Section A- Research paper

SCREENING OF PHYTOCHEMICALS AND *IN VITRO* ANTIMICROBIALACTIVITYHYDROALCOHOLICEXT RACT OF *GARDENIA RESINIFERA*

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Abstract

Life-threatening infections can be caused by microorganisms such bacteria, viruses, protozoa, fungus, and a few different types of worms. Antibacterial or antibiotic medications are used to stop or slow the growth of bacteria. Yet, these microorganisms have developed antibiotic resistance, which makes the situation worse. Thus, using medicinal plants is a safe alternative. Often referred to as "dikamali," *Gardenia resinifera* Roth. is an anthelmintic and a remedy for children's toothaches and teething issues. This study further evaluates the anti microbial activity of *Gardenia resinifera*. The plant material was collected & sujected to maceration followed by various qualitative & quantitative tests. Results showed that the pet ether extract was found to be enriched with diterpenes, phenol & carbohydrate while hydroalcoholic extract contain flavonoid, diterpene, phenol, proteins, carbohydrate & saponins. Total phenol & flavonoid content in hydroalcoholic extract was found to be 0.65 mg/ 100 mg and 0.72 mg/ 100 mg respectively. Maximum antimicrobial activity of extract was found to be against E.coli wiith the zone of inhibition 20 ± 0.47mm. While for *Bacilus subtilis*&*C. albicans* it was observed to be 15 ± 0mm & 19 ± 0.47mm respectively. From the results it can be concluded that Gardenia resinifera have convincing anti microbial activity against screened microorganisms.

Keywords: *Gardenia resinifera*, anti -microbial activity, Medicinal plants, drug resistance, *Bacillus subtilis Escherichia coli Candida albicans*

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Introduction:

Microbes, often known as microorganisms, are tiny, unicellular organisms that are invisible to the unaided eye. While certain microorganisms are beneficial to our daily lives, others are unhealthy for us. Pathogens are the dangerous microbes. Microorganisms that can cause disease include bacteria, viruses, protozoa, fungus, and a few different kinds of worms. These illnesses come from very different places and spread through very different methods. Unlike viruses, bacteria do not use the same channels to propagate disease. Viral infections are primarily transferred through the air, whereas contaminated food and water are the primary sources of bacterial infection. Therefore, viral and bacterial illnesses are usually communicable (Wilson, 2005; Lederberg, 2003).

Antibiotics and other medications are used to stop or slow the growth of microorganisms. Nevertheless, viral infections are not treatable with these antibiotics. The quantity and velocity of microbial dissemination determine the depth or severity of an illness. Our ability to cure common diseases is still under danger due to the creation and spread of bacteria that are resistant to drugs and have developed new resistance mechanisms. The increasing global development of multi- and pan-resistant bacteria, commonly referred to as "superbugs," which cause diseases that cannot be treated with currently available antimicrobial medications like antibiotics, is particularly concerning (Michael *et al*., 2014; Bush *et al*., 2011).

Hence, using medicinal plants to treat microbial infections is a safer alternative strategy. Alkaloids, saponins, tannins, flavonoids, and steroids are examples of phytochemical substances that have been proven to be physiologically active and hence partly responsible for the antibacterial properties of plants, which explains their usage in traditional medicine (Rios and Recio, 2005; Saranraj and Sivasakthi, 2014). The plant *Gardenia resinifera* Roth, often known as dikamali, is a member of the Rubiaceae family. It grows up to 6-7.5 m high and is a big, glabrous shrub or small tree that can be found throughout India's hills and ghats. Outside uses it has antibacterial, healing, and analgesic properties. It helps children with toothaches and teething issues (robbing latex to the gums) (Lakshmi and Reddy, 2012; Manjula *et al*., 2020). Thus, this deals with examining anti- microbial potential of *Gardenia resinifera*.

Materials and methods Collection of plant materials

Leaves of *Gardenia resinifera*was collected from Subham nursery, Bhopal (M.P) in month of September, 2021. The fresh leaves parts of this species were washed under running tap water, shade dried at room temperature and powderedfollowing which they were ground to a fine powder, sieved through a 500-µm sieve, and stored until the extraction.

Defatting of plant material

188 grams of shade dried powdered of leaves of *Gardenia resinifera* subjected to extraction with petroleum ether by maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction with hydroalcoholic solvent using maceration

Defatted powder was measured and mixed with hydroalcoholic solvent (ethanol: water, 80:20v/v) (Kokate, 1994). This was left for 2 days in sterile environment. The liquid extract was then filtered through Whatman filter paper no. 40. The filtrate was kept in water bath at 80-90ºC till the extract was dried out.

Biochemical Assays

Preliminary screening of biochemical tests of all three extracts were done for testing various phytochemicals found in plants (Mukherjee, 2007).

Quantitative studies of phytoconstituents estimation of total phenol content

The total phenol content of the extract was determined by the modified folin-ciocalteu method (Gaur Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Parkhe and Bharti, 2019). 10 mg Quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol.10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm.

In vitro*antimicrobial activity of leavesextract of *Gardenia resinifera

The well diffusion method was used to determine the antimicrobial activity of extract using standard procedure (Bauer *et al.,* 1966).

There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in studies. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

Results & Discussion

The pet ether extract was found to be enriched with diterpenes, phenol & carbohydrate while hydroalcoholic extract contain flavonoid, diterpene, phenol, proteins, carbohydrate & saponins. Total phenol & flavonoid content in hydroalcoholic extract was found to be 0.65 mg/ 100 mg and 0.72mg/ 100 mg respectively. Maximum antimicrobial activity of extract was found to be against E.coli wiith the zone of inhibition 20 ± 0.47mm. While for *Bacilus subtilis*&*C. albicans* it was observed to be 15 ± 0mm & 19
± 0.47mm respectively.

Table 1: Result of phytochemical screening of extractof *Gardenia resinifera*

S. No.	Constituents	Pet. ether extract	Hydroalcoholicextract
1.	**Alkaloids** Wagner's Test:	-ve	-ve
2.	**Glycosides** Legal's Test:	-ve	-ve
3.	**Flavonoids** Alkaline Reagent Test:	-ve	+ve

	Lead acetate Test:	-ve	+ve
4.	**Diterpenes** Copper acetate Test:	+ve	+ve
5.	**Phenol** Ferric Chloride Test:	-ve	+ve
6.	**Proteins** Xanthoproteic Test:	-ve	+ve
7.	**Carbohydrate** Fehling's Test:	+ve	+ve
8.	**Saponins** Froth Test:	-ve	+ve
9.	**Tannins** Gelatin test:	-ve	-ve

(+ve =positive; negative=-ve)

Table 2: Estimation of total phenol and flavonoids content of *Gardenia resinifera* extract

S. No.	Extract	Total phenol content	Total flavonoids content
		mg/ 100 mg	
1.	Hydroalcoholic	0.65	0.72

Table 3: Antimicrobial activity of standard drugagainstselected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	**20 µg/ml**	**30 µg/ml**
1.	Ciprofloxacin	*Bacillus subtilis*	12±0.5	17±0.74	20±0.15
		Escherichia coli	22±0.47	26±0.47	30±0.47
2.	Fluconazole	*Candida albicans*	26±0.47	30±0.47	32±0

Table 4: Antimicrobial activity of *Gardenia resinifera*extract

Sr. No.	Name of Organism	Zone of Inhibition(mm)		
		25 mg/ml	**50mg/ml**	**100mg/ml**
1.	*Bacillus subtilis*	6± 0.74	10 ± 0.5	15 ± 0
2.	*Escherichia coli*	12 ± 0.47	16 ± 0.47	20 ± 0.47
3.	*Candida albicans*	15 ± 0.47	17 ± 0.47	19 ± 0.47

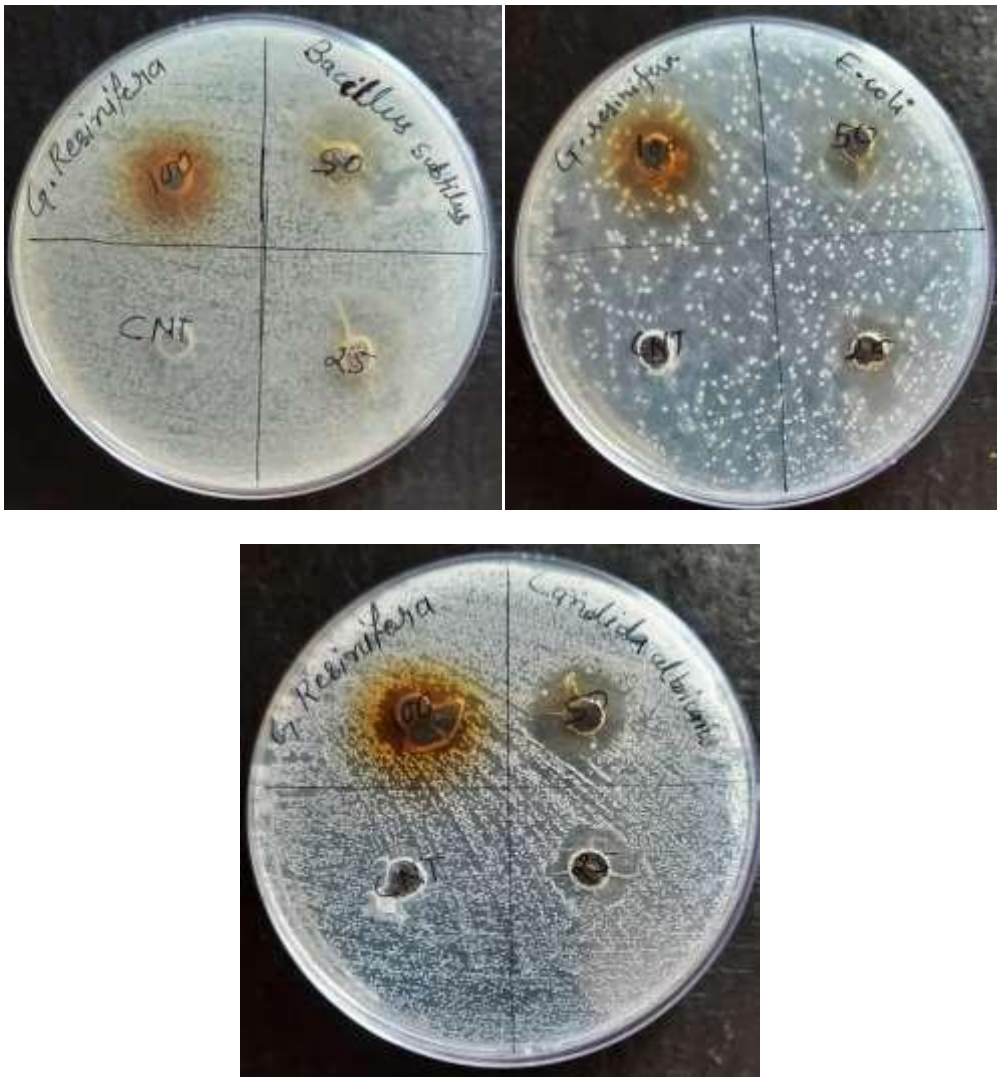

Figure 1: Image of antimicrobial activity of *Gardenia resinifera*extract (Leaf)

Conclusion

The presence of phytochemical components and their antibacterial properties support the therapeutic uses of *Gardenia resinifera* plants. The plant has been found to have bioactive chemical components and to have antibacterial properties against *Bacillus subtilis, Escherichia coli*, and *Candida albicans,* according to the study's findings. The therapeutic and selective potential of plant-based antimicrobials is considerable, and they can achieve the desired results with fewer adverse effects than the synthetic antimicrobials now in use. These results suggest that *Gardenia resinifera* has the potential to be a strong contender in the hunt for a natural antibacterial agent.

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