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Original Article

PHYTOCHEMICAL, ANTIMICROBIAL AND HEMOCOMPATIBILITY EVALUATION OF HERBAL PLANT *RHAZYA STRICTA* - AN IN-VITRO STUDY

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ABSTRACT

Plants are among the most important and common sources of potentially valuable drugs. Rhazya Stricta Decne is a traditionally important plant in Oman with medicinal properties. The aim of this study was to determine the antimicrobial activity and hemocompatibility of leaves and stem extracts of R. stricta. Phytochemical investigation of different solvent extracts of the leaves and stems of this plant resulted in the identification of various chemical constituents, such as alkaloids, saponins, glycosides and amino acids. The extracts of leaves and stems were tested against pathogenic bacterial strains using disc diffusion method and agar dilution method. Based on minimum inhibitory concentration (MIC) of the present study, the MIC value of 133 µg/ml was inhibitory to most of the organisms tested. R. stricta has considerable antifungal activity towards skin pathogen Candida albicans. The hemolytic potential of all extracts were evaluated by means of an in-vitro hemolysis assay. Most of the extracts displayed hemolytic effect less than 5%. Our present study supports the traditional claim and usefulness of this plant in Oman.

KEYWORDS: Rhazya Stricta, Extract, Antimicrobial, Hemolytic & Minimum Inhibitory

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1. INTRODUCTION

"Nature Is the Best Physician" portrayed by father of medicine. Since humanity began on the earth, they have had strong relationship with nature. The first great source they can interact with is especially plant. Medicinal plants are the most exclusive source of life-saving drugs for humans, hence all natural products play an important role in modern therapy.

Rhazya stricta Decne (R. stricta) belongs to Apocynaceae family, popularly known as Harmal in GCC. It is widely distributed throughout the world and abundantly found in Western Asia from Yemen to KSA, India and some regions of Pakistan. It is abundant in sandy areas and wadis in Oman. The plant is glabrous shrub with smooth central stem, dense erect branches, often woody. Dry plant is more effective than fresh one, which is generally used in traditional medicine as reputed tonic for diabetes, inflammatory condition and fever, and also as a painkiller and purgative. The plant extract is composed generally of alkaloid, glycoside, tannins, flavonoids and triterpenes (Al-Yahya *et al*., 1990). Phytochemical analysis of *R. stricta* collected from different regions reveals the presence of more than 100 alkaloids with several pharmacological properties (Atalay, 2001). *R. stricta* is considered as an important resource of natural biological compounds useful in human medicine (Baeshin *et al.*, 2008). *R. stricta* has the potential to interact with other drugs that are biotransformed by cytochrome P450, when given additionally with it (El-Kadi *et al.*, 2003). *Rhazya stricta* has been proven to have toxic effect on oral administration when mixed with *N. Oleander* leaves and have proven fatal to animals within 24 hours, with toxic

symptoms (Adam, 1998). In addition, many studies on mice and rats reported the biological effect of the *R. stricta* leaf extracts (El-Kadi *et al.*, 2003).

The spread of microorganisms and their antibiotic resistance is a great threat to the worldwide medical community. There are limited choices of antimicrobial agents for the treatment of many serious life-threatening infections, leading to prolonged stays of victimized individuals in the hospitals and increased care costs (Drew *et al*., 2009). Alternative therapeutic solutions, such as medicinal plants for the treatment of infections and diseases caused by resistant strains may solve the problem of antibiotic drawbacks. Medicinal plants can also be used alongside antibiotics in certain cases as well as alone, depending on the underlying causes and types of diseases. This may consequently lead to the reduction and rationalization of the antibiotics usage. The use of medicinal plants had originated a long time ago for the treatment of infections and diseases (Evans *et al.*, 2007). Therefore, researches and studies of these local plants are essential to reveal more information regarding their therapeutic benefits and potential side effects. Thus, the aim of this preliminary study was to explore the potential antimicrobial activities of the extract of *R. stricta* along with the biocompatibility evaluation of the crude extracts.

2. MATERIALS AND METHODS

2.1 Plant Material and Extraction

The aerial part of *R. stricta* was collected from Muscat region of Oman. It was identified, confirmed and authenticated by a botanical center in Oman. Herbarium is prepared and stored for future reference (BIOL-03-2017). The leaves and stem portions of *R. stricta* were cut into pieces and shade dried at room temperature. The dried leaves and stems were subjected to size reduction (coarse powder) by using dry grinder. Weighed amount (986 g) of the powdered sample was extracted with ethanol, methanol, ethyl acetate, chloroform, hot water and cold water using Soxhlet and simple maceration technique (Azwanida, 2015). The supernatant was concentrated by using Rota vapor and the dried extract was stored in an airtight container.

2.2 Phytochemical Test

The extracts were tested for the presence of glycosides, saponins, flavonoids, proteins, free amino acids, carbohydrates and alkaloids using conventional standard protocols for identifying the presence of phytochemical constituents in the extracts (Harborne, 1998).

2.3 Hemocompatibility Assay

Measuring hemolytic activity is important as it is the best indicator of cytotoxicity. The samples were made by preparing a stock solution of extract using phosphate buffer (PBS) as solvent followed by incubation. Various concentrations of the extract was used for study. Freshly collected RBC were taken and washed three times by 150 mM sodium chloride (NaCl) at 2500 rpm for 5 min. After removing NaCl at last **wash step** the cells were suspended in 100 mM sodium phosphate buffer. The test samples were mixed with 200 µl of RBC solutions and final reaction mixture volume was made up to 1 ml by adding sodium phosphate buffer. The reaction mixture was centrifuged again at 2500 rpm for 5 min. Measure the supernatant absorbance at 492 nm and 595 nm keeping sodium phosphate buffer as blank. The percentage of hemolysis was calculated using a formula. The in-vitro hemolysis test has also been employed by many different groups for their toxicological evaluation. It gives a quantitative measure of the hemoglobin release based on the compatibility of the extract with hemoglobin (Dhanalekshmi *et al*., 2010).

2.4 Antimicrobial Study

All the extracts were tested with solvent control and positive control for antimicrobial activity. Dimethyl formamide was used as a solvent control. The microbes used were gram negative bacteria viz., *E. coli, B. subtilis, P. vulgaris* and gram positive *S. aureus* and the fungi *C. albicans, A. flavus, A. fumigatus and T. mentagrophyte.* All the cultures used in this experiment were obtained from the ATCC Bacteriology Collection.

2.5 Disc Diffusion Method

Disc diffusion method was used for the determination of antimicrobial activity of the extract and the MIC was calculated by agar dilution method. The drug Ciprofloxacin 5 µg/ml was used as a reference drug. Briefly, a suspension of the tested organism was swabbed on Mueller-Hinton agar (MHA) in order to obtain a lawn culture. A filter paper (Whatman no.1) disc of 6 mm diameter, which contained 10 µl of the plant extract was placed on the inoculated plates. The inoculated plates were subsequently incubated at 37˚c for 18 hours. The zone of inhibition was measured in millimeters and compared with standard drug (Mau *et al*., 2001, Smullen *et al*., 2007, NCCLS 2002). The control consists of filter paper disc covered with dimethyl formamide and evaporated to dryness.

2.6 MIC Assay

The agar dilution method recommended by National Committee for Clinical Laboratory Standards was used in this experiment. A series of two-fold dilutions of each extract with dimethyl formamide at a final concentration ranging from 66 µg/ml to 333 µg/ml were prepared in MHA at 37˚C for antibacterial activity. A final concentration ranging from 83–333 µg/ml was prepared in Sabouraud's dextrose agar (SDA) slant to check antifungal activity. The plates were spot inoculated with 3 µl aliquots of culture containing approximately 10^5 bacteria/ml of each organism. The plates were incubated at 37˚C for 18 hours and observed for the presence or absence of growth. A 5 ml of extract at different concentrations were taken into sterile test tube and mixed with 1 ml of each fungus to be tested. Then 0.5 ml of mixture (culture with extract) was added to 2.5 ml of SDA in the tubes. Afterwards, all the tubes were incubated at 30˚C for 15 days. The tubes were observed for visible growth of fungi. The highest dilution revealing no visible growth was regarded as minimal inhibitory concentration during 15 days. Antimicrobial studies were done in triplicates (Floral *et al.*, 2003, Dhanalekshmi *et al*., 2010).

3. RESULTS

Totally, 12 extracts of leaf and stem were prepared and the percentage yields are displayed in Figure 1. Extracts of Ethyl acetate, Chloroform, Ethanol and Methanol revealed the highest yield when compared with aqueous extract. The abbreviations HLE – Hot Leaves extract, CWLE – Cold Leaves Extract, HSE – Hot Stem Extracts, CWSE – Cold Stem extracts, CSE – Chloroform Stem Extracts, ESE – Ethanol Stem Extracts, MSE – Methanol Stem Extracts, EASE – Ethyl Acetate Stem Extracts, CLE – Chloroform Leaves Extracts, ELE – Ethanol Leaves Extracts, MLE – Methanol Leaves Extracts, and EALE – Ethyl Acetate Leaves Extracts. Phytochemical study reveals that different extracts contain different phytoconstituents. All the extracts reveal the presence of saponins. Details of positive and negative reports are displayed in Table 1. The data obtained in hemolytic assay gives a qualitative indication of the damage caused by natural products in red blood cells. The result obviously declared that the natural products are more hemocompatible, and the results are depicted in Figures 2, 3 and 4. Moreover, the natural products revealed lysis less than 5% in the whole experimental concentration range indicates the biocompatibility. In the present study, Chloroform Leaf Extract, Cold Water Leaf Extract

and Stem Extract revealed hemolysis more than 5%. Further studies are needed to recheck this effect on red blood cells. All the tested extracts revealed highest activity against *S. aureus* and *P. vulgaris* when compared to standard drug ciprofloxacin (32 mcg), and the results are tabulated in Table 2. Moderate activity was observed against *B. subtilis.* CLE and ELE revealed maximum activity against *S. aureus* and EALE revealed maximum activity against *P. vulgaris.* EALE, HSE, ELE did not respond against *E.coli.* The MIC value ranging from 66.6–333.3 mcg/ml of each extract was tested by agar dilution method, and the results are presented in Table 3.

S. aureus inhibited at MIC 66.6 mcg/ml for most of the tested extracts. MIC 333.3 mcg/ml was found to be inhibitory for *B. subtilis,* whereas the inhibition of *E. coli* responded differently when compared with disc diffusion method. Regarding the antifungal activity, all the extracts were effective against *Candida albicans* with the MIC values of 166.6 and 333.3 mcg/ml, but most of the extracts are not effective against the fungus *A. flavus, A. fumigatus and T. mentagrophyte.*

4. DISCUSSIONS

The present study focused on the medical applications of *R. stricta* extracts, especially antimicrobial activity, including toxicity associated with their use. The prospective for developing antimicrobials from higher plants appears rewarding, as it may pave the way for the development of many lead compounds for phytomedicine against infectious diseases. In this study, preliminary phytochemical screening of different extracts of leaves and stems of *R. stricta* revealed the presence of various phytochemicals, and these results were similar to those obtained by other research groups (Mohammed *et al.*, 2015). There may be some variation in the phytochemical constituent's report of this study when compared with other research reports because these plant materials are collected from the wadis of Muscat region.

To our knowledge, toxicity study in detail has not been reported for this plant. Hence, there is an outstanding need of research to find whether they are safe and compatible when administered orally. Despite the widespread use of natural products, understanding of the toxicity and potential health risks associated with the use of these are extremely limited. Thus, along with the development of natural products, experts in related scientific fields are calling for a simultaneous assessment of the toxicological effects of natural products. Hemolytic assays were performed because natural products possessing potent activity may not be useful in pharmacological aspects if they possess hemolytic effect. The extent of hemolysis is an important factor of toxicity of natural products to red blood cells. If properly validated, the advantages of the early screening tests like hemotoxicity would be useful. The data obtained in hemolytic assay gives a qualitative indication of the damage caused by natural products in red blood cell. The result in the present study obviously declared that the natural products are more hemocompatible. Moreover, the natural products displayed lysis less than 5% in the whole experimental concentration range. The extent of hemolysis is an important parameter of toxicity of natural products to erythrocytes. Natural products with different types of extracts have different functional groups. The charge is crucial for toxicity, including hemotoxicity. The present study does not reveal any observational hemolytic toxicity in the red blood cells and the reported hemolytic percentage is independent of its concentration. Most of the extracts displayed hemolytic percentage, which was significantly lower than 5%. These results suggested that natural products were suitable for a wide safety margin in blood-contacting applications and oral administration.

5. CONCLUSIONS

R. stricta is an important medicinal species that is rich in alkaloids, containing mostly anticancer alkaloids and few non-

alkaloidal compounds (Gilani *et al*., 2007). Table 2 and 3 reveals the antibacterial activity of different extracts against both gram negative as well as gram positive bacteria. The results reveal that the extracts are effective against *S. aureus* and *P. vulgaris*, but the comprehensive nature of the active principle responsible for antimicrobial activity is not explored in this study. The antimicrobial activity of ethanolic extract of *Rhazya Stricta* was tested against gram positive and gram negative bacteria, and results displayed that the leaves' extract was more active compared to other parts; however, seeds' extract has maximum inhibitory activity against both types of bacteria when compared to other parts (Ahmed *et al*., 2004). The non-alkaloid extract from the leaves of the medicinal plant *R. stricta* proved to have antimicrobial activities against MRSA clinical isolates based on TEM and 1% agarose well-diffusion method (Raziuddin *et al*., 2016). In the present study, we found that antimicrobial activity was more towards gram-negative than gram-positive bacteria. Genus staphylococcus was found to be more susceptible to the extract among the tested bacteria. The extracts cover the enteric pathogen, namely E.coli. In table 3, *R. stricta* has considerable antifungal activity towards skin pathogen Candida, in Table 4. Our study also revealed that all the extracts were effective against the fungi Candida, but they are not effective against other fungi tested in this study.

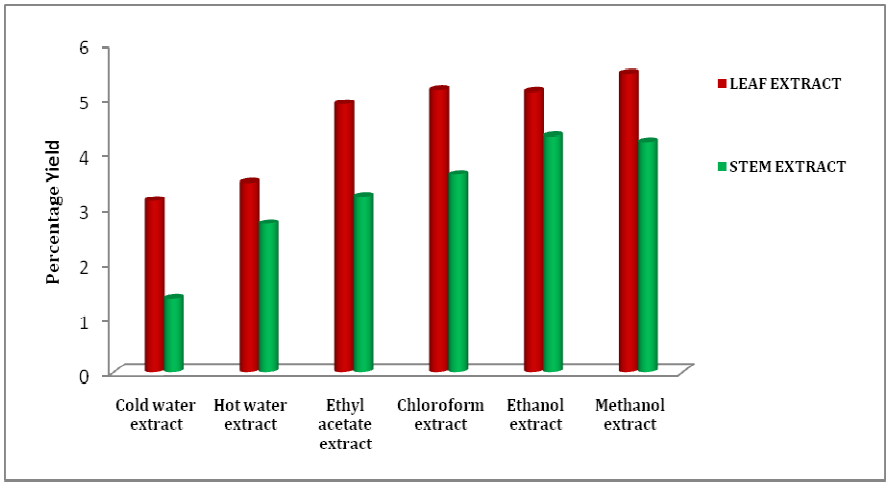

Figure 1: Percentage Yield of different Extracts.

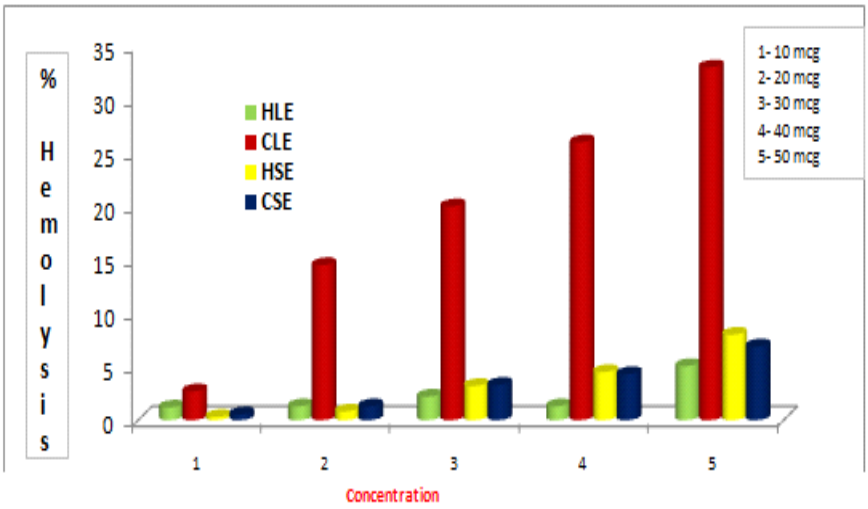

Figure 2: Hemocompatibility Test.

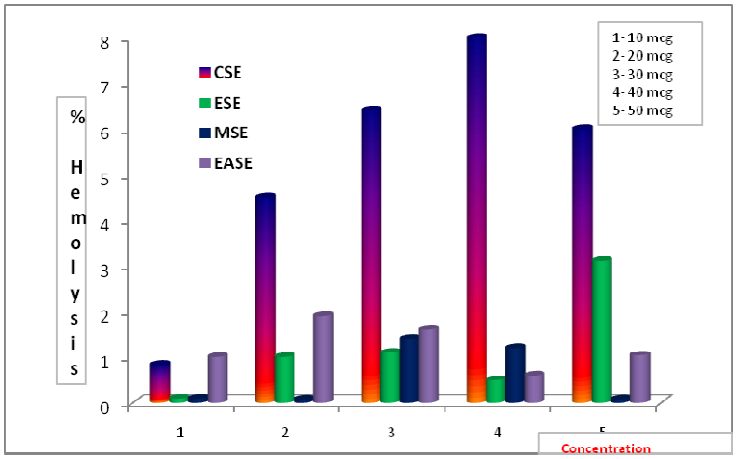

Figure 3: Hemocompatibility Test.

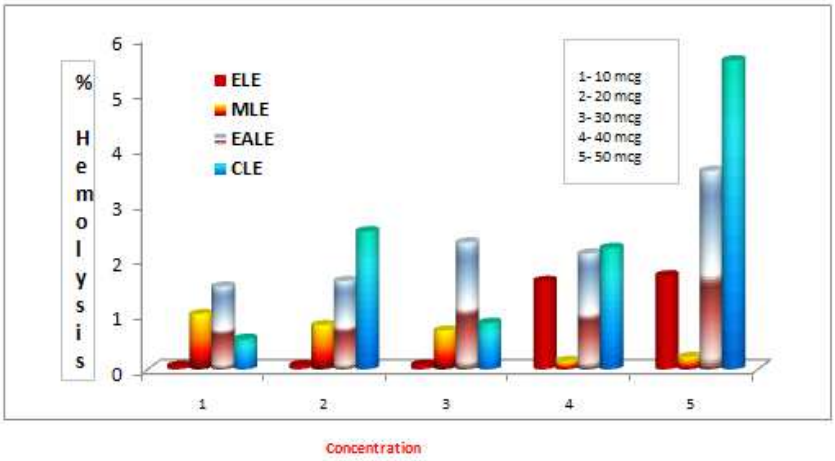

Figure 4: Hemocompatibility Test.

Table 1: Phytochemical Results of Different Extracts

	LEAF EXTRACT							STEM EXTRACT					
TEST	**A**	**B**	**C**	**D**	**E**	**F**		**A**	**B**	**C**	**D**	**E**	**F**
Glycosides	+	+	-	-	+	+		+	+	-	-	+	+
Saponins	+	+	+	+	+	+		+	+	+	+	+	+
Flavonoids	+	+	-	-	-	-		-	+	-	-	+	+
Proteins	-	-	-	-	-	-		-	-	-	-	-	-
Free amino acids	-	+	-	-	+	+		+	+	-	-	-	+
Carbohydrate	-	-	-	-	-	-		-	-	-	-	-	-
Alkaloids	+	+	+	+	-	-		+	+	+	+	-	-

Table 2: Zone of Inhibition in cm for the Concentration of 3000 mcg/ml of different Extracts

Organism	MSE	MLE	EASE	EALE	HSE	ELE	ESE	CSE	CLE	STD 32 mcg
S. aureus	2.5±0.8	2.3±0.6	2.2±0.4	2.3±0.2	2±0.6	2.8±0.3	2.4±0.7	2.2±0.5	3.1±0.8	2.4±0.5
E. coli	1.9±0.2	2±0.8	1.5±0.8	NI	NI	NI	1.4±0.8	1.2±0.2	2±0.2	1.4±0.3
B. subtilis	2.2±0.9	1.8±0.2	1.7±0.5	2.2±0.8	1.6±0.7	2.5±0.8	1.6±0.8	2±0.8	1.8±0.8	1.6±0.8
P. vulgaris	2.3±0.9	1.5±0.9	3±0.4	2.8±0.8	2±0.3	2±0.2	2±0.7	2±0.8	1.4±0.6	2±0.2

Table 3: Minimum Inhibitory Concentration for Antibacterial Activity of different Extracts

Organism	MSE	MLE	EASE	EALE	HSE	ELE	ESE	CSE	CLE
S. aureus	133.3	200	66.6	133.3	333.3	66.6	133.3	66.6	66.6
E. coli	66.6	133.3	133.3	200	200	200	200	200	200
B. subtilis	66.6	333.3	333.3	66.6	333.3	333.3	333.3	333.3	333.3
P. vulgaris	133.3	333.3	66.6	200	333.3	133.3	133.3	333.3	133.3

Table 4: Minimum Inhibitory Concentration for Antifungal Activity of different Extracts

Organism	MSE	MLE	EASE	EALE	HSE	ELE	ESE	CSE	CLE
Candida albicans	166.6	166.6	333.3	333.3	333.3	166.6	166.6	166.6	166.6
Aspergillus flavus	200	ND	200	ND	ND	ND	ND	ND	ND
Aspergillus fumigatus	ND	ND	ND	333.3	ND	ND	333.3	ND	ND
T.mentagrophyte	ND	333.3	ND	ND	ND	ND	ND	200	ND

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