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Preliminary phytochemical screening and various phytoconstituent of leaves extract of Rhynchoglossum notonianum wall

Sana Farzeen
Department of Biotechnology, Faculty of Engineering and Technology, Rama University, G.T. Road, Kanpur - 209217, India

Ajay Kumar
Department of Biotechnology, Faculty of Engineering and Technology, Rama University, G.T. Road, Kanpur - 209217, India
*Corresponding author email: ajaymtech@gmail.com

***Abstract*---**This plant belongs to the Gesneriaceae family. Rhynchoglossum notonianum Wall. Wild and developed varieties may be found across the country. The locals call it "kundru." All parts of the plant are historically used to treat different ailments. In Indian folk medicine, the leaves of this plant are used to cure a variety of diseases, including diabetes, wounds, ulcers, inflammation, skin eruptions, fever, asthma, and coughing coughs. They are also used to treat diabetes. Ethanolic extracts were subjected to a qualitative phytochemical screening to investigate the scientific foundation of ethnomedicinal potential. Many phytochemicals, such as glycosides, steroids, flavanoids, tannins, saponins, and terpenoids, were found in different extracts, and this study validated their existence. Wild edible leafy plants with greater phenolic and flavonoid content were found to be major antioxidant sources in this research, according to the evaluation of phytoconstituent. Rhynchoglossum notonianum Wall. was shown to be a rich source of many medicinally significant phytochemicals, which supports its usage as a remedy. Isolation and identification of medicinally useful active biochemical compounds may be accomplished by further investigation of this method.

***Keywords*---**rhynchoglossum notonianum wall, estimation of phytoconstituents, phytochemical screening.

Introduction

Rhynchoglossum belongs to the Gesneriaceae family of plants. There are other Klugia species listed in recent times. More than 100 species of the genus are

located in Asia, with three species discovered in tropical America [1]. As the name suggests, the leaves of the genus are alternate-distichous and asymmetrical in form. There are two mouths on the blooms. According to molecular data, Klugia from southern India are remarkably similar to those from the earlier genus Klugia, which had four stamens instead of two [2]. The pharmacopoeia nowadays is dominated by medicinal plants that have been proved to be both safe and effective. Solubility, poor permeability, limited bioavailability, and instability in the biological milieu are only some of the issues that must be taken into account when using plant/herbal medicinal substances as medications [3]. Nanoparticles may be attached to or encapsulated with herbal treatments in order to improve their pharmacokinetics and hence boost their overall effectiveness. They are worried about nanoparticles because medicinal plants' capping layers allow pharmaceutical corporations to modify the size and structure of nanoparticles, making them a specific source of worry. AgNPs were synthesised using a variety of medicinal and spice plants [4].

The therapeutic characteristics of *Rhynchoglossum notonianum* make it a valued spice in a wide variety of cuisines across the globe. A broad variety of ailments may be treated with any of these spices, according to traditional medicine. They have antibacterial, cancer-fighting and antioxidant properties as well as antidiabetic and antiemetic properties. They also reduce blood pressure and have hypoglycemia and immunomodulatory effects. According to phytochemical studies, these spices include a variety of antioxidants and alkaloids, which may aid in the reduction of silver to nanoparticles. In plants, you may find tannic acid and carotenoids as well as saponin-rich saponins, phenols, and flavonoids [5]. *Rhynchoglossum notonianum* is being studied for its phytochemical screening and different antioxidant content as part of our ongoing inquiry into the medical, nutraceutical/economical, and environmental uses of these spices.

Fig. 1. Rhynchoglossum notonianum

Materials and Methods

Collection of plant materials

The leaves of *Rhynchoglossum notonianum* (Wall.) B.L. Burtt. Were collected from local park. The leaves were identified and authenticated by Dr. Sanjeev Kumar Regional Ayurveda Research Institute (RARI) Jhansi, Uttar Pradesh, India. A

voucher specimen of the plant was kept in the herbarium of RARI; Jhansi with accession number of the specimen is 28696, on date 06/02/2022.

Preparation of plant extract [6]

A mortar and pestle were used to grind the dried leaves into a fine powder. Two hundred millilitres of each of methanol and water were used to combine 20 grammes of powder sample. The combination was left at room temperature for 24 hours. Using Whatman No.1 filter paper, the floral extract was filtered for 24 hours. For future usage, the filtrate was kept at 4 degrees Celsius.

Phytochemical Studies

Secondary metabolites generated by plants, their chemical structures, biosynthesis, metabolism, natural distribution, and biological roles are the focus of phytochemistry, which is closely connected to organic chemistry and plant biochemistry. Purification, separation and identification must be performed in order to carry out these processes. Advances in phytochemistry research are thus closely linked to both the effective use of existing methods and the continuous development of new approaches to address emerging issues. In phytochemistry, one of the difficulties is completing all of the aforementioned activities with such a tiny quantity of material [7]. To get *Rhynchoglossum notonianum* leaves, we went to the park. The Taxonomist verified the authenticity of the plants that had been collected. The plants were properly cleaned and dried in the shade after they had been harvested. These early phytochemical assays were carried out on the powdered shadow dried leaves [9].

Organoleptic evaluation

The following chemical tests were performed on powdered plant material and extracts, and the findings are shown in table 1.

Qualitative phytochemical examination of the leaf of *rhynchoglossum notonianum*

Wet chemical tests may be used to identify the chemical composition, specific identification, polarity, etc. of the components in the crude extract. A precipitate or colour reaction occurs when a certain component, generally a series of compounds, is present in the solution. An extraction process' efficiency may be monitored by doing such a test on various chemical substances. Qualitative chemical analysis was conducted on the petroleum ether, ether, ethyl acetate, methanol, ethanol, and aqueous extracts. Extracts were subjected to a variety of assays in order to determine the presence of steroid hormones, terpenoids, flavones, anthraquinones, sugar glycosides alkaloids quinones phenols tannin and saponins (table 2) [10].

Test for sterols

Initially, the powdered plant material was dissolved in petroleum ether and then evaporated to leave behind just the residue. Then, the residue was dissolved in chloroform and analysed for sterols.

- Salkowski's Test
  It was shook well, and the solution was let to cool for a few minutes before being used. Sterols were found in the bottom chloroform layer of the solution, which became red in colour as a result.
- Liebermann – Burchard's Test
  An acidic solution of concentrated sulfuric acid was introduced to the chloroform solution via the walls of the test tube and let to stand for a time. A brown ring created at the intersection of two layers. Sterols may be seen in the top layer, which became green.

Test for Terpenoids

Noller's test: Chloroform was used to extract and filter a small amount of the powdered crude substance. Tin and thionylchloride were used to gradually warm the filtrate. Terpenoids may be detected by the colour of the solution changing from clear to pink.

Test for carbohydrates

- Molisch's Test
  Sulphuric acid and alcohol were used to provide a purple tint that indicated that carbohydrates were present in the powdered crude drug extract when it was mixed with aqueous extract.
- Fehling's Test
  Over an hour and a half, Fehling solutions I and II were used to treat the powdered crude drug aqueous extract. Free reducing sugars were detected in the form of a crimson precipitate.

Test for Flavonoids

- Magnesium turning- con HCl test:
  It was cooked with a little amount of alcohol and filtered. A few drops of strong hydrochloric acid and magnesium turnings were added to the test solution and allowed to boil for five minutes. The presence of flavonoids is indicated by a red or magenta tint.
- Alkali Test
  A 10% solution of aqueous sodium hydroxide was added to the little amount of test solution. Flavonoids may be seen in the colour of the fruit, which is yellow or orange.
- Acid Test
  A few drops of strong sulphuric acid were added to the little amount of test solution. Flavonoids are indicated by their yellow to scarlet hue.

Test for Proteins

- Millon's Test
  Millon's reagent was used to heat a little amount of the powdered drug's acidic – alcoholic extract. Proteins may be seen in the white precipitate that becomes red when heated.
- Biuret Test
  Sodium hydroxide solution 10% was added to another part of acidous – alcoholic extract of the powdered medication, followed by one drop of weak copper sulphate solution. Proteins were detected by the violet hue, which was acquired.

Test for Alkaloids

- The powdered material was combined with 1gm of calcium hydroxide and 5ml of water into a homogeneous slurry and allowed to sit for 5 minutes before being pulverised. A porcelain plate on a water bath was used to evaporate it to a fine powder. Chloroform was added, mixed well, and heated on a water bath for 30 minutes. The chloroform was then removed by filtering and evaporation. To this, we added 5 ml of diluted hydrochloric acid and 2 ml of each of the following reagents.
  - Mayer's Reagent - No Cream precipitate
  - Dragendorff's Reagent - No Orange brown precipitate
  - Hager's Reagent - No Yellow precipitate
  - Wagner's Reagent - No Reddish brown precipitate.
  - These negative results indicate the absence of alkaloids.
- Test for Purine group (Murexide test)
  In a porcelain dish, 1 ml of hydrochloric acid and 0.1 gramme of Potassium chlorate were added, and the mixture was heated to dryness on a water bath until the acid had evaporated. Next, a diluted ammonia solution's vapour was sprayed over the residue. The lack of purple hue indicates that the purine group of alkaloids is not present.

Test for Glycosides

- Borntrager's Test
  To the filtered filtrate, benzene was added and agitated vigorously after boiling the leaf powder with dilute sulphuric acid. After separating the organic layer, a gradual addition of ammonia solution was made. Anthraquinone glycosides were not found in the ammonical layer, indicating that they were not present.
- Modified Borntrager's Test
  Dilute hydrochloric acid and a few drops of ferric chloride were used to cook 0.1 grammes of powdered medication, which was then filtered and allowed to cool. The benzene layer was then removed from the filtrate using benzene. The benzene extract was diluted with an equal amount of ammonia solution. Anthraquinone glycosides were found in the ammonical layer, resulting in a pink tint.

Test for Cardiac Glycosides (for Deoxysugar)

- Keller Kiliani Test
  The powdered leaf was cooked for two minutes in 10ml of 70% alcohol, chilled, and then filtered. 5 drops of a lead acetate solution were added to the filtrate, which was then evaporated to dryness with 10ml of water. In 3 cc of glacial acetic acid, the Residue was dissolved. Two drops of ferric chloride solution were added to the mixture at this time. It was then carefully added 3 ml of strong sulphuric acid and inspected. Cardiac glycoside deoxy sugars were not found in the reddish brown layer.
- Test for Cyanogenetic
  Glycosides Only a little amount of water was used to cover the powder in a stoppered conical flask. When the cap of the flask was placed, a sodium picrate paper strip was hung in the flask for two hours. cyanogenetic glycosides were not detected since the paper was not brick red.

Test for saponins

A little amount of the powdered medication was dissolved in 20 ml of water and gently heated for 2 minutes before being filtered and allowed to cool. This was followed by shaking 5 ml of the diluted filtrate briskly. In the lack of saponins, there was no foaming.

Test for Tannins

Water was used to extract a little amount of the powdered medication. A few drops of ferric chloride solution were added to the aqueous extract. There was a noticeable bluish black tint, which indicated the presence of tannins.

Test for the presence of Volatile oil

Hydrodistillation of fresh leaves (250 gm) was performed using a volatile oil estimation device (BP 1980). The fact that no oil was recovered suggests that new leaves and stems lack volatile oil.

Test for mucilage

Extracts from the powdered crude medication were treated with ruthenium red in a few ml of water. Mucilage may be seen by the colour of the mucilage.

Quantitative estimation of phytoconstituents

Using a standard or reference marker compound, a specific group of compounds present in crude extracts may be quantitatively estimated and reported as being comparable to the quantity of compound contained in that extract per standard compound.

Estimation of total phenolic content

Plant secondary metabolites are dominated by phenol. Natural phenols are abundant and play a vital role in the healing properties of many plants. Tannings, flavonoids, anthraquinones, and coumarins are all examples of polymeric compounds containing aromatic rings. Antioxidant activity is one of the many biological effects of phenolic compounds, which are water soluble [11].

Total flavanoid content estimation

As the Latin term flavus implies, "Flavonoid" is derived from the Latin word "yellow." It's true that most flavonoids are yellow. They may be found in abundance throughout the natural world. One benzene–gamma–pyrone structure is all it has. Proteins like structural proteins and enzymes may attach to these molecules, making them powerful antioxidants. Anthocyanins, leucoanthocynadins, chalcones, and aurones are a few of the many classes within each category that may be differentiated by the presence of heterocyclic rings with extra oxygen and hydroxyl groups [12].

Estimation of total tannin content

Seguin coined the name "tannin" in 1796. Organic, non-nitrogenous plant compounds known as tannins have astringent qualities because of their complex, organic structure. There are a lot of these chemicals in plants, and they're all over the place. The majority of the tannins have a rather high molecular weight. polymerization of simple polyphenols, which is what they are [13].

Result and Discussion

Organoleptic Evaluation

Table 1
Organoleptic Evaluation

S. No.	Organoleptic property	Appearance
1	Nature	Coarse powder
2	Colour	Dark green in colour
3	Odour	Characteristic odour
4	Taste	Bitter

Preliminary phytochemical screening

Wet chemical tests can detect the chemical composition, specific identity, polarity, etc. of crude extract components. a component, usually a sequence of compounds, is present in the solution. This test can detect chemical components and determine if an extraction procedure is performing properly. The aqueous and petroleum ether extracts were analysed chemically. The extracts were tested for steroid hormones, terpenoids, flavonoids, and anthraquinones, among other chemicals.

Table 2
Preliminary phytochemical screening for the leaf powder of *rhynchoglossum notonianum*

"S. no.	Test	MERN
1	TEST FOR STEROLS	
	a. Salkowski's test	+
	b. Libermann- Burchard's test	+
2	TEST FOR CARBOHYDRATES	
	a. Molisch's test	+
	b. Fehling's test	+
	c. Benedict's test	+
3	TEST FOR PROTEINS	
	a. Millon's test	+
	b. Biuret test	+
4	TEST FOR ALKALOIDS	
	a. Mayer's reagent	-
	b. Dragendroff's reagent	-
	c. Hager's reagent	-
	d. Wagner's reagent	-
	e. Test for Purine group (Murexide test)	-
5	TEST FOR GLYCOSIDES	
	a. Anthraquinone glycosides	-
	i) Borntrager's test	
	ii) Modified Borntrager's test	+
	b. Cardiac glycosides	-
	i) Keller Killiani test	-
	c. Cyanogenetic glycosides	-
	d. Coumarin glycosides	-
6	TEST FOR SAPONINS	-
7	TEST FOR TANNINS	
	$FeCl_3$ test	+
8	TEST FOR FLAVONOIDS"	
	a. Shinoda test	+
	b. Alkali test	+
	c. Acid test	+
9	TEST FOR TERPENOIDS	+
10	TEST FOR VOLATILE OILS	-
11	TEST FOR MUCILAGE	+

+ presence; - absence

Quantitative estimation of phytoconstituents
Estimation of Total Phenolic Content

Table 3 shows the total phenolic content of MERN and MERNAgNPs.

Table 3
Total phenolic content in mern & mernagnps in terms of gallic acid equivalents

Conc. of gallic acid in µg/mL	Absorbance at 760nm	Conc. of ethanolic extract in µg/mL	Absorbance at 760nm*		Amount of total phenolic content in terms mgGAE/g of extract*	
			MERN	MEAgNPs	MERN	MEAgNP
2	0.231 ± 0.012	50	0.532±0.003	0.544±0.004	93.08±1.4	95.15±0.5
4	0.454 ± 0.008	100	0.982±0.010	0.993±0.003	85.84±0.7	86.79±0.9
6	0.697 ± 0.007		Average		89.46±1.05	90.97±0.7
8	0.920 ± 0.033					
10	1.164 ± 0.030					

* mean of three readings ±SEM

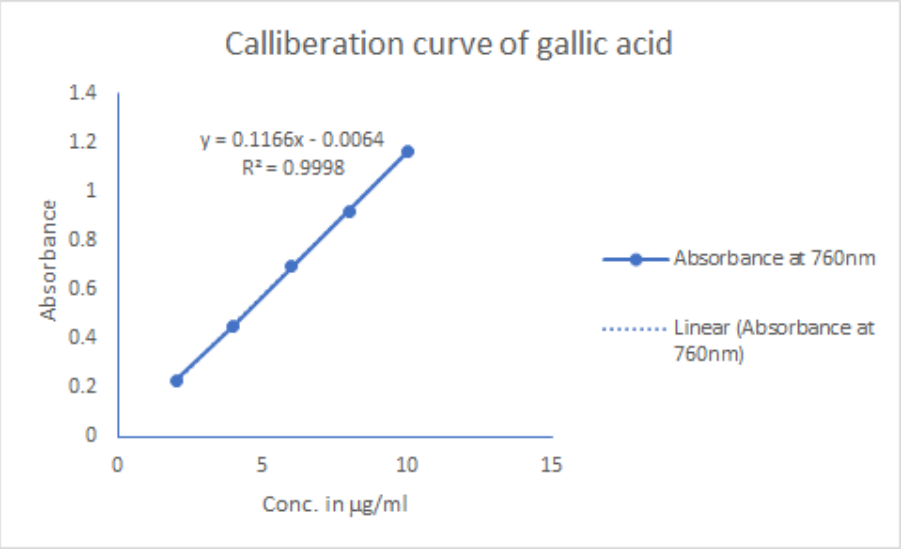

Fig. 2. Calibration curve of gallic acid

There was a correlation of 0.9998 between the linear regression equation y = 0.1166x-0.0064 and the correlation coefficient. Using the aforementioned linear regression equation, we calculated the mg GAE/g extract phenolic content in MERN & MERNAgNPs to be 89.46±1.05 & 90.97±0.7 respectively.

Estimation of Total Flavonoid Content

Table 4 shows the findings for the total flavonoid content of MERN and MERNAgNPs.

Table 4
Total flavonoid content in mern & mernagnps per gram of in terms of quercetin by aluminium chloride method

Conc. of quercet in in µg/mL	Absorbance at 415nm	Conc. of extract in µg/mL	Absorbance at 415nm*		Amount of total flavonoid content in terms mgQE/g of extract*	
			MERN	MEAgNPs	MERN	MEAgNPs
20	0.591 ± 0.03	100	0.339±0.013	0.37±0.013	116.98±0.28	119.47±0.39
40	1.153 ± 0.06	200	0.671±0.019	0.67±0.019	124.78±3.71	130.09±0.39
60	1.712 ± 0.11		Average		120.88±1.99	124.78±0.39
80	2.392 ± 0.05					
100	3.114 ± 0.05					

* mean of three readings ±SEM

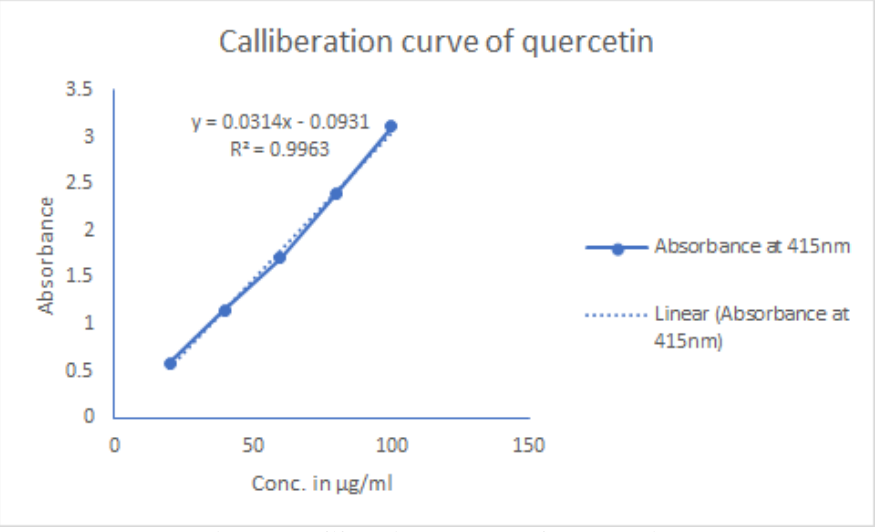

Fig. 3. Calibration curve of quercetin

In the linear regression equation, y = 0.0314x-0.0931 and the correlation was 0.9963. The aforementioned linear regression equation was used to determine the mg quercetin equivalent/g of extract flavonoid concentration in MERN & MERNAgNPs to be 120.88±1.99 & 124.78±0.39 mg/g.

Estimation of total Tannin Content

Table 5 shows the findings for the total tannin content of MERN and MERNAgNPs.

Table 5
Total tannin content in mern & mernagnps in terms of tannic acid equivalents

Conc. of Tannic acid in µg/mL	Absorbance at 700nm	Conc. of extract in µg/mL	Absorbance at 700nm*		Amount of total Tannin content in terms mg tannic acid/g of extract*	
			MERN	MEAgNPs	MERN	MEAgNPs
4	0.098 ± 0.04	10	0.061±0.017	0.09±0.004	248.44±5.17	297.42±9.18
8	0.185 ± 0.03	20	0.142±0.010	0.147±0.007	306.77±1.98	318.68±2.70
12	0.205 ± 0.03		Average		277.60±3.57	308.05±5.94
16	0.363 ± 0.22					
20	0.417 ± 0.12					

* mean of three readings ±SEM

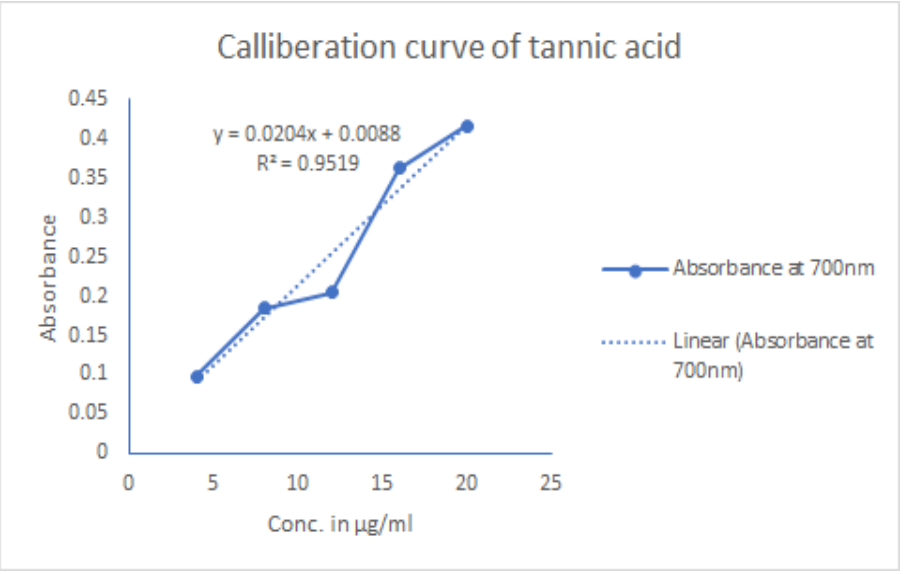

Fig. 4. Calibration curve for tannic acid

After oxidising the analyte using the Folin–Denis reagent in an alkaline solution, spectrophotometry is used to determine the total tannin content. Samples may be reduced using redox reactions and other reducing agents. y = 0.0204x + 0.0088 was determined to be the linear regression equation, and the correlation coefficient was found to be 0.9519. MERN and MERNAgNPs had a total tannin concentration of 277.60±3.57 and 308.05±5.94 mg tannic acid/g extract, respectively, according to the linear regression equation.

Conclusion

The chapter on phytochemical evaluation deals with the preliminary phytochemical evaluation and quantitative estimation of phytoconstituents present in the methanolic extract of the plant which gives information on the identify the presence of the secondary metabolites present in *Rhynchoglossum Natanianum* Wall. The amount of phenols, tannins and flavonoid content of MERN

and MERNAgNPs was compared. These determination and quantification gives the information about the amount of secondary metabolites present in the MERNAgNPs was higher than the MERN which is responsible for the therapeutic or pharmacological activity of the plant.

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