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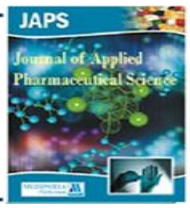

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Evaluation of phytochemical analysis and antibacterial activity of *Bauhinia purpurea* L. and *Hiptage benghalensis* L. Kurz

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ABSTRACT

Phytochemicals from the leaves and stem bark of *Bauhinia purpurea* and *Hiptage benghalensis* were extracted using different solvents of various polarities such as petroleum ether, chloroform, acetone, methanol and water. The antibacterial activity was carried out against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* by disc diffusion method. Among the solvent extracts, methanol extract was most effective against the tested microorganisms. Phytochemical analysis revealed the presence of alkaloids, coumarin, flavonoids, phenols, tannins and terpenoids.

Keywords: Phytochemicals, antibacterial activity, disc diffusion, *Hiptage benghalensis, Bauhinia purpurea.*

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant based traditional medicine system continues to play an essential role in health care, with about 80% of the worlds inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.,* 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.,* 2000). Recently, scientific interest in medicinal plants has burgeoned due to the increased efficiency of plant derived drugs and raising concern about the side effects of modern medicine. The efficacy of current antimicrobial agents has been reduced due to the continuing emergence of drug resistant organisms and the adaptations by microbial pathogens to commonly used antimicrobials. Therefore, the search for new drugs from plants continues to be a major source of commercial drugs. Plant based antimicrobials represent a vast untapped source of medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur (Parekh *et al.,* 2007). The screening of plant extracts and their products for antimicrobial activity has shown that, higher plants represent potential sources of novel antibiotic prototypes (Afolayan, 2003). Even though hundreds of plants species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated

(Balandrin *et al.,* 1985). Considering this, an attempt has been made to investigate the phytochemical screening and antibacterial activity of petroleum ether, chloroform, acetone, methanol, and aqueous extracts of leaf and stem bark of *Bauhinia purpurea* L. and *Hiptage benghalensis* (L.) Kurz.

MATERIALS AND METHODS

Collection of plant materials

The leaf and stem bark materials of *Bauhinia purpurea* (L.) and *Hiptage benghalensis* (L.) Kurz. were collected from the well grown plants in Grizzled Giant Squirrel Wildlife Sanctuary, Western Ghats, Srivilliputhur, Tamil Nadu. They were shade dried at room temperature for 10-15 days.

Extraction of plant material

Various organic solvents were used for the extraction of bioactive compounds. The leaf and stem bark powders (10g) of *Bauhinia purpurea* and *Hiptage benghalensis* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Bauhinia purpurea* and *Hiptage benghalensis* were dried and successfully extracted with petroleum ether, chloroform, acetone, methanol and then water in a Soxhlet apparatus. The extracts obtained were completely evaporated by using vacuum rotary evaporator. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Brindha *et al*. 1981, Anonymous 1996, Lala 1993*).*The concentrated extracts were used for antibacterial activity.

Microorganisms

Bacterial strains of *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 109), *Bacillus subtilis* (MTCC 441), *Escherichia coli* (MTCC 424), *Pseudomonas aeruginosa* (MTCC 443) and *Salmonella typhi* (MTCC 531) were produced from microbial type culture collection, Chandigarh. The bacteria were incubated on a nutrient agar-slant (stationary cultures) for 48h at 37^{o}C followed by inoculation in Muller Hinton Agar (MHA) medium.

Antibacterial assay

Antibacterial activity was demonstrated using a modification of the method originally described by (Bauer *et al.* 1966) which is widely used for the antibacterial susceptibility testing (Barry and Thornsberry 1985). A loopful bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20µl) various crude solvent extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram positive and Gram negative bacteria. Respective solvents without plant extracts served as negative control. Standard antibiotics of chloramphenicol and tetracycline were used as reference or positive control. Plates were incubated at 37^{o}C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs.

RESULT AND DISCUSSION

Phytochemical study of the methanol extract of leaf and stem bark of *Bauhinia purpurea* indicated the presence of alkaloids, coumarin, flavonoids (Table 1) whereas, the methanol extract of leaf and stem bark of *Hiptage benghalensis* revealed the presence of alkaloids, anthraquinones, catechin, coumarin, flavonoids, phenols, steroids, tannins, terpenoids and xanthoprotein (Table 2).

Table 1: Preliminary phytochemical screening of leaf and stem bark extract of *Bauhinia purpurea.*

Presence/absence of bioactive componens	Name of the extract									
	Petroleum ether		chloroform		Acetone		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	+	-	-	-	-	+	+	-	-
Anthraquinones	-	-	+	+	+	-	-	-	+	-
Catechin	-	-	+	+	-	-	-	-	+	-
Coumarin	+	+	-	-	-	-	+	+	-	-
Flavonoids	-	-	-	-	+	+	+	+	-	-
Phenols	+	+	+	+	-	-	+	+	-	-
Quinones	+	+	-	-	-	-	+	+	+	+
Saponins	+	-	+	-	+	+	+	+	-	-
Steroids	+	+	+	+	-	-	+	+	-	-
Tannins	-	-	-	-	+	+	+	+	+	+
Terpenoids	-	-	+	+	-	+	+	+	+	-
Xanthoprotein	-	-	+	+	-	-	+	-	-	-
Sugar	+	+	+	-	+	+	+	+	+	+

+ denotes: presence; - denotes: absence

Table2: Preliminary phytochemical screening of leaf and stem bark extract of *Hiptage benghalensis.*

Presence/absence of bioactive componens	Name of the extract									
	Petroleum ether		chloroform		Acetone		Methanol		Water	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Alkaloids	-	-	-	-	-	-	+	+	-	-
Anthraquinones	-	-	-	-	+	+	+	+	-	-
Catechin	-	-	-	-	+	-	+	+	-	-
Coumarin	-	-	+	+	+	+	+	+	-	-
Flavonoids	-	-	-	-	-	-	+	+	-	-
Phenols	-	-	+	+	+	+	+	+	-	-
Quinones	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	-	-	+	-	-	-	+	-
Steroids	+	+	+	+	-	-	+	+	-	-
Tannins	+	+	-	+	-	+	+	+	-	-
Terpenoids	-	-	-	-	-	-	+	+	-	-
Xanthoprotein	+	+	-	+	+	+	+	+	+	-
Sugar	-	-	+	+	+	+	+	-	-	-

+ denotes: presence; - denotes: absence

The leaf and stem bark extracts of *Bauhinia purpurea* and *Hiptage benghalensis* were tested for their antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus*

Table 3: Antibacterial activity of leaf and stem bark of *Bauhinia purpurea* and *Hiptage benghalensis.*

Name of the extract	Plant Name	Plant part & Antibiotic	Zone of inhibition (mm)					
			S.aureus	***K.pneumoniae***	***B.subtilis***	***E.coli***	***P. aeruginosa***	***S. typhi***
Petroleum ether	*Bauhinia purpurea*	L	0	1	3	3	4	0
		SB	2	2	2	1	1	0
		T	10	9	10	9	8	10
		C	8	10	11	10	10	11
	Hiptage benghalensis	L	3	0	1	0	1	2
		SB	0	2	0	4	0	3
		T	9	8	9	9	9	9
		C	9	9	9	9	9	9
Chloroform	*Bauhinia purpurea*	L	0	2	3	3	0	1
		SB	2	0	6	0	4	0
		T	10	10	8	10	9	9
		C	10	9	9	10	9	10
	Hiptage benghalensis	L	5	4	0	6	3	5
		SB	2	4	0	1	0	3
		T	9	9	9	8	9	9
		C	9	9	9	9	9	9
Acetone	*Bauhinia purpurea*	L	3	0	2	1	0	2
		SB	1	2	0	3	0	4
		T	9	8	9	8	9	9
		C	9	9	8	9	9	9
	Hiptage benghalensis	L	2	4	0	2	2	1
		SB	0	2	1	0	1	3
		T	8	8	8	9	9	9
		C	9	9	8	9	9	9
Methanol	*Bauhinia purpurea*	L	2	2	6	4	1	2
		SB	6	3	5	3	3	4
		T	9	9	10	9	9	9
		C	9	10	9	9	10	9
	Hiptage benghalensis	L	4	5	3	3	0	4
		SB	4	3	2	4	1	3
		T	9	9	8	9	9	9
		C	9	9	9	9	9	9
Water	*Bauhinia purpurea*	L	2	0	1	0	0	1
		SB	0	1	3	2	0	2
		T	8	8	9	8	9	9
		C	9	8	9	9	9	9
	Hiptage benghalensis	L	1	2	1	1	2	1
		SB	0	1	0	0	2	0
		T	9	9	8	9	9	8
		C	9	9	9	9	9	9

L- leaf SB- Stem Bark T- Tetracycline C- Chloramphenicol

subtilis, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* and the result are presented in table 3. All the extracts have exhibited different degrees of antibacterial activity. Petroleum ether extract of *B. purpurea* stem bark showed activity against all the tested pathogenic bacteria, whereas, leaf extract did not inhibit the growth of *S. aureus.* Chloroform extract of *B. purpurea* leaf failed to inhibit the growth of *S. aureus* and *P. aeruginosa;* whereas, stem extract did not inhibit the growth of *K. pneumoniae*, *E. coli* and *S. typhi*. Acetone extract of *B. purpurea* leaf showed moderate antibacterial activity against *S. aureus*, *B. subtilis*, *E.coli, S. typhi* and stem bark extract was moderately effective against *S. aureus, K. pneumoniae, E. coli* and *S. typhi*. Methanol extracts of leaf and stem bark of *Bauhinia purpurea* exhibited activity against all the tested pathogenic bacteria. Methanol extracts of leaf and stem bark of *B. purpurea* showed the highest inhibition zone against *B. subtilis* and *S. aureus* respectively. Chloroform extract of stem bark of *B. purpurea* showed maximum activity against *B. subtilis.*

Petroleum ether extract of *Hiptage benghalensis* leaf showed antibacterial activity against *S. aureus, B. subtilis, P. aeruginosa and S. typhi;* whereas, stem bark extract showed activity against *K. pneumoniae, E. coli* and *S. typhi.* Chloroform extract of leaf of *H. benghalensis* exhibited antibacterial activity against *S. aureus, K. pneumoniae, E. coli, P. aeruginosa* and *S. typhi,* whereas, stem bark extract did not inhibit the growth of *B. subtilis* and *P. aeruginosa.* Acetone extract of leaf showed the activity against all the tested pathogenic bacteria except *B. subtilis*, whereas, stem bark extract did not inhibit the growth of *S. aureus*, and *E. coli*. Methanol extracts of leaf and stem bark of *Hiptage benghalensis* exhibited activity against all the tested pathogenic bacteria. Chloroform extract of leaf of *Hiptage benghalensis* showed the maximum inhibition zone against *E. coli.* Aqueous extracts of both the plants were observed least inhibition against all the tested pathogenic bacteria. Antibacterial activity was comparable with that of standard antibacterial agent tetracycline and chloramphenicol against the organisms tested.

The results of the present investigation clearly indicated the antibacterial efficacy of *Bauhinia purpurea* and *Hiptage benghalensis* leaves and stem bark extracts. This activity may be due to the presence of bioactive compounds in the leaves and stem bark. Several phytochemicals like flavonoids, phenols, tannins and terpenoids are effective antimicrobial substances against a wide range of microorganisms. (Tsuchiya *et al.,* 1996; Mason and Wasserman, 1987; Ya *et al.,* 1988). Thus, the study ascertains the value of the plants, which could be of considerable interest to the development of new drugs.

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