Qualitative Phytochemical Screening of *Rhazya Stricta* stem and leaves extracts

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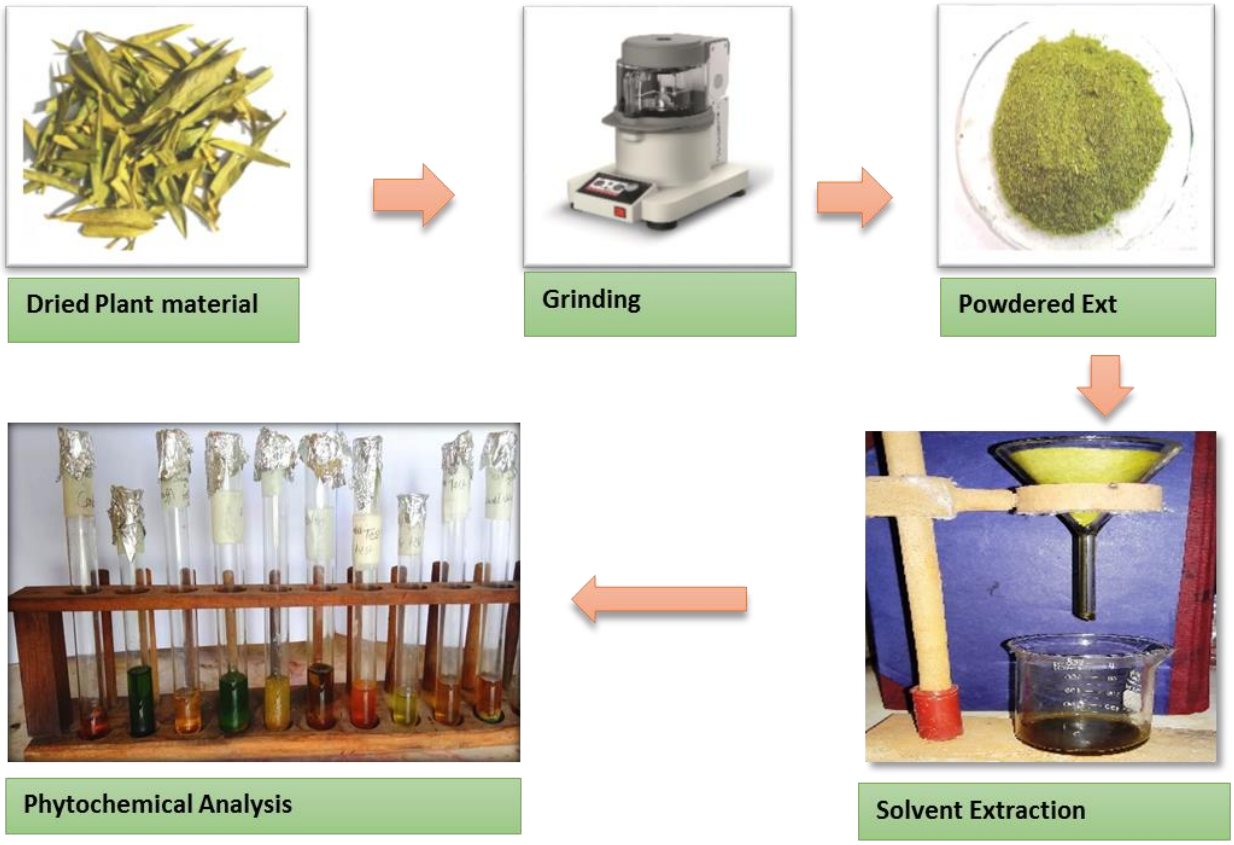
Abstract

Plants are essential source of medicines and drugs, due to containing various bioactive compounds. These plants are used as home remedies in many places. *Rhazya stricta* is a rich plant of biologically active compounds that have important pharmacological properties and are used as a medicinal plant globally. This research is based on the phytochemical screening of *Rhazya stricta's* leaves and stems ethanolic extract by doing various qualitative tests such as Selwinoff's test, Barfoed's test, Wagner's test, Molish test, Shinoda test, Fehling test etc. this screening has unmasked the presence of primary and secondary metabolites in *Rhazya stricta*. The yield for ethanolic extracts of *Rhazya stricta's* leaves and stem were noted to be 17.5% and 12.5% respectively. Both parts (leaves & and stem) of *Rhazya stricta* contain all primary metabolites except protein which is absent in the stem. Secondary metabolites including alkaloids, cardiac glycosides, terpenoids, flavonoids, phenols, saponins, steroids, tannins and caumarin are present in both parts (leaves & stem). The qualitative analysis eases the way for *Rhazya stricta* plant to be used as a medicine for human beings.

Key words: *Rhazya stricta*, Pharmacological, Metabolites, Alkaloids, Bioactive, Ethanolic extract.



Graphical Abstract

1. Introduction

Plant kingdom is very essential origin for synthesizing medicines and drugs. According to World Health Organization (WHO), 80 percent people even now trusted on natural herbs and using them as a home remedies [1]. There are many bioactive compounds exist in medicinal plants which are used for preserving several human ailments as well as take important part in healing [2]. Many medicines that are used now a days are either extracted from plants or generated using their chemical structure as a model [3]. These bioactive compounds can be extracted from any part of the plant such as stem, seeds, flowers, fruit, leaves, roots and barks etc. [4]. Medicinal plants getting more importance day by day due to their countless advantages, specifically in the medicinal and pharmacological line and curing human beings. These phytochemicals combine with other fibers and nutrients and work together as a defense system of the human body against various ailments and provide protection to the body [5]. Approximately more than five thousand phytochemicals have been recognized however, huge portion is still unrevealed [6]. Chemicals isolated from plants are known as phytochemicals. On the basis of their activities in plant metabolism these metabolites are distributed into two classes; primary metabolites and secondary metabolites. Primary metabolites contain amino acids, proteins, common sugar, chlorophylls, purines and pyrimidines of nucleic acid etc. secondary metabolites includes phenolic (lignin, tannins, flavonoids, isoflavonoid, coumarins and furano-coumarins), Terpenes (monoterpenes, diterpenes, sesterpenes, triterpenes, sesquiterpenes), Sulfur containing compounds (glucosinolates, thionins, allinin, glutathione, phytoalexins and defensins) and Nitrogen containing compounds (cyanogenic glycosides, alkaloids and non-protein amino acids). Antioxidants are also secondary metabolites in nature created by plants that stops oxidation and neutralized oxidants because oxidation causes various ailments in human body such as cardiovascular diseases and cancers etc. plant generates stunning arrangement of antioxidants including benzoic acid, flavonoids, cinnamic acid, carotenoids, folic acids, tocopherols, ascorbic acid and tocotrienols [7]. Medicinal plants contain a huge collection of secondary phytochemicals which have numerous biological activities and are more responsible for medicinal effects [8]. This group phytochemicals are non-nutrients however, they have biological activity against chronic diseases [9]. Medicines derived from plants are of low cost, easily accessible, safe, less side effects and effective [10]. The different colors of vegetables and fruits are because of phytochemicals, according to United States National Cancer Institute and American Institute advice; we should take one serving each of the seven color groups daily to prevent different types of cancers [11]. Cohort studies suggested that the maximum intake of natural foods like whole grains, fruits and vegetables reduces the risk of coronary artery diseases and stroke [12].

1.2 Botanical description

The *Rhazya stricta* was first discovered by Muslim scientist Abu Bakar Muhammad bin Zakariya Ar-Razi in 925, known in Europe mainly in Latinized name of 'Rhazes'. Rhazya was titled after the name of this Muslim scientist. *Rhazya stricta* belongs to Apocynaceae family, one of the important and biggest family in angiosperm [13]. *Rhazya* genus contains two kinds of plants that are: *Rhazya Stricta* Decaisne and *Rhazya Orientalis* [14]. *Rhazya stricta* is dispersed all over South Asia (Pakistan, Afghanistan and India) and also on the boarder of the Arabic gulf countries including the United Arab Emirates (UAE), Saudi Arabia, Qatar, Iraq and Iran [15]. The botanical description of the *Rhazya stricta* is shown in the inset of Table-1.

Table-1: Botanical description of *Rhazya stricta* [16]

Parts	Details
Duration & habitat	Glabrous shout raised evergreen shrub with numerous stems arising from base to upward direction, perennial stems are covered by many leaves.
Stem	Main central smooth stem raised from base and divided into various wide sub-branches near to the base.
Leaves	Leaves are stalked and plain, elongated or oval shaped, raised closely, with entire margin and sever vertex, dense, fibrous and alternative sharp edge pointed toward base, almost 10cm lengthy and 1.5cm away, to 12cm on little trunks, with noticeable axil and having smooth surface area.
Inflorescences	Inflorescence grow on the top of stems and are axillary cymes.
Flowers	Flowers are bisexual, snowy color, 2 to 2.5cm long, quinary, small pedicel, white petalled flowers ,4 millimeter lengthy cylex, with embedded stamens, extremely wedge-shaped lobed, petals 1 to 1.4 cm.

1.3 Phytochemicals in *Rhazya stricta*

Several parts of *Rhazya stricta* contains phytochemicals in valuable quantities including alkaloids, tannins, flavonoids, phenolic compounds, terpenoids, glycoside, steroids, saponin, proteins, amino acid, carbohydrates, oil and fats. Calcium (17360mg/kg), potassium (1257.6mg/kg), iron (437.8mg/kg), sodium (1736mg/kg), magnesium (336.9mg/kg) are in maximum content present in leaves of Rhazya stricta. Likewise, vital amount of zinc ranging from (8.48-38.48mg/kg), Cobalt (0.00-8.76mg/kg), Manganese (0.00-40.96mg/kg), Lead (0.32-25.28mg/kg), Cadmium (0.00-4.88mg/kg), Copper (0.00-6.96mg/kg), Nickel (3.04-3.58mg/kg), Chromium (0.00-4.88mg/kg) are also present in various parts of *Rhazya stricta* plant [17]. Naturally occurring initial 10 indole alkaloids are found in *Rhazya stricta* which are antirhine, 3-epi-antirhine, condylocarpine, eburnamine, eburnamonine, taberonine, ursolic acid, stigmasterol, olenaolic acid, (+)-vincadiformine, (−)-vincadiformine, (−)-16R,21R-omethyleburmanine, 15-hydroxy-

vincadifformine, and dihydroburnamenine [18]. Due to the presence of large amount of phytochemicals, the *Rhazya stricta* possess significant biological activities.

1.3.1 Antioxidant activity

The antioxidant activity of *Rhazya stricta* is due to the presence of alkaloids. It may be include for cure of different ailments like chronic rheumatism, tumors, arteriosclerosis etc. [19]. The methanolic extract of *Rhazya stricta* is the important source for natural antioxidants that have ability to remove free radicals and anion radicals [15].

1.3.2 Anticancer activity

The extract of *Rhazya stricta* has dominant effect on ER-positive but also significantly stops the ER-negative best cancer cells growth. *Rhazya stricta* is used as a chemopreventive agent especially for the ER-negative breast cancers which have weak diagnosis and less survival [20]. Several extracts of Punica granatum and Rhazya stricta were used against Hepato-cellular carcinoma (HepGII) cell lines and Colon cancer (CACO) [21].

1.3.3 Anti-diabetic activity

For cure the of diabetes mellitus *Rhazya stricta* leaves are used. Baeshen et al., figures out the effect of *Rhazya stricta* aqueous extract of on adiponectin protein and insulin resistance, which increase the adiponectin levels concentration and capable them to treat diabetes [22].

1.3.4 Anti-fungal activity

Rhazya stricta root fragments of methanol and chloroform show antifungal activities against Aspergillus flavus, Aspergillus terreus and Candida albicans. Similarly Methanolic and chloroformic samples show antifungal activities against Fusarium solani, C. albicans, A. flavus and Trichophyton longifusis [15].

1.3.5 Antibacterial activity

Wide-spectrum antimicrobial activity is shown by isolated component Tetrahydrosecamine from *Rhazya stricta*, another strictanol component is also very active against Pseudomonas aeruginosa and E. coli. *Rhazya stricta* ethanolic fruit extract sample has antibacterial, acetylcholinesterase and lipoxygenase activities [23].

1.3.6 Allelopathic activity

The phenomena in which beneficial or harmful effects of one plant on another plant occur is known as Allelopathy. To review the allelopathic potential of *Rhazya stricta*, the aqueous extracts of stem and leaves are used on germination and seedling development of maize. From other study of allelopathy the seedling of Vicia faba on dealing with *Rhazya stricta* extracts indicated numerous types of mitotic irregularities and chromosomal aberrations [22].

1.3.7 Effect on blood pressure

The *Rhazya stricta* alkaloidal fraction show remarkable performance in decreasing blood pressure (BP). The lyophilized extract of *Rhazya stricta* dosage of 5 to 100 mg per kg, show an unpredictable effect on heart rate, and a dose dependent reduce up to 40% in mean blood pressure (MBP) of urethane-anaesthetized rats [14].

1.3.8 Effect on Mosquitoes

To stop mosquito's population *Rhazya stricta* aqueous extract is used because of its significant toxicity which reduced the growth and development of C.pipiens larvae. Four days after at the 600 µg extract concentration; the larvae undergo 100% mortality [24].

Due to these numerous biological activities of *Rhazya stricta* plant we selected it for phytochemical screening.

2.0 Plant material & Methods

Locally available shrub of the family Apocynaceae, genus Rhazya Decne and specie *Rhazya Stricta*, commonly known as Gandechar leaves and stem were collected from the mountains of Malakand Khyber Pakhtunkhwa Pakistan, in the month of April. The plant is then identified by Professor Rab Nawaz Khan Govt. Postgraduate College Dargai. Then the leaves and stem first washed with tap water then by distilled water and were kept in shadow for 3 weeks to dry (Fig.2). After drying the sample were grinded into fine powder of 1mm (Fig.3). Then 40 grams of each sample is added to 250ml of ethanol in separate beakers and kept in room temperature for 13 days. After 13 days the solvent is filtered by Whatman no. 42 (125mm) filter paper (Fig.4). Then the ethanolic extracts were kept at room temperature for few days to evaporate the ethanol and get a viscous thick crude which some dissolved in ethanol and some in water and numerous phytochemical tests were performed.

The yield values of both extracts are determined by following formula:

$$\frac{\text{Extracts obtained}}{\text{Total amount of crude Drug}} \times 100$$

For ethanolic macerated extract

First sample (leaves) Yield value = (7g / 40g) × 100 = 17.5%

Second sample (stem) Yield value = (5g / 40g) × 100 = 12.5%

The chemical and reagents used in this work are listed in Table-2.

Table-2: list of chemicals used in phytochemicals analysis

S.No.	Chemicals	S.No.	Chemicals

1	Ethanol	9	Barfoed's Reagents
2	Million's Reagents	10	Benedict's reagent
3	Sulphuric Acid	11	Wagner's Reagent
4	Selwinoff's Reagents	12	Hager's reagent
5	Methyl Alcohol	13	Fehling A & B solution
6	Alpha Nepthol	14	HCl
7	Ferric Chloride	15	Ethyl Acetate
8	Acetic Acid	16	Ammonia

3.0 Phytochemical screening

In the current study phytochemical analysis of *Rhazya stricta's* leaves and stem ethanolic extracts were done qualitatively according to the procedures described by [25, 26], which unmasked numerous essential biologically active constituents in *Rhazya stricta* plant and results are shown in table 3 & 4.

4.0 Results

The results revealed by phytochemical analysis of *Rhazya stricta's* leaves and stem are shown in Table-3 and 4.

Negative symbol (-) shows absence while Positive symbol (+) shows presence of the consistent phytochemicals in corresponding leaves and stem ethanolic extracts of *Rhazya stricta*.

Table 3: Qualitative analysis of primary phytochemicals

S.No	Primary phytochemicals	Tests	*R.stricta's* Leaves	*R.stricta's* Stem
1	Test for carbohydrates	Molish's test	+ive	+ive
2	Test for reducing sugar	Benedict's test	+ive	+ive
		Fehling's test	+ive	+ive
3	Test for monosaccharaides	Barfoed's test	+ive	+ive
4	Test for hexose sugar	Cobalt chloride test	+ive	+ive
		Selwinoff's test	+ive	+ive
5	Test for non-reducing sugar	Benedict's test	-ive	-ive
		Fehling's test	-ive	-ive

6	Test for non-reducing polysaccharide	Iodine test	-ive	-ive
7	Test for protein	Biuret's test	+ive	-ive
		Million's test	+ive	-ive
8	Test for amino acid	Ninhydrin test	+ive	+ive
9	Test for fixed oils & lipids	Filter paper test	+ive	+ive

Table-4: Qualitative analysis of secondary phytochemicals

S.No	**Secondary phytochemicals**	**Tests**	***R.Stricta's* Leaves**	***R.Stricta's* Stem**
1	Test for Alkaloids	Hager's test	+ive	+ive
		Wagner's reagent	+ive	+ive
		Mayer's reagent	+ive	+ive
2	Test for Anthocyanin	Sodium hydroxide test	+ive	-ive
3	Test for Betacyanin	Sodium hydroxide test	-ive	+ive
4	Test for Cardiac glycosides	Acetic acid test	+ive	+ive
5	Test for Cyanogenic glycoside	Sodium picrate test	-ive	+ive
6	Test Flavonoid	Sulphuric acid test	+ive	+ive
7	Test for Phenols	Ferric chloride test	+ive	+ive
8	Test for Phlobotannins	HCL test	-ive	-ive
9	Test for Saponins	Foam test	+ive	+ive
10	Test for Saponins glycoside	Foam test	-ive	-ive
11	Test for Steroids	Sulphuric acid test	+ive	+ive
12	Test for Tannins	Ferric chloride test	+ive	+ive

13	Test for Terpenoids	Chloroform test	+ive	+ive
14	Test for Anthraquinone glycoside	Borntrager's test	-ive	+ive
		Modified Borntrager's test	-ive	+ive
15	Test for Chalcones	NH_4OH test	-ive	+ive
16	Test for Caumarin	NaOH test	+ive	+ive
17	Test for Emodins	NH_4OH test	-ive	-ive

5.0 Discussion

Phytochemical screening is very important process because it found and isolate those constituents from plants which have essential biological activities and are very necessary for treatment, cure and healing of different human ailments. In current study we have done phytochemical analysis of *Rhazya stricta's* leaves and stem ethanolic extracts which revealed the presence of different primary and secondary bioactive phytochemicals. *Rhazya stricta* leaves and stem extracts give positive results for all primary phytochemicals except protein which is absent in stem. Secondary phytochemicals including alkaloids, steroids, cardiac glycoside, flavonoids, phenols, saponins, tannins, terpenoids and caumarin are present in both leaves and stem while emodins, saponin glycoside and phlobotannins are absent in both leaves and stem of *Rhazya stricta*. Furthermore chalcone, anthraquinone glycoside and betacyanin are absent in leaves and present in stem while anthocyanin absent in stem.

6.0 Conclusion

Present study concluded that *Rhazya stricta* is immense source of bioactive constituents. *Rhazya stricta* contains all primary and secondary metabolites which have numerous biological functions and suggested for commercial and medicinal uses. *Rhazya stricta* shows presence of distinctive alkaloid content which is also acknowledge by different scientific studies. Appreciable activities of *Rhazya stricta*, including anti-bacterial, anti-fungal, anti-cancer, allelopathic, anti-oxidant, anti-diabetic, effect on blood pressure and effect of mosquitos etc. increase its importance in field of medicines and need to be further study and attention.

Acknowledgement

We would like to thank Mr. Hazrat Bilal Chairman of Department of Chemistry Government Postgraduate College Dargai for giving us research facilities and support.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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