Screening on Phytochemical Analysis, Antimicrobial And Antioxidant Activities of Floral Parts of *nerium Sp*

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Abstract- *A medicinal plant is any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for chemo pharmaceutical semi synthesis. Many investigations have demonstrated to elucidate the chemical components of flower origin. In this study, the preliminary phytochemical group test of the aqueous extract of dried flowers of Nerium oleander extract was performed for the presence of various active principles. Antimicrobial activity is determined by agar diffusion method while antioxidant activity is explained by DPPH method. Due to its potency against oxidant and microbial activity, these oleander flowers can be used in pharmacology field in future to treat various diseases in human*.

Keywords- Antimicrobial, Antioxidant, Nerium oleander, Pharmaceuticals, Phytochemicals.

I. INTRODUCTION

1.1 Medicinal plants

Medicinal plants are of great importance to the health as they are used to treat different diseases.When a plant is designated as 'medicinal', it is implied that the said plant is useful as a Drug or Therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants possess special virtues for drugs, therapeutic agents and for medicinal purposes.

1.2 Nerium oleander

Nerium oleander (common name: Areli) is an evergreen shrub in the dogbane family Apocynaceae, It is about 2-5m in height. It is especially suited to sunny and dry localities (Lokesh *et al.*, 2010) [1]. Oleanders are drought tolerant evergreen plants of this family.

Nerium oleander shows terminal flower clusters with milky latex with red, pink and white coloured. Each flower is about 5cm in diameter with 5 petals and rich in colour. Due to their potency, they are used in different medicinal fields.

1.2.1 Toxic effect and Medicinal uses

Nerium oleander is one of the most poisonous plants in the world. The most significant toxins are oleandrin, oleandrigenin and nerine. (Sabira Begum *et al*., 1999) [2].

In Turkish folk medicine, oleander flowers are used against rheumatic pain as a home remedy (Yesilda, 2002) [3] . The flowers are used against corns, warts, cancerous ulcers, carcinoma, ulcerating or hard tumours.

Fig. 1.1 *Nerium oleander* (Red)


Fig. 1.2 *Nerium oleander* (White)

1.3 Phytochemicals

Phytochemicals are bio-active chemicals of plant origin. They are regarded as secondary metabolites because the plant that manufactures them may have little need for them. They are naturally synthesized in all parts of the plant

body (Hill, 1952) [4]. Most important bioactive constituents of plants are alkaloids, flavonoids, glycosides and tannins.

1.3 Antimicrobial Activity

Globally, researchers are using extracts of plants for their antiviral, antibacterial, and antifungal activities. The characteristics of the plants that retard the growth of microorganisms have been investigated in different laboratories around the world since 1926 (Bakht *et al.*, 2012) [5]. Antimicrobial activity of many plants against microorganisms is due to the presence of active components present in the plant.

1.5 Antioxidant Activity

All aerobic organisms, including human beings, have antioxidant defences that protect against oxidative damages, numerous damage removal and repair enzymes to remove or repair damaged molecules. Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective (Duh *et al.,* 1999) [6].

In the present investigation, we studied flower extracts of *Nerium oleander* and for determining the presence of phytochemicals, Antimicrobial and Antioxidant activity.

II. MATERIALS AND METHODS

2.1 Sample Collection

Fresh White, Red flowers of *Nerium oleander* was collected from different healthy plants in and around the area of Anthiyur, Erode District**,** Tamilnadu.

2.2 Sample Extraction

The fresh flowers were cleaned thoroughly and dried at 2-3 days in the sunlight under sterile conditions. The dried samples were made into fine coarse powder and stored in screw cap bottles until further analysis.

Nerium oleander (Red)

Nerium oleander (White)

Fig. 2.1 Powdered Oleander flowers samples

Sixteen gram of each sample powder was taken, to which 80ml of different solvents (Acetone, Ethanol and Diethyl ether) were added, mixed, and kept for one day. The contents were periodically shaken using an electric shaker. After a day, the contents were filtered using Whattman No.1 filter paper (pore size 25µm) to get a clear solution in a conical flask and they are stored for future use.

2.3 Phytochemical Analysis

Preliminary screening of the extracts and identification of major phytochemical was done by colour tests adapting standard methods. (Raman, 2006) [7].

2.4 Antimicrobial Activity

Antibacterial and antifungal activity studies were carried out by agar diffusion method (Barry *et al.,* 1976) [8].

2.4.1 Test organisms

Two Gram-positive bacteria as *Bacillus sp*., *Staphylococcus sp*. and three Gram negative bacteria as *Escherichia coli, Salmonella sp*., and *Pseudomonas sp*. were used for antibacterial activity. One Fungi *Aspergillus sp*.was used for antifungal activity.

2.4.2 Methodology

The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours.In an aseptic room, 20ml of the Nutrient agar medium is poured into each Petri dish and allowed to solidify at room temperature.After solidification,0.1ml of the test organisms culture were inoculated with the help of a sterile cotton swab soaked in a bacterial culture or suspension, providing an uniform surface growth of bacterium**.** The wells were bored with 8mm borer in seeded agar, and then the particular concentrations (20µl) of the extracts (Ethanol) were added in each well. Soon after the plates were then kept at 10°C for

30min. After it normalized to room temperature plates were incubated at 37°C for 24hrs. After incubation period is completed, the zone of inhibition was measured and recorded.

2.5 Antioxidant Activity

The detection of anti-radical substances of the extracts was performed by the method of DPPH (Mensor *et al.*, 2001) [9].

2.5.1 DPPH free radical scavenging activity

The diluted working solutions of the test extracts were prepared in ethanol. Ascorbic acid was used as standard in 530µg/ml solution. 0.002% of DPPH (Diphenyl-2-picrylhydrazyl) was prepared in ethanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV spectrophotometer. Ethanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank.

Calculation

The optical density was recorded and % inhibition was calculated using the formula,

Equation 1: Percent (%) inhibition of DPPH activity = 100-(A-B/A) x100

Where,

A = optical density of the blank and
B = optical density of the sample
.

III. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Various phytochemicals can be obtained from plants which are very beneficial for mankind and used in manufacturing of traditional drugs.

This study shows the presence of phytochemical constituents such as Carbohydrate, Alkaloids, Tannins, Saponins, Amino acids and also Proteins in all the extracts of Red and White flowers. It also revealed the presence of Flavonoids and Steroids. Steroidal compounds are used in pharmacy due to their relationship with sex hormones. Phenols are determined only in Ethanolic extract of White flower and in both ethanol and Diethyl ether extract of red flower. The phenolic products of medicinal plants have a great pharmacological interest. Phytochemical screening of Red and white Nerium oleander is explained in Table 3.1 and 3.2.

3.2 Antimicrobial Activity

` Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action. They are effective in the treatment of infectious diseases.

In the present study, the ethanolic extract of *Nerium oleander* showed maximum zone of inhibition (28mm) against *Pseudomonas sp*. Maximal activity of the ethanol extract point out that the active components present in flower extract can prove to be a great remedy for treating diseases. The antifungal activity of the ethanolic *Nerium oleander* flower extracts against *Aspergillus sp.* Table 3.3 explains the antimicrobial activity of Oleander flowers.

Table 3.1 Phytochemical screening of *Nerium oleander* (Red and white flower)

		Nerium oleander (Red)			*Nerium oleander* (White)		
Constituent	**Chemical Test**	**Acetone**	**Ethanol**	**Diethyl ether**	**Acetone**	**Ethanol**	**Diethyl ether**
Carbohydrate	Molisch's Test	+	+	+	+	+	+
Reducing sugar	Fehling's Test	+	+	+	+	+	+
Alkaloids	Hager's Test	-	-	-	-	-	-
	Wagner's Test	+	+	+	+	+	+
	Mayer's Test	+	+	+	+	+	+
Saponins	Foam Test	-	+	+	-	-	-
Tannins	Lead Acetate Test	+	+	+	+	+	+
Flavonoids	Acid Test	+	+	+	+	+	+
Terpenoids	Acetic acid Test	+	+	+	+	+	+
Phlobotannins	-	-	-	-	+	+	+
Coumorins	-	+	+	+	+	+	+
Cycloglycosidase	-	+	+	+	+	+	+
Phenol	Ferric Chloride Test	-	+	+	-	+	-
Quinone	-	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-	-
Steroids	-	+	+	+	+	+	+
Carotenoids		+	+	+	+	+	+
Fatty acids	Transparency Test	+	+	+	+	+	+
Amino acids	Ninhydrin Test	+	+	+	+	+	+
Proteins	Millon's Test	+	+	+	+	+	+
Cholesterol	-	-	-	-	-	-	-
Glycosides	Liebermann's Test	+	+	+	+	+	+
Anthocyanin	-	-	-	-	-	-	-
Leucoanthocyanin	-	+	+	+	+	+	+
Emodin	-	-	-	-	-	-	-
Cardiac glycosides	-	+	+	+	+	+	+

+ = Positive; - = Negative

Table 3.3 Antimicrobial effect of *Nerium oleander* flowers

Microorganism	Zone of Inhibition (mm)	
	Nerium oleander (Red)	*Nerium oleander* (White)
Bacteria		
Bacillus sp.	17 ± 0.5	9 ± 0.2
Staphylococcus sp.	21 ± 1.2	12 ± 1. 5
E.coli	20 ± 1.5	10 ± 0.5
Salmonella sp.	25 ± 3.0	15 ± 1.5
Pseudomonas sp.	28 ± 2.6	18 ± 1.5
Fungi		
Aspergillus sp.	18 ± 1.5	17 ± 1.5
Penicllium sp.	18 ± 1.5	17 ± 0.5

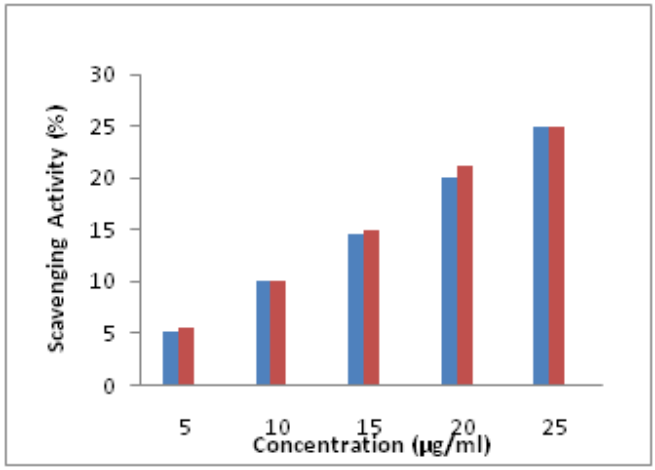

Fig.3.1 DPPH Free radical scavenging activity

IV. CONCLUSION

Nearly 80% of the world populations depend on the traditional medicine for primary health care. Use of flowers as a source of medicine has been inherited in India. Flower extracts are given as concoctions for various ailments. In the present study, phytochemical, antimicrobial and antioxidant activities of the flower extract was carried out to find out the major activities.

The flowers *Nerium oleander* (Red, White) has got certain active phytochemicals such as Carbohydrates, Proteins, Amino acids, Cardio glycosides, Flavonoids and Alkaloids in all the extracts (Acetone, Ethanol, Diethyl ether) which are used to treat various human diseases

The ethanolic extracts of flowers show effective inhibition against two Gram-positive bacteria as *Bacillus sp.*, *Staphylococcus sp*. and three Gram negative bacteria as *Escherichia coli, Salmonella sp.*, and *Pseudomonas sp*. along with two fungi *Aspergillus sp*. and *Penicillium sp*. The Reactive Oxygen Species (ROS) released by the human body are eliminated by molecules with antioxidant properties. The ethanolic extracts of *Nerium oleander* plants show effective scavenging activity.

The study shows the pharmacological importance of flower of *Nerium oleander* showing antimicrobial and antifungal activity and thus substantiates traditional medicinal use. After further purification and characterization of the active metabolites present in flower of *Nerium oleander* followed by a detailed study of toxicity and pharmacological effects of the compound, the flower extracts may be used as remedy against various diseases without any side effects and the plant species can be a good pharmacophore source in future. Thus, the study provides a strong direction for proper investigation of various plants to explore molecules having antimicrobial and antifungal properties against human pathogens.

REFERENCES

[1] Lokesh, R., E. Leonard Barnaba, P. Madhuri, K. Saurav and Sundar K. 2010. Larvicidal activity of Trigonella foenum and Nerium oleander leaves against mosquito larvae.

[2] Sabira Begum, Bina S. Siddiqui, Razia Sultana, Atiya Zia and Amin Suria. (1999). Phytochemistry Volume 50, Issue 3, 10: pp. 435-438.

[3] E. Yesilada, (2002). Biodiversity in Turkish folk medicine. Kluwer Academic/Plenum Publishers. London, U.K.

[4] Hill AF (1952). Economic botany. A textbook of useful plants & plant products. 2nd edn. McGarw-Hill Book Company Inc., New York.

[5] Bakht, J., G. Azra and Shafi, M. 2012. Antimicrobial activity of Nicotiana tabacum using different solvents extracts. Pak. J. Bot., 44(1): 459 - 463. Barry

[6] Duh, P.D., Y.Y. Tu and Yen, G.C. 1999. Antioxidant activity of aqueous extract of Harnjyur (Chrysanthemum morifolium Ramat). Lebensmwiss Technol., 32: 269 - 277

[7] Raman, N. 2006. Phytochemical Technique. New Indian Publishing Agencies: New Delhi, pp.19.

[8] Barry, A.L. 1976. Principle and Practice of Microbiology Lea & Fabager. Philadelphia

[9] Mensor, L.L., F.S. Menezes, G.G. Leitao, A.S. Reis, T.S. Santos and Coube, C.S. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother. Res., 15: 127– 130.'