IJBPAS, March, 2019, 8(3): 491-499

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

PHYTOCHEMICAL AND ANTIBACTERIAL EVALUATION OF *RHAZYA STRICTA* DECNE

HAROON KHAN[1], MUHAMMAD ASIF SHAHZAD [1], FAZAL UR REHMAN MARWAT [1,] HASHMAT ULLAH [1], ALTAF ALI MANGI[*1], SHUMAILA PARVEEN ARAIN[2], ESHWA DAR [3], FAKHRA BATOOL [4], ANILA ALAM [4], SALEEM AHMED JOYO[5], MEHIR-E-HIRA [1]

[1]Faculty of Pharmacy Gomal University Dera Ismail Khan, KPK

[2]Faculty of Pharmacy University of Sindh Jamshoro, Pakistan

[3]Islam College of Pharmacy Sialkot, Pakistan

[4]Sardar Bahadur Khan Women University Quetta Balochistan, Pakistan

[5]Department of Pharmacy Shaheed Mohtarma Benazeer Bhutto Medical University Larkana Sindh, Pakistan

***Corresponding Author: Altaf Ali Mangi: Phd Scholar Faculty of Pharmacy Gomal University Dera Ismail Khan KPK Pakistan, E Mail: Pharmacistsindh41@gmail.com +923313421263**

Received 16th Oct. 2018; Revised 17th Nov. 2018; Accepted 22nd Nov. 2018; Available online 1st March 2019

https://doi.org/10.31032/IJBPAS/2019/8.3.4649

ABSTRACT

Background: *Rhazya stricta* is an erect glabrous shrub widely distributed in Pakistan. The extract of its leaves has been prescribed in folk medicine in the treatment of various diseases. The present study aims to evaluate *Rhazya stricta* Decne leaves for its phytochemical and antibacterial properties.

Study Site and Date: The study site was Dera Ismail Khan KPK Pakistan and sample was collected from federally administered tribal area, Mohmand Agency, Pakistan

Methods: Plant was shade dried at room temperature for four weeks. Leaves (dried) of the plant were comminuted by using electric grinder with final dry weight of 750 g

Results: It was observed that total phenolic content in relation to gallic acid equivalent (GAE) of extract was 189.9 µg GAE/mg and in methanolic extract of *Rhazya stricta* Decne flavonoid with respect to quercitin equivalent (QE) was determined to be 27.7 µg QE/mg. It was also noted that methanolic extract exhibited scavenging potential of 38.39 % and the total antioxidant capacity was found to be 273.80 µg ascorbic acid equivalent/mg of extract.

Conclusion: The plant showed highest activity against *subtilis aureus* while least against Escherichia coli, thus ensuring its phytochemical and therapeutic importance.

Keywords*: Rhazya stricta,* Total phenolic contents, Phytochemicals, Antibacterial activity

INTRODUCTION

The folkloric medicinal herbs are generally considered as a key source of novel drugs. These herbs contain many ingredients which are used for treating different diseases. The federally administered tribal areas of Pakistan possess rich medicinal plants. Some of these medicinally important plants are widely used for various diseases and disorders but they need to be evaluated scientifically. *Rhazya stricta* Decne is one of the plants among them. *Rhazya* belongs to the family of *Apocynaceae* which have two species namely *Rhazya stricta* and *Rhazya orientlis* (Ali et al, 2000). *Rhazya stricta* is widely used for its medicinal purposes in Pakistan, India, Saudi Arabia, Iran and Iraq (Gilani et al, 2007). It is noted that the plant in dried form is believed to be more effective than the fresh plant. This plant is used widely in many places of the world as folkloric medicinal agent. Secondary metabolites of this plant are mostly found to be alkaloids and flavonoids. Mostly all parts of this plant are used in treatment of different disorders like diabetes, hypertension and foot burning. It is also found to be effective in toothache, syphilis, colic disorders, wound and urinary tract diseases (Mukhopadhyay et al, 1983). Moreover it is general tonic and has antimicrobial, anti-inflammatory, anticancer, stimulant, analgesic, anthelmintic, purgative and antihypertensive activities (Khan, 2007; Ahmed et al, 2014). During normal cellular metabolism, reactive oxygen species are produced causing oxidative stress and damage to the body lipids, nucleic acids and proteins (Akhtar and Mirza, 2015). To get rid of oxidative stress, natural antioxidants are recommended due to toxic carcinogenic effects of synthetic antioxidants (Botterweck et al, 2000). Therefore current study focused to evluate *Rhazya stricta Decne* leaves for its phytochemical and antibacterial properties.

MATERIALS AND METHODS

Reagents

Methanol (Merk, Germany), Folin-coicalteu (Aldrich), Gallic Acid (Sigma), Quercitin (PDH), DPPH (2,2 diphenyl-1-picrylhydrazyl) (Fluka), ascorbic acid (Sigma), Sodium Phosphate (Sigma), Ammonium Molybdate (Fluka), Ferric Cyanide (Sigma), Dimethyl Sulfoxide (Sigma), Potassium Ferricyanide (Sigma), phosphate buffer (Aldrich), different strains of bacteria (*P. aeruginosa, E. coli*, *K. pneumoniae*, *S. aureus, B. subtilis*) and antibiotic (cefixime).

Instruments:

Centrifuge machine (H-200, Kokusan, Ensink-Company Japan), Digital Balance (Japan), Grinder (Germany), Magnetic Stirrer (Denver, USA), Micro Pipettes, 96 well plates and Petri Dishes (Pyrex, Germany), UV Spectrophotometer (Model 1601, Shimadzu, Japan).

Collection and identification of plant

This plant was collected from federally administered tribal area, Mohmand Agency, Pakistan and was authenticated by Dr. Mushtaq Ahmad, department of plant science, faculty of biological science, Quaid-i-Azam University, Islamabad.

Preparation of extract

Plant was shade dried at room temperature for four weeks. Leaves (dried) of the plant were comminuted by using electric grinder with final dry weight of 750 g. The extract was prepared by macerating 100 g of powder in methanol. Plant material was soaked in volumetric flask separately for period of three days and mixed occasionally six times a day. Subsequently the obtained extract was passed through filter and residue was put in the solvent again. This procedure was repeated three times., in order to obtain crude extract.

Phytochemical analysis

1). *Total phenolic content determination*

The phenolic contents were determined as describe previously (Ul-Haq et al., 2012). Positive control used was Gallic Acid and Folin-coicalteu was used as a reagent. The reaction mixture which was composed of 20 µl sample and 90 µl Folin-coicalteu reagent was incubated for 30 minutes followed by the addition of 90 µl of sodium bicarbonate. The Absorbance of resultant mixture (reaction mixture) was observed at λ_{max} of 630 nm using microplate reader. The reagent was used as blank sample, and the results were taken as an average of triplicate experiments. Calibration curve was obtained in parallel employing gallic acid (positive control).

Phenolics obtained were expressed as gallic acid equivalent (µg/mg extract).

2). *Total flavonoid content determination*

In extract, total flavonoid contents were determibed by method as described previously (Ul-Haq et al., 2012. Quercitin was used as positive control. A mixture of 20 µl sample, 4 mg/ml DMSO, 10 µl of 10 % aluminium chloride, distilled water 160 µl and potassium acetate 10 µl was incubated. Absorbances were measured at 415 nm with microplate reader. The final concentrations of quercitin used in calibration curve were 3.12, 6.25, 12.5, 25 and 50µg/ml. Total Flavonoid Content were shown as µg QE/mg of extract after analyzing in triplicate.

Evaluation of antioxidant potential

i. Free radical scavenging assay

The process is also termed as DPPH assay, and is based on the discoloration of purple colored DPPH solution (2, 2 diphenyl-1-picrylhydrazyl free radical, 3.92 mg/100 ml of methanol). Antioxidant activity was determined using standard previous procedure (Bibi et al, 2011). Briefly, 180 µl of DPPH aliquots and 20 µl sample solution were added to each well of 96 well plates respectively. Vitamin C was employed as reference standard. The absorbances of both the reaction mixture and the standard were recorded at 517 nm. The percentage radical scavenging was found by using following equation.

$$\% \text{ scavenging activity} = (Abc - abs)/Abc \times 100$$

- While, Abc indicates absorbance of negative control which is reagent solution without sample)
- Where, abs indicating absorbance of DPPH solution having samples.

ii. Phosphomolybdenum based total antioxidant potential determination

Total antioxidant activity of the sample was assessed while incubating stock solution in DMSO (100 µl of 4mg/ml DMSO) having reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate) and 4 mM ammonium molybdate for 90 minutes at 90°C (Prieto, Pineda and Aguilar, 1999). Absorbance of this mixture (reaction mixture) was evaluated at 645nm spectrophotometrically. Comparison was made with ascorbic acid (positive control) and reagent having no sample (negative control), and the results were taken as an average of triplicate experiments.

Total reducing power assay

The reducing power assay was carried out using previously adopted procedure with minor modifications (Jafri et al., 2014). Ascorbic acid was employed as standard. A mixture of 100 µl sample, potassium

ferricyanide (1 %) and phosphate buffer pH 6.6 (0.2 ml) was incubated at 50ºC. Trichloroacetic acid 10 % for 30 minutes was mixed with above mixture and the resultant mixture was centrifuged for about 10 minutes at 3000 rpm. Reaction mixture supernatant of was transferred to 96 well plate followed by the addition of distilled water and ferric cyanide 0.1%. Phosphate buffer was used as blank. Spectrophotometrically absorbances of reaction mixture, standard and blank were recorded at 760 nm, and the results were shown as µg of Ascorbic acid that was equivalent per mg of extract after triplicate analysis.

Antibacterial assay

The antibacterial activity of plant extract was determined using disc diffusion method with minor modifications (Bibi et al, 2011). Before the sensitivity determination the bacterial strains *Staphylococcus aureus, bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli, Klibesella* pneumoniae were inoculated from stored strains (4°C) into nutrient broth. The prepared inocula were incubated at 37°C for 24 hours. 100 µl bacterial inocula were swabbed onto petri dishes which contains fresh nutrient agar. The test sample comprising of 5 µl of 20mg/ml DMSO infused filter paper discs that were placed over the swabbed surface. In the same way discs were impregnated with reference standards 5 µl (4 mg/ml DMSO) used as positive control (cefixime was used as standard antibiotic) while discs impregnated with DMSO acted as negative control. Incubation of these plates was done at 37°C for 24 hours. Zones of inhibition of the sample and discs having standard were recorded using calipers.

DATA ANALYSIS

Each assay in this experiment was performed in triplicates and the results were shown as Mean ± standard deviation. Analysis of variance was applied to the resultant mean values of different assays.

RESULTS

Total phenolic and flavonoid content

In methanolic extract the phenolic content of *Rhazya Stricta Decne* in relations to gallic acid equivalent (GAE) was 189.9 µg GAE/mg. Flavonoid content in methanolic extract of the plant in relations to quercitin equivalent (QE) was found to be 27.7 µg QE/mg. Flavonoid and phenolic contents have been reported to be work in close association in biological systems due to antioxidant potential, getting one oxygen and other free radicals. It is also observed that antioxidant capacity of phenols is due to presence of groups like hydroxyl, methoxy, and ketonic in a molecule having phenols.

The antioxidant activity of phenols could be due to the presence of methoxy, hydroxyl, double bond conjugation or ketonic group (Afshar et al, 2012).

Antioxidant potential:

The antioxidant potential of the plant extract was determined using several methods (Yıldırım et al, 2000) as:

Free radical scavenging Assay

The DPPH method is preferred over other methods because it is easy, reliable and fast. The basic mechanism for DPPH antioxidant capacity is established on the capability of antioxidants to decolorize 2, 2-diphenul-1-picryl-hydrazyl. This occurs due to fact that from donor antioxidant (sample) one electron is gained and decolonization occurs. It could be determined quantitatively from variation appears in absorbance. *Rhazya stricta* Decne, methanolic extract exhibited 38.39 % DPPH scavenging activity. Antioxidant compounds are usually in phenolic form. Phenols have the ability to destroy the radicals due to the presence of hydroxyl group. The leaf of *Rhazya stricta* Decne leaf has good pharmacological importance and its isolation, purification, and characterization is strongly suggested to be investigated on the basis of above finding.

Total reducing power assay:

In sample extract, reducing power is related with antioxidant activity and might function as significant consideration of the antioxidant activity. The methanolic extract of *Rhazya stricta* Decne total reducing power was obtained and that was 55.382 µg AAE/mg. It was observed that a direct relationship is observed in many plant extracts between reducing power and free radical scavenging activity. The main reason behind reducing power is that reducing agent donates a hydrogen atom to any non-reducing species that depicts antioxidant potential. Reductants are associated with reducing power potential thereby donating a hydrogen atom and reducing free radical into non-reactive species and hence anti-oxidant action is produced (Wang et al., 2008). Results are given in Table 1.

Antibacterial assay:

The growth inhibition of extract of *Rhazya stricta* Decne against different bacterial strains is given in Table 2.

Table 1: Summary of results of phytochemical analysis

TPC	TFC	Antioxidant potential		TRP
GAE/mg	µg QE/mg.	DPPH assay (%)	TAC (µg AAE/mg)	µg AAE/mg
189.9	27.7	38.4	273.8	55.4

Table 2: Growth inhibition of different bacterial strains by extract of *Rhazya stricta* Decne

Extract	Diameter of growth inhibition zone (mm)				
	Gram Negative			Gram positive	
Rhazya stricta Decne	*P. aeruginosa*	*K. pneumonia*	*E.Coli*	*S. aureus*	*B. subtillis*
Rhazya stricta Decne	7 ± 02	8 ± 01	_	25 ± 1.5	7 ± 1.5
Negative control	_	_	_	_	_
Key; Not active: (-); Negative control: DMSO					

DISCUSSION

The methanolic extract of the leaves of medicinal plant *Rhazya stricta* Decne proved to have antibacterial activities based on disc diffusion method. It also shows that the plant species has excellent antioxidant potential. The plant species contains phenols and flavonoids. It has been used against different diseases but still more work needs to be done regarding its medicinal importance for the betterment of human health. This plant extracts exhibited different growth inhibition against various bacteria strains. In this assay gram negative bacteria and gram positive were used. *Rhazya stricta* Decne methanolic extracts indicated highest activity against strains of S. aureus (25 mm) where lowest activity of both the extracts was revealed by E.coli that was 0 mm or no activity. DMSO was nontoxic as there were no growth inhibition zones around DMSO impregnated discs. Phosphomolybdenum method was use to determine total antioxidant capacity. In this method green colored phosphate molybdate complex appears in acidic environment that gives absorbance 645 nm (Prieto et al., 1999). *Rhazya stricta* Decne methanolic extract showed total antioxidant capacity of 273.80 µg AAE/mg. It has been observed that positive correlation exist between flavonoids, phenolics and antioxidant activity. These results are in confirming previous of positive correlation between TFC, TAC and radical scavenging activity (Erel et al., 2012

Acknowledgment: We thank all students who helped to collect and analyze data.

REFERENCES

[1] Ali BH, Alqarawi AA, Bashir AK, Tanira MO. Antioxidant action of extract of the traditional medicinal plant Rhazya stricta Decne. in rats. Phytotherapy Research. 1; 14(6): 469-71.

[2] Gilani SA, Kikuchi A, Shinwari ZK, Khattak ZI, Watanabe KN. 2007 Phytochemical, pharmacological and ethnobotanical studies of Rhazya stricta Decne. Phytotherapy Research. 1; 21(4): 301-7. (2000).

[3] Mukhopadhyay S, El-Sayed A, Handy GA, Cordell GA.

Catharanthus alkaloids XXXVII. 16-Epi-Z-isositsirikine, a monomeric indole alkaloid with antineoplastic activity from Catharanthus roseus and Rhazya stricta. Journal of natural products; 46(3): 409-13. (2008).

[4] Khan S. In vitro antifungal activity of Rhazya stricta. Pak J Pharm Sci..; 20(4): 274-9. (2007).

[5] Ahmed N, Mahmood A, Mahmood A, Tahir SS, Bano A, Malik RN, Hassan S, Relative importance of indigenous medicinal plants from Layyah district, Punjab Province, Pakistan. Journal of ethnopharmacology. 8; 155(1): 509-23. (2014).

[6] Botterweck AA, Verhagen H, Goldbohm RA, Kleinjans J, Van den Brandt PA. Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. Food and Chemical Toxicology. 31; 38(7): 599-605. (2012).

[7] Ul-Haq I, Ullah N, Bibi G, Kanwal S, Ahmad MS, Mirza B. Antioxidant and cytotoxic activities and phytochemical analysis of *Euphorbia wallichii* root extract and its fractions. Iranian journal of pharmaceutical research: IJPR. 11(1):241-49. (2012).

[8] Bibi G, Ullah N, Mannan A, Mirza B. Antitumor, cytotoxic and antioxidant potential of Aster thomsonii extracts. African Journal of Pharmacy and Pharmacology. 28; 5(2): 252-8. (2015).

[9] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry. 1; 269(2): 337-41. (2016).

[10] Jafri L, Saleem S, Haq IU, Ullah N, Mirza B. In vitro assessment of antioxidant potential and determination of polyphenolic compounds of Hedera nepalensis K. Koch. Arab. J. Chem., 19:158-163(2104)

[11] Afshar FH, Delazar A, Nazemiyeh H, Esnaashari S, Moghadam SB.; Comparison of the total phenol, flavonoid contents and antioxidant activity of methanolic extracts of Artemisia spicigera and A. splendens growing in Iran.

Pharmaceutical Sciences. 18(3):165-70. (2103).

[12] Yıldırım A, Mavi A, Oktay M, Kara AA, Algur ÖF, Bilaloğlu V. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf ex DC), sage (Salvia triloba L.), and Black tea (Camellia sinensis) extracts. Journal of Agricultural and food chemistry.; 48(10): 5030-4. (2016)

[13] . Wang H, Gao XD, Zhou GC, Cai L, Yao WB. In vitro and in vivo antioxidant activity of aqueous extract from Choerospondias axillaris fruit. Food Chemistry. 106(3): 888-95.(2015).