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ANTIBACTERIAL ACTIVITY OF PAKISTANI *RHAZYA STRICTA*

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The crude ethanolic extract of *Rhazya stricta* Dcne; (Family, Apocynaceae) was tested, on the basis of medicinal and folklore reports for antimicrobial activity against a wide range of gram - positive and gram - negative organisms. Leaves extract was found to be more active as compared to other parts exhibiting 69.23% and 66.66% activity against gram - positive and gram - negative organisms respectively. Seeds extract exhibited maximum inhibitory activity ("A" category zone) i.e. 23.07% and 16.66% against gram - positive and gram - negative organisms respectively as compared to other parts of *Rhazya stricta*. Reference standard i.e. co-trimoxazole exhibited only 7.69% "A" category zones against gram - positive organisms only.

Key words: *Rhazya stricta*, Apocynaceae, Antibacterial activity.

Introduction

Recognition, importance, necessity and potentiality of medicinal plants in present day practice cannot be overlooked. They are still being used as a source of medicament for a variety of diseases, not only by rural population but also by socio - cultural system of people with fair amount of success having no scientific information.

Rhazya stricta Dcne., commonly known as Sundwar, Sewar or Gandera, belongs to the family Apocynaceae - an alkaloids bearing family. It comprises of 300 genera and 200 species. Found abundantly in Pakistan and is an endemic flora of Sind (Hooker 1875, Salimuzzaman *et al* 1966, Baqar 1967 & 1989 and Khan *et al* 1979). The plant is well known for its antitumor activity (Watt 1892 & Sarfaraz *et al* 1972). In indigenous system of treatment, the plant is used as an effective remedy for boils, eruption, syphilis, sore throat, fever, rheumatism, pain and painful affections and in general debility (Watt 1892, Kirtikar *et al* 1933 & Chopra *et al* 1956).

Earlier studies indicates that the plant has been a subject of number of chemical investigations, which have revealed the presence of a large number of indole alkaloids whose structure have been elucidated using modern spectroscopic techniques (Chatterjee *et al* 1961, Salimuzzaman 1966, Mukhopadhayay *et al* 1981, 1983 and Atta -ur- Rahman *et al* 1982, 1989 & 1995; Gerasimenko *et al* 2001, Stockigt *et al* 2002). These alkaloids are reported to exhibit hypotensive, antispasmodic, antidiabetic (Tanira *et al* 1996 and Ali *et al* 1997) anti-inflammatory (Wasfi *et al* 1995) antimicrobial (Mariee *et al* 1988, Bashir *et al* 1992 & 1994) and antineoplastic (Mukhopadhyay *et al* 1981 & 1983) activities.

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Keeping in view, the reported medicinal properties related to this plant, it was thought to exploit *Rhazya stricta* plant biologically for its antimicrobial activity. The present work involves the antibacterial activity of different parts i.e. leaves, roots, fruits and seeds of *Rhazya stricta* plant. This plant is also available in other parts of the world. On the other hand, it will also give a new challenge to strike new sources of medications as claimed by folklore practitioners on firm footed scientific evaluations.

Materials and Methods

Collection and identification of plant material. The plant material was collected from the suburbs of Karachi. Properly identified by a taxonomist and a voucher specimen was deposited in the herbarium of Applied Biology Research Centre, PCSIR Laboratories Complex, Karachi.

Preparation of extract. Each part of the collected plant material i.e. leaves, roots, fruits and seeds were individually washed and dried in oven at 45˚C ± 5˚C. The dried and milled part of *Rhazya stricta* was extracted with 95.00 % ethyl alcohol (100 g per 1.25 lit) at room temperature. Solvent was allowed to remain in contact with each part of plant material for 48 h with continuous agitation for 6 h per day, then decanted and pooled. Process was repeated thrice in order to obtain the maximum quantity of the extract. Pooled solvent was removed under vacuum at 40˚C ± 1˚C. This afforded a crude ethanolic extract. Percentage yield of each part i.e. leaves, roots, fruits and seeds was calculated as 7.40%, 5.20%, 3.20% and 2.10% respectively.

Preparation of solution. All extracts were dissolved in an aqueous ethanolic solution (25.00%) with 2.50% Triton as an

emulsifier to give a strength of 100 mg / ml. Co-trimoxazole was used as a reference standard due to its wide range of effectivity against gram - positive and gram - negative group of organisms.

Micro-organisms used. The said activity was assessed against gram - positive (13) and gram - negative (24) organisms. All the test organisms used in the present study were clinical isolates and obtained from the Department of Microbiology, University of Karachi. The organisms were maintained on Tryptic soya agar slants (Merck) at 4°C prior to testing. Test culture inocula was prepared by using Tryptic soya broth culture medium maintained at 37°C ± 1°C for 24 h.

Antibacterial activity. Antibacterial was carried out by hole plate diffusion method using 0.5 ml of the inoculum containing 10^5 bacterial cells, respectively. The inoculum was thoroughly mixed with 25 ml of melted sterile liquid Tryptic soya agar and poured into pre - sterilized petri dishes respectively. Plates were left to set at 4°C for one h. Holes of 6mm diameter were made from the centre of each seeded plate. Holes were then filled aseptically with 0.2 ml (20mg) of test solution (various parts of plant extracts), reference standard and negative control (solvent only) respectively and marked accordingly. All plates were then incubated at 37°C ± 1°C for 24 h and zones of inhibition were measured and recorded accordingly. The above experiment was repeated thrice.

Results and Discussion

Alcoholic extracts of various parts of *Rhazya stricta* showed a varied degree of antibacterial activity against wide range of gram - positive and gram - negative organisms as shown in Table 1 and 2, while Table 3 represent a comparative data on different parts of *Rhazya stricta* according to the zone of categorization in percentage. Negative control exhibited no activity against gram - positive and gram - negative organisms. Extracts exhibiting zone of inhibition less than 9mm were taken as negative.

The study gives a preliminary account of antibacterial substances present in different parts i.e. leaves, roots, fruits and seeds of *Rhazya stricta* plant. The considerable difference observed in the potency of activity amoung different parts of *Rhazya stricta*, clearly indicates that there exists a marked difference in active component in various parts of the plant. In general, leaves extracts exhibited a pronounced maximum activity i.e. 66.66% against gram - negative group and 69.23% against gram - positive groups, while root extract exhibited miximum activity i.e. 33.33% against gram - negative and 23.07% against gram - positive organisms respectively. Seed portion exhibited 46.15% and 54.16% and fruit 46.15% and 41.66% activity respectively against gram - positive and gram-negative organisms. Negative control was found to be non-active against both gram positive and negative group of orga-

Table 1

Comparative antibacterial study of different parts of *Rhazya stricta* (leaves, roots, fruits & seeds) and co-trimoxazole against gram - positive organisms according to zone of inhibition (mm)

S.No.	Name of organism	Zone of inhibition					
		Rhazya stricta				Standard	Negative
		Leaves	Roots	Fruits	Seeds	Co - trimoxazole	control
1.	*Bacillus subtilis*	B	-	-	-	-	-
2.	*Cornybacterium diptheriae*	C	-	-	-	B	-
3.	*Cornybacterium xerosis*	-	-	-	-	-	-
4.	*Micrococcus roseus*	-	-	-	-	C	-
5.	*Micrococcus variance*	A	-	-	-	A	-
6.	*Staphylococcus aureus*	D	-	-	-	B	-
7.	*Staphylococcus aureus*	B	D	C	B	B	-
8.	*Staphylococcus aureus*	-	-	C	C	-	-
9	*Staphylococcus epidermidis*	-	-	-	-	C	-
10.	*Staphylococcus saprophyticus* (coagulase + ve)	C	-	B	D	C	-
11.	*Streptococcus faecalis*	C	-	B	A	C	-
12.	*Streptococcus pyogenes*	B	B	B	A	C	-
13.	*Streptococcus viridans*	B	B	A	A	C	-
Total	Percent Activity	69.23%	23.07%	46.15%	46.15%	76.92%	

Categorization of zone of inhibition in mm: A = 40 - 49; B = 30 - 39; C = 20 - 29; D = 10 - 19.
Zone of inhibition from 6 - 9 mm were considered as negative.

Table 2

Comparative antibacterial study of different parts of *Rhazya stricta* (leaves, roots, fruits & seeds) and co - trimoxazole against gram - negative organisms according to zone of inhibition (mm)

S.No.	Name of organism	Zone of Inhibition					
		Rhazya stricta				Standard	Negative
		Leaves	Roots	Fruits	Seeds	Co - trimoxazole	control
1.	*Acinetobacter calcoaceticus*	-	C	B	C	-	-
2.	*Aeromonas hydrophilia*	-	D	D	C	-	-
3.	*Actinobacillus lignierersii*	-	-	-	-	-	-
4.	*Branhamella catarrhalis*	B	B	B	B	-	-
5.	*Citrobacter diversican*	-	-	-	A	-	-
6.	*Citrobacter freundii*	B	-	-	B	D	-
7.	*Escherichia coli (Communior)*	D	-	-	-	D	-
8.	*Escherichia coli (Communis)*	-	-	-	-	D	-
9	*Klebsiella pneumoniae*	A	B	B	A	-	-
10.	*Klebsiella ozaenae*	B	-	-	-	-	-
11.	*Proteus mirabilis*	B	B	B	A	C	-
12.	*Proteus vulgaris*	-	-	-	-	D	-
13.	*Pseudomonas aeroginosa*	A	D	D	C	B	-
14.	*Pseudomonas mallei*	-	-	-	-	-	-
15.	*Pseudomonas maltophilia*	A	C	C	A	-	-
16.	*Salmonella para typhi A*	B	-	C	D	-	-
17.	*Salmonella para typhi B*	C	-	-	-	B	-
18.	*Salmonella typhi*	C	-	-	-	B	-
19.	*Shigella bodydii*	C	-	-	-	B	-
20.	*Shigella dysenteriae*	C	-	-	D	C	-
21.	*Shigella flexaneriae*	C	B	-	-	C	-
22.	*Shigella sonnei*	-	-	D	B	C	-
23	*Vibrio cholera eltor*	B	-	-	-	D	-
24.	*Vibrio cholera inaba*	B	-	-	-	D	-
Total	Percent activity	66.66	33.33	41.66	54.16	54.16	Nil

Categorization of zone of inhibition in mm A= 40 - 49; B = 30 - 39; C = 20 - 29; D = 10 - 19.
Zone of inhibition from 6 - 9 mm were considered as negative.

nisms. Reference standard exhibit 76.92% and 54.16% activity against gram - positive and gram - negative group of organisms respectively (Table 1 & 2).

According to Table 3, based on zone of categorization, the highest activity was exhibited by seeds extract i.e. 23.07% and 16.66% against gram - positive and gram - negative organisms respectively, while reference standard i.e. co-trimoxazole fails to exhibit 'A' category activity against gram - negative group of organisms and 7.69% against gram - positive group of organisms. It is evident from the collected data that activity of various parts of *Rhazya stricta* plant when categorized according to the zone of inhibition falls mostly in A and B category while on the other hand reference standard i.e. co-trimoxazole mostly in "C" category (Table 3).

The difference in activity among test i.e. *Rhazya stricta* and reference standard i.e. co - trimoxazole is basically due to the fact that most of the organisms are gradually becoming less sensitive due to wide spread use of antibiotics while on the other hand, various parts of *Rhazya stricta*, present a good potency of antibacterial activity. Furthermore, the antibacterial activity assessed also confirms the folklore use of the plants and its various parts against various infectious diseases such as boils, eruption, fever, sore throat, inflammation and in anti - neoplastic diseases. (Watt 1892, Kirtikar *et al* 1993

Table 3

Comparative study of different parts (leaves, roots, fruits and seeds) of *Rhazya stricta* against gram - positive and gram - negative organisms according to zone of categorization.

Categorization index	% Zone of inhibition									
	Gram positive					Gram negative				
	Leaves	Roots	Fruits	Seeds	Ref.Std.	Leaves	Roots	Fruits	Seeds	Ref.Std.
A	7.69	-	7.69	23.07	7.69	8.33	-	-	16.66	-
B	30.76	15.38	23.07	7.69	23.07	29.16	16.66	16.66	12.50	12.50
C	23.07	-	15.38	7.69	46.15	25.00	8.33	12.50	16.66	16.66
D	7.69	7.69	-	7.69	-	4.16	8.33	12.50	8.33	25.00
Total activity (%)	69.21	23.07	46.14	46.14	76.91	66.65	33.32	41.66	54.15	54.16

and Chopra *et al* 1956). The antibacterial activity exhibited can be attributed due to the wide spread occurrence of alkaloids, tannins, chrysophenol and trace metal contents present in *Rhazya stricta* plant (Philipson *et al* 1984; Atta - ur - Rehman *et al* 1989 and Kaneez *et al* 1999). It can be concluded that different parts of *Rhazya stricta* may be used as a good antibacterial agent drug in the treatment of infectious diseases, provided it is found effective and safe in *in - vivo* study.

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