Assessment of *In-Vitro* Antibacterial Activity of Gargles of *Catharanthus Roseus* Linn.

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***Abstract*: This research work aimed to find out the antibacterial activity and phytochemical analysis of leaf extract of *Catharanthus Roseus* Linn.**
Antibacterial activity was studied against four bacterial strains such as *E. coli, S. aureus, S. typhi* & *Pseudomonas aeruginosa.* The Catharanthus roseus leaves extract was most potent for the bacterial strain of *E coli* and *S. typhi* and a little less potent for *S. aureus* and *Pseudomonas aeruginosa.* These bacterias are the main cause of various throat infections strep throat, sore throat, etc, and also cause a bad odor in the mouth.
The current study showed that aqueous extract of *C.roseus* leaves shows significant antimicrobial activity. The

***Keywords*: Antibacterial, *Catharanthus roseus*, Evaluation, Extraction, Gargles.**

Introduction - Herbal drugs which are in simple words termed crude drugs are an essential part of our lives for ages. They are the oldest form of health care known to mankind. Herbals contain several chemical constituents that have been used to treat various health issues for the upliftment of humankind[1-6]. *Catharanthus roseus* commonly known as vinca is one such herbal plant that has been used for its antibacterial, antidiabetic, anti-arrhythmic, antioxidant, antimutagenic, and anticancer activity. Yohimbine, an alkaloidal compound is responsible for plant antibacterial activity[7,8].
Gargles are aqueous solutions used to prevent or treat throat infections. These are the concentrated solution which is diluted with warm water before use. They are brought into intimate contact with the mucous membrane of the throat. Herbs have been used for centuries to prevent and control the disease. Herbal extracts are effective because they interact with specific chemical receptors within the body and are in a pharmacodynamic sense, drugs themselves. The antibacterial activity of *Catharanthus roseus* is useful in treating sore throats[9].

A. **Pharyngitis** -Pharyngitis is a sore throat that is caused by inflammation in the area behind the mouth. Pharyngitis can be caused by various bacteria or microbial infections. Pharyngitis caused by *Streptococcus pyogenes* is among the most concerning owing to its associated severe complications such as acute rheumatic fever and glomerulonephritis[10].
B. **Tonsillitis** - Tonsillitis is swelling and redness of the tonsils, the soft tissue in the back of the mouth. It is a common sore throat condition associated with acute upper respiratory tract infection, and recurrent episodes of infection may result in chronic tonsillitis[11].
C. **Laryngitis** - Laryngitis is swelling and redness of the voice box or larynx. It may be acute or chronic, infective or inflammatory, an isolated disorder, or part of a systemic disease, and often includes symptoms such as hoarseness[12].

Upper respiratory tract infection is a widespread infection that causes various problems such as scratchy or sore throat, sneezing, stuffy nose, and cough. These infections are generally caused by bacteria. Antibacterial drug gargling helps to treat and prevent such bacterial infections.
The advantage of taking a herbal drug for formulating gargles is that gargles are not only used for antibacterial effects but are widely used for hygienic purposes also. So prolonged use of such gargles will be safe and effective for use[13].

Drug profile
Catharanthus roseus commonly known as Vinca is the dried plant of *Catharanthus roseus* Linn., belonging to the family Apocynaceae. The plant was formerly designated as *Vinca rosea* L.[14,15].
Introduction of sample

Sample Name - *Catharanthus roseus*

Figure:1 The whole plant of *Catharanthine roseus*

Main Constituents - Vinblastine, Vincristine, Vindoline, Catharanthine, Yohimbine[16,17]

Uses [14-16, 18]

- Relieving muscle pain, depression of the central nervous system, also used for applying to wasp stings and to heal wounds.
- It is used as an antibacterial, antifungal, and antioxidant. In cases of scurvy and for relaxed sore throat and inflamed tonsils, it may also be used as a gargle.
- Vinblastine is an anti-tumor alkaloid used in the treatment of Hodgkin's disease. Vincristine is a cytotoxic compound used to treat leukemia in children. Vinca is used in herbal practice for its astringent and tonic properties in menorrhagia and in hemorrhages generally.
- For bleeding piles, it may be applied externally, as well as taken internally. It is also used in the treatment of diabetes. Another alkaloid of importance is ajmalicine is a vasodilator and is used as a hypotensive agent.

Scientific Classification
Kingdom- Plantae

Division- Magnoliophyta (Flowering plants)

Class- Magnoliopsida (Dicotyledons)

Order- Gentianales

Family- Apocynaceae

Genus- Catharanthus

Species- Roseus

Botanical Name: *Catharanthus roseus*

Synonyms - Vinca rosea, *Catharanthus,* Madagascar periwinkle, Barmasi.

Distribution

The plant is a native of Madagascar and is found in many tropical and subtropical countries, especially in India, Australia, South Africa, and North and South America. It is now widely distributed throughout warm regions and is much cultivated as an ornamental. It grows profusely in southern Florida. The plant is cultivated as a garden plant in Europe and India.

Material and Method

Preparation *C roseus* leaves extraction

The fresh leaves of *C. roseus* were collected from Dehrakhas, Dehradun, and authenticated from the Botanical Survey of India (BSI), Union Ministry of Environment and Forests, Dehradun, Uttarakhand, India under the Accession No. 461 dated on 19/05/021. The leaves were washed with water to remove dust particles and then shade dried at room temperature and coarsely powdered and stored until used.

Procedure of Extraction

Extraction for the gargles of *C. roseus* was carried out by decoction extraction method. 100 gm of dried powder of leaves of *C. roseus* was taken in a beaker and placed in a heating mantle. Distilled water is used as a solvent for the decoction extraction method. The drug after completely submerging insolvent is boiled at a low temperature for 20 min. Then by using a filter paper the extract is filtered out and marc is separated[19].

Synthesis of Herbal Gargles

Add grated fresh drug powder in a cup of warm water for 20min. Decoction of the drug is prepared. Strain the liquid by using filter paper. Take a portion of *C. roseus* leaves extract mix it with a portion of honey and make up the volume to 100ml with chloroform water IP.

Table No.1 Ingredients used for gargle formulation

S. No	Ingredients	Quantity
1.	Plant extract	5ml
2.	Honey	1ml
3.	Chloroform water IP	q.s to 100ml

Fig No 2 Gargles of *C. roseus* leaves extract

Study of Organoleptic Characters

The organoleptic character's color, odor, and taste are characterized by the gargle formulation using sensory organs of the body.

Evaluation of gargles

- **Visual inspection / Organoleptic evaluation**

  a. **Nature** - Crushed
  b. **Colour** - Yellowish-brown
  c. **Odour** - Characteristic Odour
  d. **Taste** - Palatable taste

- **Ph measurement** - The Ph of the gargles was observed to be 6.6 which is slightly acidic. The normal Ph of the throat is 7.0

- **Determination of phase separation** - Phase separation was determined by observing the prepared gargle for 24hrs. No phase separation was observed.

Why gargles?

1. **Easy to Prepare or formulate:** Gargles are used not only to treat but also to prevent any type of bacterial infection. Gargles are easy to prepare by simply mixing with lukewarm water and are self administratively by rinsing the throat with it.
2. **Soothe Inflammation and Infection**: The gargle is quite helpful with throat inflammation caused by seasonal allergies, colds, and sinus infections. They help to soothe inflammation, infection and also ease the throat pain caused by bacterial infection.
3. **Decrease of Harmful Bacteria in the Mouth**: Gargle is a neutralizer of acids that cause bacteria to multiply in the mouth. Regular use of a gargle is done to rinse the throat and mouth, it prevents any bacterial growth from occurring and removes the bad odor too.
4. **Nasal Passageways Cleared:** A gargle is one of the best things to clear your passages from head and chest congestion-based flu. The gargle rinses the throat of the mucus buildup in respiratory tracts and the nasal cavities[20].

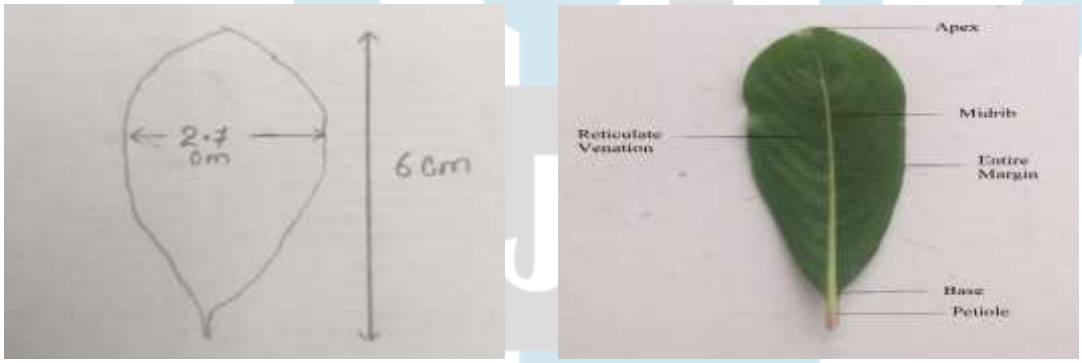
Antimicrobial Activity

It can be defined as all the active principles or agents which inhibit the growth of bacteria, prevent the formation of bacterial colonies, and may destroy them[21]. Antimicrobial activity is carried out against different bacterial strains which are most potent to cause throat infections such as sore throat, stuffy nose, cough, and other upper respiratory tract infections. The determination of the antimicrobial activity of gargles aqueous extract of *C. roseus* is done by the Agar well variation method[22,23].

- **Agar diffusion well-variant** -Agar well diffusion assay method was used to determine the inhibition of bacteria by gargles. Nutrient Agar media was prepared by dissolving 0.65gm Nutrient Broth and 0.8gm of agar into 50ml distilled water and sterilized by autoclaving at 121˚C, 15 lbs pressure for 15 minutes, then aseptically poured the medium into sterile Petri plates and allowed to solidify. The bacterial broth culture was spread on each Petri plate using a sterile L- shaped spreader. Then wells are cut in the Petri plates using a sterilized borer. And with the help of micropipette gargles of *C roseus* extract are being poured into the wells in equal concentration. The procedure was repeated for each Petri plate, with different bacterial strains. Then the Petri plates were incubated at 37˚C for 24 hrs. Incubated plates were observed for the zone of inhibition.

Results And Discussion

- **Pharmacognostic Evaluation of *C.roseus* leaves**



(a) **(b)**

Fig. No.3 Organoleptic/ Macroscopic Evaluation (a) Size of the leaf; (b) Macroscopy of leaf

Table No.2 Organoleptic/ Macroscopic Evaluation of *C. roseus* leaf

Organoleptic/ Macroscopic Evaluation	Observation
Colour	Green
Odour	Characteristic
Taste	Bitter
Shape	Ovate to oblong
Size	6cm (length); 2.7cm (width)
Apex	Acute or acuminate
Margin	Entire
Venation	Reticulate
Base	Asymmetric

- **Physicochemical Evaluation**

Table No.3 Physicochemical Parameters

S. No.	Physicochemical Parameters		Observation	
			Test	**Standard[82,85]**
1.	Loss on drying		4.07%	5.01%
2.	Ash value	Total ash value	0.5%	NMT 14.6%
		Water-soluble ash value	1.09%	1.68%
		Acid- insoluble ash value	0.71%	NMT 1.0%
3.	Extractive values	Alcohol- soluble extractive value	4.2%	NLT 12%
		Water-soluble extractive value	6.0%	NLT 40%

- **Phytochemical Investigation**

Table No. 4 Phytochemical Investigation of Extract

S. No.	Phytochemicals		Chemical Tests	Observation
1.	**Alkaloids**		Dragendroff's test	+
			Mayer's test	+
			Wagner's test	+
			Hager's test	+
			Tannic acid test	+
2.	**Tannins & Phenols**		Ferric Chloride test	+
3.	**Saponin**		Foam test	+
4.	**Carbohydrates**		Molisch's test	+
			Benedict's test	-
			Fehling's test	+
			Barfoed's test	-
5.	**Flavonoids**		Shinoda test	+
			Alkaline reagent test	+
6.	**Glycosides**	**Cardiac Glycosides**	Killer- killani test	-
			Legal's test	-
			Baljet's test	-
			Bromine water test	-
			Raymond's test	-
		Anthraquinone Glycosides	Borntager's test	-
			Modified Borntager's Test	-
		Cyanogenetic Glycosides	Grignard's test	-

- ***In- Vitro* Antibacterial Activity of Gargles**

The In-Vitro antibacterial activity of gargles of C.roseus leaves was determined by measuring Zone of inhibition. The radius of the zone inhibited by gargles was calculated, and by using the area of the circle, the area of bacterial inhibition in the petri plates was determined.

Area of the circle = πr^2

(a)

(b)

(c)

(d)

Fig No.4 Antibacterial activity of gargles of C. roses (a) *E. coli*;

(b) *S. typhi*; (c) *S. aureus* (d) *Pseudomonas aeruginosa*

Table No. 5 Result of preliminary antibacterial test of gargles of C.roseus leaves extracts by Agar well diffusion method

S No	Bacterial Strain	Zone of Inhibition (mm^2) of Gargles	Classification of Inhibition
1.	*E.coli*	490 mm^2	Strong
2.	*S. typhi*	379.9 mm^2	Active
3.	*S. aureus*	490.6 mm^2	Strong
4.	*Pseudomonas aeruginosa*	314 mm^2	Active

The antibacterial studies were carried out on the dried plant extract of *C.roseus* using four bacterial strains i.e *E. coli*, *S. typhi*, *S. aureus* & *Pseudomonas aeruginosa* by Agar well diffusion method. The significant antibacterial activity is determined by measuring the radius of the zone of inhibition and calculating the area inhibited by the extract.

Conclusion

The present study was designed to search for newer, safer, and more potent dosage forms for antibacterial effects which may accomplish our present needs. Various standardization parameters of the *C. roseus* leaf such as organoleptic macroscopic parameter,

microscopic parameters, and physicochemical evaluation were determined, which showed the different characteristics of the leaf. Dried powder of leaves of *C. roseus* was subjected to extraction using distilled water as a solvent by decoction method of extraction. The percentage yield of water-soluble extractive value and alcohol-soluble extractive value was found to be 6.0% and 4.2%.

This indicates that water-soluble extractives are present in maximum quantity and signifies the effectiveness of the water extract as an antibacterial agent. The preliminary phytochemical investigation shows the presence of

The gargle formulation of leaf extract was prepared by decoction method. Antibacterial activity against four bacterial strains such as *E. coli*, *S. typhi*, *S. aureus* & *Pseudomonas aeruginosa* is reported to be 490mm, 379.9mm, 490mm & 314mm respectively. This antibacterial activity signifies that bacterial strains *E. coli and S. aureus* are more susceptible to the antibacterial formulation than *S. aureus* & *Pseudomonas aeruginosa.*

Further investigation has to be performed in order to recommend the leaves of *C. roseus* extracts in animal or human phytotherapy.

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