Evaluating the Antibacterial Activities of *Rhazya stricta* Decne

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ABSTRACT

Current study was conducted to discover better and safer plant oriented chemotherapeutic agent. *Rhazya stricta* Decne is widely used for medicinal purposes throughout the world and we examined the effect of methanolic, ethyl acetate and chloroform extracts of *Rhazya stricta* stem and leaves on growth inhibition of various bacterial strains viz. *Escherichia coli*, *Staphylococcus aurous* and *Bacillus subtilis*. Agar well diffusion method was followed in this study. The ethyl acetate fraction of leaves and chloroform fraction of stem exhibit high zone of inhibition against *E. coli*. The chloroform fraction of stem of *Rhazya stricta* showed least activity against S*taphylococcus aurous.* The entire fractions showed no significant activity against *Bacillus subtilis.* It was thus concluded that the methanolic extract of *Rhazya stricta* possesses strong antibacterial capacity and plant can be efficiently used for curing bacterial diseases.

Keywords: *Rhazya stricta*, methanol, *Escherichia coli*, *Staphylococcus aurous*, *Bacillus subtilis.*

Introduction

Plants have been used throughout the world (including Asian and Pacific countries) in folk medicine and as local cures for common ailments since ancient times. Recently, the World Health Organization reported that 75-95% of the world's population of developing countries were chiefly rely on traditional medicines and major part of traditional therapies involved the use of plant extract products or their active constituents [13]. Traditional medicine usage is a common practice in developed and developing countries at the primary healthcare level [9].

Plant extracts and essential oils have been used as alternatives to antibiotic due to their antimicrobial activities [3]. All plants produce chemical

compounds as part of their normal metabolic activities. These are divided into primary metabolites, such as sugars and fats, found in all plants, and secondary metabolites, compounds not essential for basic function found in a smaller range of plants, some useful ones found only in a particular genus or species.

Bacterial resistance to currently used antibiotics is becoming a concern to public health [14]. The development of bacterial super resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the research is going on for new antimicrobial agents, either by designing and synthesis of new agents, or through search of natural sources for as yet undiscovered antimicrobial agents [4]. Herbal medications in particular have seen a revival of interest [5] due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals.

Rhazya stricta Decne, locally known as Harmal, is a member of family Apocynaceae. It is widely distributed throughout Western Asia from Yemen to Arabia, to the North-West of Pakistan. *Rhazya stricta* Decne is a small glabrous erect shrub with a smooth central stem and dense semi-erect branches. *Rhazya stricta Decne* is a stiff-growing plant with erect stems, 2-3 feet high and upright thickish smooth leaves placed close together on the stem [16]. The plant is used in this region, mostly in the form of decoctions, for a variety of unrelated illnesses that include diabetes mellitus, fever, sore throat, inflammatory conditions and helminthiasis [2]. In the rural areas of Saudi Arabia, the leaves of *R. stricta* are used in folk medicine as a reputed bitter tonic and a curative for *syphilis* [1]. Its infusion is a good tonic with peculiar bitter taste. Phytochemical analysis has identified more than 100 alkaloids [10]. These alkaloids have several pharmacological properties. It is also used for throat sour, in fever, general debility and as curative for chronic rheumatism and tumor. *Rhazya stricta* is used traditionally in Asia for the treatment of different types of diseases such as skin diseases, stomach diseases and antihypertensive [15]. The leaves, flowers and fruit are also used in joint

infections and for cancer (Rahman and Qureshi, 1990).

Materials and Methods

Collection of Plant Material

The plant material (stem and leaves) was collected from a village Garu situated in Nizampur, District Nowshera, Khyber Pakhtunkhwa, Pakistan. Plant specimens were identified in the Department of Botany, Abdul Wali Khan University, Mardan. Leaves and stem of *Rhazya stricta* was collected and then shade dried at room temperature. The dried plant was finally grinded by grinder machine. The powdered plant obtained was then soaked in Methanol for extraction. After this period extract was concentrated by evaporating solvents using rotary vacuum evaporator under reduced pressure at temperature below 55°C. This process was repeated two times until the extraction was completed and residue was obtained. The residue was then fractionated by using different organic compounds i.e. Chloroform, n-hexane and Ethyl acetate for evaluating antimicrobial activity.

The experiments were performed in microbial free environment in laminar air flow cabinet and all glassware was properly sterilized. Solvent free plant extracts were stored at room temperature. Microbial strains were collected from Phytomedicine and Biochemistry Lab, Department of Chemistry, University of Peshawar. These strains were already identified from National Institute of Health; Islamabad. The following work was carried out in the PNRL (Phytomedicine and Organic Biochemistry Lab) Department of Chemistry, University of Peshawar.

Antibacterial Assay:

Antibacterial assay was carried out by agar well diffusion method [Atta-ur-Rehman *et al*., 1999]. Crude extract and fractions were used in dose of 3 mg/mL of Di Methyl Sulpho Oxide (DMSO) and pure compounds were used in dose of 1 mg/mL.

Antibacterial activity was studied against various human pathogens including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. In this bio assay, three types of media were used viz. solid medium (nutrient agar), semi-solid medium

(soft agar) and liquid medium (nutrient broth).

Nutrient Agar:

28 gms nutrient agar was dissolved in distilled water and volume was made up to 1 liter. It was then placed in autoclave at 121^{o}C for 15 minutes. Media was then cooled to 4^{o}C and poured in sterile petriplates and media was then allowed to solidify at room temperature.

Soft Agar:

0.8 gms soft agar was dissolved in distilled water and volume was made up to 100 ml. it was then dispensed in 7 ml quantity to screw capped test tubes. These test tubes were placed in autoclave at 121^{o}C for 15 minutes and then refrigerated.

Nutrient Broth:

0.8 gms nutrient broth was dissolved in distilled water and volume was made upto 100 ml. prepared broth was dispensed in 3 ml quantity to screw capped test tubes, which were placed in autoclave at 121^{o}C for 15 minutes and then refrigerated.

Procedure:

The culture of bacterial organisms was maintained on stock culture agar. A colony of bacterial culture was inoculated in nutrient broth and incubate at 37±1^{0}C for 24 hours. Next day soft agar was melted and cooled to 40^{0}C and then added 100 µL of bacterial culture, shaken well and then poured it on nutrient agar containing plate. The plate was rotated to make even distribution of culture and allowed to solidify the lawn. Wells (6 mm diameter) were made in medium in each plate using a sterile metallic borer with centers at least 24 mm apart. Samples (3 mg/ml of DMSO) were then added in respective well using sterilized dropping pipettes. Other wells supplemented with DMSO and reference anti bacterial drug served as negative and positive control, respectively. Plates were then incubated at 37±1^{0} C for 24 hours. Activity was determined by measuring diameter of zones depicting inhibition (mm). Antibacterial potential of sample was then determined as per criteria mentioned in Table 2.8. Percent growth inhibition was calculated with reference to the positive control.

Table 2.8: Criteria for determining antibacterial assay

S.No	Diameter	Status of Activity
1	Below 9 mm	No activity
2	9-12 mm	Non-Significant
3	12-15 mm	Low
4	16-18 mm	Good
5	Above 18 mm	Significant

Determination of antibacterial activity:

Agar well diffusion method was used and in this method wells were drugged in the media with the help of sterile metallic borer with centers at least 24mm apart. 2-8 hrs old bacterial culture approx.. 10 colony forming units were spread on the surface of nutrient agar with help of sterile cotton swab. Recommended concentration of the test sample is then added in the respective wells. The plates were incubated at 37^{o}C for overnight.

Results and Discussion

In current study crude extracts of *Rhazya stricta* was obtained using methanol, then further fractioned with n-hexane, ethyl acetate, chloroform, and water. These fractions were tested against gram negative and gram positive bacteria.

The chloroform fraction of plant stem showed lower activity against *Staphylococcus aureus* (Gram positive bacterial pathogens), higher antibacterial activity against *Escherichia coli* (Gram negative bacteria), and least antibacterial activity was observed for *Bacillus subtilis* (Table 1). In [12] work high antibacterial activity against *Staphylococcus aurous* was recorded in chloroform extract of *Tribulus lanuginosus, Hygrophila auriculata* and *Aerva lanata* plants.

Table 1: Effect of chloroform fraction of *Rhazya stricta* against selected bacterial species

S.No	Bacterial Species	Control DMSO	Streptomycin (Control)	*Rhazya stricta* Leaves	*Rhazya stricta* Stem
1	*Escherichia coli*	0	30	0	15
2	*Bacillus sutilis*	0	28	0	12
3	*Staphylococcus aurous*	0	28	10	13

We also observed that the chloroform extracts obtained from leaves showed no significant growth inhibition of selected bacterial species. Similarly ethyl acetate fraction of stem showed low activity against *E. coli*, while no significant activity against *Staphylococcus aureus* and *Bacillus subtilis* (Table 2). [8] studied same activity against *Staphylococcus aureus* using etanolic extracts of different medicinal plants. The ethyl acetate fraction of leaves also showed good antibacterial against *E.coli* while no activity against *Staphylococcus aureus & Bacillus subtilis.* Results of n-hexane fraction of stem and leaves showed no activity against the tested bacterial species (Table 3).

Table 2: Ethyl acetate fraction of *Rhazya stricta* induces inhibition against selected bacterial species

S.No	Bacterial Species	Control DMSO	Streptomycin (Control)	*Rhazya stricta* Leaves	*Rhazya stricta* Stem
1	*Escherichia coli*	0	30	16	13
2	*Bacillus sutilis*	0	28	10	10

3	*Staphylococcus aurous*	0	28	11	0

Table 3: n-Hexane fraction of *Rhazya stricta* induces inhibition against selected bacterial species

S.No	Bacterial Species	Control DMSO	Streptomycin (Control)	*Rhazya stricta* Leaves	*Rhazya stricta* Stem
1	*Escherichia coli*	0	30	0	08
2	*Bacillus sutilis*	0	28	08	10
3	*Staphylococcus aurous*	0	28	10	0

Methanol fraction of leaves did not show any activity against the given bacterial pathogens (Table 4). While methanol fraction of stem showed low activity against *E.coli* while no activity for the rest of the tested bacterial strains. Research work of [11] exhibited that methanol extract of three medicinal plants (*Blepharis maderasphatensis, Dopteracanthus prostrates* and *Allemandes cathartic*) showed antibacterial activity against *E.coli*. By taking the overall results we concluded that differences were observed between antibacterial activities of the extracts. These differences could be due to the differences in the chemical composition of these extracts as the secondary metabolites of plants have many effects including antibacterial and antiviral properties [17][6]. It was observed that the chloroform fraction of stem and ethyl acetate fraction of leaves of *Rhazya stricta* is more active against *E. coli* as compared to *Staphylococcus aureus* and *Bacillus subtilis.* Therefore, the inhibitory activity found herein against *E. coli* is complementary to Yagoub's [19] report. Results of [7] exhibited that *A. alboviolaceum* and *A. polyanthum*

members of genus *Aframomum* exhibited inhibitory effects against E.Coli. According to [20] antibacterial activity of the genus *Aframomum* was due to the presence of flavonoids, diterpenoids and arylalkaloids. Although ethyl acetate fractions of stem and methanol fraction of leaves also showed low activity against *E.coli* while chloroform fractions of stem showed low activity against *Staphylococcus aurous* .So it may be further investigated in this direction for isolation of useful antibacterial drugs. This study indicated the potentiality of *Rhazya stricta* extracts in curing the diseases caused by *E.coli*.

Table 4: Methanol fraction of *Rhazya stricta* induces inhibition against selected bacterial species

S.No	Bacterial Species	Control DMSO	Streptomycin (Control)	*Rhazya stricta* Leaves	*Rhazya stricta* Stem
1	*Escherichia coli*	0	30	10	13
2	*Bacillus sutilis*	0	28	08	09
3	*Staphylococcus aurous*	0	28	12	10

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