ANTIBACTERIAL ACTIVITY OF *MUCUNA ATROPURPUREA.* DC.

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ABSTRACT

In this study, an attempt was made to evaluate the antibacterial assay of hexane, petroleum ether, benzene, methanol and water extracts of root and seed of *Mucuna atropurpurea* used in ethnomedicine by the Palliyar tribals of Grizzled Giant Squirrel Wildlife Sanctuary, Western Ghats, Tamil Nadu. The extracts were tested against *Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* using disc diffusion method. The extracts tested possess various degree of antibacterial activity.

Keywords: *Mucuna atropurpurea, Staphylococcus aureus,* antibacterial activity.

INTRODUCTION

In many parts of the world, medicinal plants are used for antibacterial, antifungal and antiviral activities. These plant extracts were used as a source of medicinal agents to cure urinary tract infections, cervicitis, vaginitis, gastrointestinal disorders and skin infections such as herpes simplex virus type I (Cacers *et al.,* 1990; Meyer *et al.,* 1996). It is necessary from the scientific point of view, to establish a rational relationship between chemical, biological and therapeutical activities of folklore medicine (Gentry, 1993). Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. In recent years, there has been a growing interest to evaluate plants possessing antibacterial activity for various diseases (Clark and Hufford, 1993).

Most of the *Mucuna* species are medicinally valuable. Tribal sectors utilize the leaves, roots, pods, seeds, pod hairs etc for treating their ailments. About five hundred grams of dried seeds of *Mucuna atropurpurea* are made into fine powder and boiled in water along with equal quantity of the powder made from the dried seeds of tamarind to make a paste. This paste is applied on the fractured area and bandaged tightly using a clean cloth. After a week, the bandage is removed and the paste is applied for rebinding. This procedure is repeated for three to four times for proper setting of the fractured bone by the Palliyar tribals of Grizzled Giant Squirrel Wildlife Sanctuary, Tamil Nadu (Muthukumarasamy, 2004). In review of the above said medicinal properties, the present study was designed to investigate the antibacterial activity of root and seed extracts of *Mucuna atropurpurea*.

MATERIALS AND METHODS

Collection of Plant Materials

The root and seed materials of *Mucuna atropurpurea* 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 root and seed powders (10g) of *Mucuna atropurpurea* were first extracted with petroleum ether for defatting in a Soxhlet apparatus. The defatted powdered sample of *Mucuna atropurpurea* were dried and successfully extracted with hexane, petroleum ether, benzene, 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, 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*., (1996) which is widely used for the antibacterial susceptibility testing (Barry and Thornsberry, 1985). A loopful bacterium 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.

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extracts of root and seed of *Mucuna atropurpurea* revealed the presence of alkaloid, anthraquinone, coumarin, phenols, steroids, tannins, saponins and flavonoids (Table 1). The results of the antibacterial activity of the plant extracts were tabulated in table 2.

Table 1: Preliminary phytochemical screening of root and seed extracts of *Mucuna atropurpurea*

Presence/absence Of bioactive componens	Name of the extract									
	Hexane		Petroleum ether		Benzene		Methanol		Water	
	Root	Seed	Root	Seed	Root	Seed	Root	Seed	Root	Seed
Alkaloids	+	+	+	+	+	+	+	+	-	-
Anthraquinones	-	-	-	-	+	-	+	+	-	-
Catachin	-	-	-	-	-	-	-	+	-	-
Coumarin	-	-	+	-	+	+	-	-	-	-
Flavonoids	+	-	-	-	-	-	+	-	-	-
Phenols	+	+	+	-	+	+	+	+	-	+
Quinones	+	-	-	-	-	-	-	-	+	-
Saponins	-	-	-	-	-	-	+	-	+	-
Steroids	+	+	+	+	-	-	-	+	-	-
Sugar	+	-	-	-	+	+	+	-	+	+
Tannins	+	-	-	-	-	-	+	+	-	-
Terpenoids	+	+	-	-	-	-	+	+	-	-
Xanthoprotein	+	+	+	+	-	-	+	+	-	-

+ Presence - Absence

Table 2: Antibacterial activity of root and seed extracts of *Mucuna atropurpurea.*

Name of the extract	Plant part & (Antibiotic)	Zone of inhibitor (mm)					
		S.aureus	K. pneumoniae	B.subtillis	P.aeruginosa	S.typhi	E.coli
Hexane	R	3	-	3	-	3	1
	S	3	3	2	-	3	2
	T	8	9	8	9	9	9
	C	9	9	9	9	9	8
Petroleum ether	R	2	3	3	-	-	4
	S	4	-	-	2	3	1
	T	9	9	8	8	9	8
	C	9	8	9	8	9	8
Benzene	R	3	4	6	2	-	3
	S	2	2	3	4	3	5
	T	9	9	9	9	8	9
	C	9	8	9	9	9	9
Methanol	R	3	2	2	-	2	4
	S	6	5	4	6	3	2
	T	8	9	9	9	9	8
	C	9	9	9	9	8	9
Water	R	1	2	1	-	-	1
	S	-	-	1	1	2	2
	T	9	8	9	9	9	8
	C	9	9	8	9	9	9

R- Root S- Seed T- Tetracycline C- Chloramphenicol

All the extracts have exhibited different degrees of antibacterial activity. Methanol and benzene extracts of seed of *M. atropurpurea* showed activity against all the tested pathogenic bacteria, whereas, the methanol and benzene extracts of root did not inhibit *Pseudomonas aeruginosa* and *Salmonella typhi* respectively. Petroleum ether extract of *M. atropurpurea* seed showed activity against all the used pathogenic bacteria except *Klebsiella pneumonia*e and *Bacillus subtilis*, while the root extract failed to inhibit the growth of *Pseudomonas aeruginosa* and *Salmonella typhi*. Similarly, the hexane extract of *M. atropurpurea* seed showed antibacterial activity against all the tested pathogenic bacteria except *Pseudomonas aeruginosa*; but the root extract failed to inhibit the growth of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* The aqueous extract of *M. atropurpurea* seed showed activity against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi,* and *Escherichia coli,* whereas, the aqueous root extract of *M. atropurpurea* did not inhibit *Pseudomonas aeruginosa* and *Salmonella typhi.* The methanol extract of *M. atropurpurea* seed showed high degree of inhibition against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The root extract of benzene also showed high degree of inhibition against *Bacillus subtilis.* Antibacterial activity was comparable with that of the standard antibacterial agent tetracycline and chloramphenicol against the organisms tested. These observations suggest that, the aqueous and organic extracts from the same plants showed different activities. There is no common rule for this, but in most cases, the organic extracts showed the same or greater activity than the aqueous extracts. In addition, the effectiveness of plant was not due to one main active constituent, but due to the combined action of other chemical compounds involved in it (Bai, 1990). The antibacterial activities of the *M. atropurpurea* plant may be attributed to the presence of bioactive compounds such as phenols,

saponins, steroids, alkaloids and flavonoids as suggested by several literatures. Hence, it is necessary for isolation and identification of the compounds of *M. atropurpurea* extracts responsible for the antibacterial activity. Therefore, the active component of the *M. atropurpurea* extract could be of interest for further development as an antibacterial agent.

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