial pathogens were *Shigella flexneri* (MTCC-9543), *Salmonella entericasertyphi* (MTCC733), *Bacillus subtilis* (MTCC1305), *Streptococcus mitis* (MTCC2897), *Klebsiella pneumonia* (MTCC-109), *Staphylococcus aureus* (MTCC-1430). These species were procured from Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India. The remaining test bacteria were procured from P.G. Department of Microbiology, OUAT, Bhubaneswar, Odisha. These organisms were identified by standard microbial methods.[18] The antibacterial screening of the extracts were carried out by determining the zone of inhibition using agar well diffusion method.[19] Ciprofloxacin was taken as reference antibiotic.

2.4 *Agar well diffusion assay*

Agar well diffusion method[30] was followed to determine the zone of inhibition of microbes in Nutrient Agar (NA, HiMedia Laboratories Ltd., Mumbai) plates which were swabbed (sterile cotton swabs) with 8 hr old broth culture of bacteria. Wells (8 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Stock solution of plant extracts were prepared at a concentration of 3 mg/ml and about 50 µl of the solvent extracts were added aseptically into the wells and allowed to diffuse at room temperature for 2 hours. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37 °C for 24 hours for bacterial pathogens. Triplicates were maintained and the diameter of the zone of inhibition (mm) was measured and the data were statistically analysed.

2.5 Phytochemical Screening

Phytochemical screening was carried out to determine the presence of flavonoid, tannin, alkaloid and triterpenoid.[20] The solvents used were methanol and distilled water in different tests.

III. RESULT AND DISCUSSION

3.1 Percentage yield

The percentage yield of n-hexane and methanol extracts of *Adenanthera populnea* bark was found to be 6.5% and 12% respectively (Table 1).

3.2 Phytochemical Studies: The phytochemical studies showed the presence of triterpenoids, alkaloid, tannin and flavonoids. Tannin was present only in the n-hexane extract of *Adenanthera pavonina*, whereas other phytochemicals were found in both solvent extracts (Table 2). This study indicated that some bioactive molecules were present in both extracts and could be used in developing of new drugs for different diseases.

3.3 Anti-microbial studies: The n-hexane and methanolic extracts of the plant *Adenanthera pavonina* were subjected to antibacterial activity ant the results were expressed in terms of zone of inhibition. Among the two extracts of the plant, the methanolic extract proved more potent than the methanolic extract. It exhibited maximum zone of inhibition against *Shigella flexneri* at 6 mg/ml which was quite comparable with antibiotic Ciprofloxacin (0.5 mg/ml). The n-hexane extract was moderately effective against all the test bacteria. (Table 3).

Table. 1: Percentage of yield of *Adenanthera pavonina* bark.

Solvent for extraction	Wt. of dry material (gm)	Wt. of extract (gm)	% yield
n-hexane	30	1.95	6.5%
methanol	30	3.6	12%

Table. 2: Phytochemical analysis of bark of *Adenanthera pavonina.*

Name of the plant	Extracts	Flavonoid	Tannin	Alkaloid	Triterpenoid
Adenanthera pavonina	n-hexane	+	+	+	+
	methanol	+	-	+	+

Table. 3: Extracts showing zones of inhibition (in mm) against bacterial species.

Name of the plant	Test agent	Concentration (mg/ml)	*E. coli*	*S. enteric typhi*	*S. paratyphi*	*S. aureus*	*Shigella flexneri*
Adenanthera pavonina	**Ciprofloxacin (RA)**	**0.5**	**30±0.816**	**32±1.69**	**28±0.81**	**20±0.816**	**29±1.69**
	n-hexane	6	10.10	11.21±	14.01±	09.02±	11.03±
		3	09±0.10	10.31±0.20	12.21±0.21	08.10±0.12	09.12±0.31
		1.5	--	--	--	--	--
		0.75	--	--	--	--	--
	methanol	6	15.30±0.264	11.06±0.35	14.20±01	16.33±0.251	16.56±0.208
		3	12.36±0.05	10.13±0.12	12.23±0.208	12.38±0.03	13.65±0.18
		1.5	12.0±0.04	11.10±0.02	10.21±0.01	11.32±0.128	13.76±0.08
		0.75	10.533±0.2	10.61±0.081	10.56±0.25	11.06±0.115	12.56±0.25

RA: Reference Antibiotic

IV. CONCLUSION

It was found that the methanolic extract of *Adennthera pavonina* showed a more potent antibacterial activity than n-hexane extract. The inhibitory effect of the plant extract against bacteria - *Shigella flexneri, Escherichia coli, Staphylococcus aureus* were very promising which indicated the capability of the plant as an effective source of medicine against pathogens of diarrhoea and dysentery. The plant can further be studied for isolation of important chemical constituents which are specific for the antimicrobial activity. The phytochemical analysis showed the presence of tannins, alkaloid, flavonoids and triterpenoids which might be responsible for its potent antibacterial activity. From the above study it can be concluded that the plant is potent for the pharmaceutical industry and needs further investigation and study.

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