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Nymphaea rubra nanoparticle bioactivation against *Streptococcus aureus*

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***Abstract*:** The utilization of antibacterial agents that are found in plants can result in a more efficient management of diseases that affect humans. An investigation into the hepatoprotective power of *Nymphaea rubra* flower extract was carried out. *Nymphaea rubra* has a variety of therapeutic applications, including having anti-inflammatory properties, being sedative, astringent, cardiotonic, and aphrodisiac, among others. Existing pharmaceuticals are derived from plants to the extent of approximately 25–50%, which paves the way for an increase in interest in medicinal plants. *Bacillus cereus, Staphylococcus aureus,* and *Pseudomonas aeruginosa* were all combatted by the flowers with great success.

***IndexTerms*: *Nymphaea rubra, Streptococcus aureus* and Phytochemicals.**

I.INTRODUCTION

The aquatic plant *Nymphaea rubra*, more commonly referred to as water lily, occurs in aquatic environments. Rhizomes are responsible for spreading the roots by anchoring themselves in the mud of the pond. The true stems are not present in these plants; instead, they have leaves that are very large and flat, and their shape can be either oval or rounded. Throughout the course of the growing season, these plants that have a short lifespan are routinely replaced. In addition to that, it is a plant that purifies water. In addition to its capacity to filter out microbes that are present in water, the root of the plant is also capable of absorbing toxic substances such as mercury, lead, and phenol, amongst others. Afforestation, water purification, and landscaping are all areas that benefit from its application (Shi, 2009).

Plants that have the ability to function as drugs. A study conducted by Raja *et al*. (2010) found that the extract of rhizomes and flowers exhibited both anti-inflammatory and anti-diabetic properties. According to work done by Mukherjee *et al*. (2010), the seeds appear to possess immunomodulatory properties. Researchers Sinha *et al*. (2000) found that the extract of stalks had an anti-pyretic effect. Research conducted by Agnihotri *et al*. (2008) found that stamens, leaves, and flowers all contain antioxidant activity.

Ambal is the name given to this plant, which is utilized as a medicinal substance in the Siddha and Ayurvedic medical systems in India. This particular plant is most commonly used for the treatment of digestive disorders such as indigestion, dyspepsia, and dysentery. In the treatment of cutaneous diseases, its seeds are utilized. Rhizome in its raw form is an effective treatment for dysentery, according to Panda and Misra (2011). Antimicrobial drugs may be helpful in treatment, but they also cause some side effects that cannot be reversed, such as damage to the liver, strokes, kidney failure, and other potentially fatal conditions. Consequently, in recent years, researchers have been focusing their attention on plant-based drugs, which have seen a significant increase in their number. According to Harish Chandra *et al.* (2017), certain medicinal plants have the potential to be effective in the treatment of certain life-threatening diseases.

Bacillus cereus, Staphylococcus aureus, and *Pseudomonas aeruginosa* were all susceptible to the effect of the flowers, according to research published by Sasikala *et al.* (2018) and Vasu *et al.* (2008). In addition to being effective against other microorganisms such as *Klebsiella pneumoniae*, *Escherichia coli,* and *Shigella dysentriae*, it was also effective against certain fungi such as *T. mentagrophytes* and *C. albicans* (Indhu *et al.,* 2014; Indhu *et al.,* 2017; Indhu *et al.,* 2023; Parimala *et al.,* 2014). Therefore, according to the findings of these studies, the crude extracts from the genus Nymphaea have the potential to act as an antimicrobial agent. This is because they contain the phytoconstituent catechin, which is what makes them useful for traditional medicine in the treatment of infections (Dash *et al.,* 2013). Secondary metabolites are found in plants, and polyphenols and flavanoids make up the largest group of phytochemicals. Plants contain a wide variety of secondary metabolites. The only method that was used by people in ancient times to treat infectious diseases was the utilization of plants, which was later demonstrated by scientific research regarding the therapeutic effects of these plants (Russo *et al.,* 2017).

II.MATERIALS AND METHODS

PREPARATION OF EXTRACT

In order to obtain a colourless extract on the top of the extractor, a sample of ten grammes of the shade dried powder of plant parts of *Nymphaea rubra* was successively extracted in a soxhlet extractor with two hundred ml of ethanol and water. Separate concentrations were performed under reduced pressure on each of the extracts of the plant. Each of these solvent extracts was weighed and then dissolved in DMSO before being put through additional tests (Mohana *et al.,* 2008). This was done after the solvent had been completely evaporated during the process.

PRELIMINARY PHYTOCHEMICALS STUDY (SOLOMON *ET AL*., 2013)

Test for alkaloids - Wagners test, Test for carbohydrate - Molisch's test, Test for Flavonoids - Alkaline reagent test, Test for phenols - Ferric chloride test, Test for Saponins - Foam test, Test for Tannins - Braymer's test, Test for Terpenoids - Salkowki's test, Test for Quinones – HCL Test, Test for steroids - Salwoski test, Tests for proteins - Millon's test.

ISOLATION OF PRESUMPTIVE *S. AUREUS* FROM MILK SAMPLES

A series of dilutions were prepared by diluting 1 mL of each sample of raw and pasteurised milk with 9 mL of saline peptone solution at a concentration of 0.1%. The dilutions ranged from 10-4 to 10-8 for raw milk and from 10-2 to 10-6 for pasteurised milk by using a diluting solution. A L rod was then used to spread the inoculum that had been applied to the surface of the nutrient agar. The incubation was carried out for a single day at a temperature of 37 degrees Celsius.

PHENOTYPIC DETECTION OF INDUCIBLE RESISTANCE TO CLINDAMYCIN BY D-TEST

On Mueller–Hinton agar plates that had been previously inoculated with 0.5 McFarland bacterial suspensions, a disc containing 15µg of erythromycin was meticulously positioned at a distance of 15 mm (edge to edge) from clindamycin, which contained 2µg. After 18 hours of incubation at 37 degrees Celsius, the plates were examined. If an isolate was resistant to erythromycin but susceptible to clindamycin, and it had a D-shaped inhibition zone around the clindamycin disc, then it was considered to be positive for inducible resistance (D test positive, iMLSB phenotype by the laboratory). It was determined that the isolate was negative for inducible resistance (D test negative, MS phenotype) if it was resistant to erythromycin and susceptible to clindamycin, and if both zones of inhibition showed a circular shape. However, the isolate was considered to have an active efflux pump.

ANTIMICROBIAL ACTIVITY OF PLANT EXTRACT

This examination was carried out in accordance with the methodology described by Jahir et al., 2011. Following the pouring of twenty ml of Mueller hinton agar (MHA) that had been sterilized, each Petri plate with a diameter of ninety ml was allowed to solidify. The inoculums contained 108 colony-forming units per ml of bacteria. Through the utilization of a sterile cork-borer, a wheel with a diameter of 6 ml was bored with the medium of each plate respectively. Ampicillin, at a concentration of 5µg/ml, was employed as a positive control, while DMSO was introduced into a separate well, serving as a negative control.

PREPARATION OF SILVER NANOPARTICLES

According to the findings of qualitative phytochemicals research, the nanoparticle synthesis of copper sulphate was applied to the phytochemicals that contained the highest counting of methanol extract. After adding 0.5 ml of extract to these solutions, the color of the solution began to change from light brown to dark brown. This change occurred as a result of the reaction between the Cu ions and the formation of u NPs. The 5 ml of 2 millimolar CuSO4 solution was prepared. The agar well diffusion method was then used to test the antimicrobial activity of the nanoparticles that had been obtained through synthesis.

III.RESULTS

ETHANOL EXTRACT OF *NYMPHAEA RUBRA*

The findings of the phytochemical analysis performed on the ethanol extract of Nymphaea rubra are presented in Table 1. The flower part that was extracted with methanol showed positive results for the presence of all of the phytochemicals that were tested, with the exception of proteins. The acetone extract contained a number of different compounds, including alkaloids, flavonoids, phenols, tannins, saponins, and sterols. There was no evidence of the protein in either of the extracts. Within the scope of this investigation, the greatest number of phytochemicals that contained methanol extract were utilized for subsequent research.

Table 1

Preliminary phytochemicals analysis of ethanol extract of *Nymphaea rubra*

S.no	Phytochemicals	Acetone	Methanol
1.	Alkaloids	+	+
2.	Carbohydrates	-	+
3.	Flavonoids	+	+
4.	Phenols	+	+
5.	Tannins	+	+
6.	Terpenoids	-	+
7.	Saponins	+	+
8.	Sterols	+	+
9.	Quinones	-	+
10.	Protein	-	-

PHENOTYPIC DETECTION OF INDUCIBLE RESISTANCE TO CLINDAMYCIN BY D-TEST

The 'D' test was performed on each and every isolate examined in this study. Three distinct types of results were discovered among the sixteen isolates. One of the isolates, which accounted for 6.2% of the total, was an iMLSB, three had an MLSB, and five had an MS phenotype. The antibiotics erythromycin and clindamycin were effective against the remaining isolates, as shown in Table 2.

Table 2

Phenotypic detection of inducible resistance to Clindamycin by D-test

S.No	Isolates	iMLS	MS TYPE	cMLSB
1.	Sa 1	-	-	-
2.	Sa 2	-	+	-
3.	Sa 3	-	+	-
4.	Sa 4	-	-	+
5.	Sa 5	-	-	+
6.	Sa 6	+	-	-
7.	Sa 7	+	-	-
8.	Sa 8	-	+	-
9.	Sa 9	+	-	-
10.	Sa 10	-	+	-
11.	Sa 11	-	-	-
12.	Sa 12	-	+	-
13.	Sa 13	-	-	-
14.	Sa 14	+	-	-
15.	Sa 15	-	-	+
16.	Sa 16	+	-	-

Antimicrobial activity of flower extract of *Nymphaea rubra*

According to the highest number of phytochemicals that were found in the methanol extract, which was chosen for its antibacterial activity. A total of five iMLS positive isolates were chosen for the purpose of determining the antimicrobial activity of *Nymphaea rubra* with regard to the present investigation. The zone of inhibition was anywhere between 10 and 18 mm in size. The *S. aureus* isolate number three was the most severely inhibited of the five isolates, and its zone of inhibition was found to be anywhere between 10 and 18 mm. After *S. aureus*, this is the second most inhibited against it. There was no evidence that the flower extract of *Nymphaea rubra* was able to inhibit *S. aureus*. Among the four concentrations, 2.5 mg and 5 mg were successfully inhibited to

a single isolate, while 7.5 mg was successful in inhibiting four isolates. It was found that the use of control agents such as methanol and ciprofloxcin did not inhibit the growth of the isolates (Table 3).

Table 3

Antimicrobial activity of flower extract of Nymphaea rubra

S.No	Isolates name	Con.of extract (mg) Zone of measurement in mm				Methanol	Ab
		2.5	5	7.5	10		
1.	*S.aureus 1*	-	-	12	14	-	-
2.	*S.aureus 2*	-	-	13	14	-	-
3.	*S.aureus 3*	12	14	16	18	-	-
4.	*S.aureus 4*	-	-	-	-	-	-
5.	*S.aureus 5*	-	-	10	14	-	-

ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES OF FLOWER EXTRACT OF *NYMPHAEA RUBRA*

Within the scope of this investigation, copper nanoparticles (CuNPs) were employed to assess the antibacterial efficacy against five iMLS positive isolates. There was a significant reduction in the amount of *S. aureus* among the five isolates, with the zone of inhibition ranging from 11 to 24 mm. The second most inhibited to *S.aureus* and followed by *S.aureus*. When compared to the five different concentrations, 2.5 mg inhibited four of the isolates, while 5 mg inhibited all of the isolates. Copper sulphate was used as a control agent, but they were not successful in inhibiting any of the isolates. Compared to flower extract that did not contain nanoparticles, this nanoparticles demonstrated superior inhibitory activity against all of the isolates.(Table 4)

Table 4

Antimicrobial activity of silver nanoparticles of flower extract of *Nymphaea rubra*

S.No	Isolates name	Con.of extract (mg) Zone of measurement in mm				copper sulphate
		2.5	5	7.5	10	
1.	*S.aureus 1*	-	12	16	19	-
2.	*S.aureus 2*	12	15	18	21	-
3.	*S.aureus 3*	14	17	20	24	-
4.	*S.aureus 4*	11	15	17	19	-
5.	*S.aureus 5*	12	16	19	22	-

III.DISCUSSION

Antibiotic resistance is a significant issue that has emerged as one of the most important concerns in the realm of public health in the 21st century. It is a problem that affects people all over the world. For the most part, this phenomenon was brought about by the inappropriate and indiscriminate use of antibiotics for the treatment of the common bacterial infection. Bacterial species that are able to survive after being exposed to one or more antibiotics are referred to as MDR, which stands for multiple antibiotic resistance. Pathogenic isolates that are resistant to multiple antibiotics are considered to be among the most dangerous. This occurrence is extremely hazardous to both humans and animals due to the fact that resistance has been demonstrated to increase with the increasing duration of treatment. (Sivanantham and *et al* 2023).

Within the scope of this investigation, a total of six different bacterial genera and two different species of candida were obtained from a clinical laboratory. These bacterial isolates were subsequently confirmed through microscopic and morphological characterization. Antimicrobial resistance patterns were examined using a number of different antibiotics on the confirmed isolates during the experiment. Among the twelve antibiotics, penicillin was resistant to all of the isolates. It was followed by ampicillin and cefotaxime, which had a resistance rate of 83.3%, and then by cefixime, ceftriaxone, and kanamycin, which had a resistance rate of 67.7%. Of the antibiotics, chloramphenicol and amikacin had the lowest resistance percentage, which was 17%. The bacteria *P. mirabilis* showed the highest level of resistance, which was 67%, followed by *K. pneumoniae*, which showed 50%.

A variety of secondary metabolites, such as sterols, alkaloids, and tannins, were found on the Nymphaea species that was isolated from flowers. In addition, the plant belonging to this genus is found to contain tannins, flavanoids, alkaloids, and saponins (Raja *et al*., 2010). The antimicrobial activity of the plant can be attributed to these substances, which are called metabolites. According to Sarma *et al*. (2008), it was also utilized for the treatment of diabetes, inflammation, urinary and liver disorders, indigestion, menstruation, and a great deal of other conditions also.

Phytochemicals analysis reveals the presence of numerous medicinally significant secondary metabolite types of phyto constituents. The extracts of ethanol and water showed positive results for the presence of a number of phytochemicals. Furthermore, the ethanol extract demonstrated the presence of a number of phytochemicals (Thangaraj and Sivanantham 2015). The results of the preliminary investigations that were carried out on the various extracts of Nymphaea species are presented herewith.

When compared to the aqueous extract, the effectiveness of the inhibitory activity of the methanol extract was demonstrated in this study. Because of the presence of phytochemical constituents, specifically alkaloids, flavonoids, saponin, tannin, glycosides, phenols, and steroids, it is known that these constituents are primarily responsible for the beneficial activity. Alkaloids, flavonoids, steroids, saponin, protein, and glycosides are all found in methanol solvent extracts, according to our phytochemical screening and antibacterial research (Siva and Savithramma, 2016). It is possible to mention that these compounds are present in the extracts. According to the findings of the current investigation, the antimicrobial effect of ethanol extracts of leaf and flower of Nymphaea species was found to be acceptable in comparison to the standard antibiotics. This was observed when the results of the investigation were compared with the standard antimicrobial drugs. There is a possibility that this is due to the fact that the plant material contains high concentrations of polar compounds, which are soluble in solvents that have a high polarity, such as water, methanol, and ethanol.

IV.CONCLUSION

The present study was carried out to explore antimicrobial, antioxidant, and anticancer potential of *Nymphaea alba* and *Nymphaea rubra*. The maximum bioactivity was observed for ethanol extract. Bioactivity guided screening of ethanol extract revealed the presence of bioactive component, which known to have useful bioactivities. This finding shows the importance of screening plants for antimicrobial agents against resistant strains. The reported results should pave the way for more investigations, aiming at determining the in vivo toxicity and pharmacological effects, and a more in depth evaluation of the plant composition.

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