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Research Article

Preliminary Phytochemical Investigations on Roots of *Nerium Oleander*, Linn.

*Jyoti S. Kawalekar, Varsha P., Vijayalakshmi N

Department of Botany, K.L.E.Society's R.L.Sc. Institute Belgaum, Karnataka, India

ABSTRACT

In most of the developing countries drugs of herbal origin have been used in traditional systems of medicines since ancient times. Traditional systems of medicine continue to be widely practiced on many accounts. Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. . This study such as ethnomedicine keenly represents one of the best avenues in searching new economic plants for medicine. Thus Herbs are staging a comeback and herbal 'renaissance' is happening all over the Globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex.. It is now very much evident that plants have bio-active chemicals called Phytochemicals which have physiological effect on human body. Today these phytochemicals have formed the bases of modern drug industries. Keeping this into mind the present investigation is carried out in the roots of *Nerium oleander* Linn

Keywords: *Nerium oleander*, Ethnomedicine, Phytochemicals, secondary metabolites.

INTRODUCTION

India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). India recognizes more than 2500 plant species which have medicinal values.(Kirtikar K.R, Basu BD (1995). Of these, most of the plants have good medicinal value. However, only few species are used for their medicinal values by traditional communities. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani, Siddha and Ayurveda since ancient times. The drugs are derived either from the whole plant or from different organs, like leaves, stem, bark, root, flower, seed, etc. Some drugs are prepared from excretory plant product such as gum, resins and latex. Traditional systems of medicine continue to be widely practiced on many accounts such as Population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments. Global estimates indicate that 80% of about 4 billion population cannot afford the products of the Western Pharmaceutical Industry and have to rely upon the use of traditional medicines which are mainly derived from plant material. In spite of the overwhelming influences and our dependence on modern medicine and tremendous advances in synthetic drugs, a large segment of the world population still likes drugs from plants. In many of the developing countries the use of plant drugs is increasing because modern life saving drugs is beyond the reach of most of the world's population. Thus Herbs are staging a comeback and herbal 'renaissance' is happening all over the Globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. The use of indigenous drugs for the alleviation of human suffering is of considerable economic importance to our country. It is now very much evident that plants have bio-active chemicals called Phytochemicals which have physiological effect on human body. Today these phytochemicals have formed the bases of modern drug industries. (Edeoga H.A, et. al.2005) There are several plants of medicinal importance and we have chosen one among them and that is *Nerium oleander* Linn.

Nerium oleander Linn. is a member belonging to Apocynaceae family. According to the literature survey it is a valuable medicinal plant. It is a herb with different colored flowers and is suited for dry locality (Lokesh .R.et.al 2010). In India they are thus the most favored plants for the road dividers, where a plant has to withstand heat and dust, and little water. *Nerium oleander*

Author for correspondence: E-mail: jskawalekar@gmail.com

Table.1. Percentage yield of extracts

Sl. No.	Extracts	Nature of Extract	Weight (mg)	%w/w Yield
1.	Petroleum ether(40^o-60^oC),	Semisolid viscous	35mg	2.87%
2.	Alcohol	Semisolid viscous	72mg	5.7%
3.	Aqueous	Semisolid viscous	85mg	6.8%

Linn. is cultivated worldwide as an ornamental plant; it is native only in the Mediterranean region (Kingsbury, 1964; Hardin & Arena, 1974). It has highly branched root system. *Nerium oleander* Linn. grows to 2–6 m (6.6–20 ft) tall, with erect stems. Stem is branched and grayish colored. Leaves are corecious, exstipulate, linear, lanceolate, dark green with unicostate, reticulate venation. The flowers grow in clusters at the end of each branch; they are white, pink to red, (Pankhurst, R-2009) with a deeply 5-lobed fringed corolla round the central corolla tube. They are often, but not always, sweet-scented.(Bingtao Li, Antony J. M. Leeuwenberg, and D. J. Middleton. -2009).The fruit is a long narrow capsule 5–23 cm (2.0–9.1 in) long, which splits open at maturity to release numerous downy seeds.Roots are bitter to taste. The plant exudes a thick white sap when a twig or branch is broken or cut (Font-Quer, 1974; Schvartsman, 1979; Lampe & McCann, 1985; Pearn, 1987). From the literature it is learnt that lot of work has been done with respect to bark, leaves, and flowers. A wide spectrum of biological activities has been reported with various constituents isolated from different parts of the plant but the work on the root is less hence the present study. It is also learnt that there is possible anti cancerous effect although reliable research in human has not been performed (Adome.R.A.et.al 2003) Historically, *Nerium oleander* Linn. has been reported in ancient texts and folklore for many years.Survey on literature on *Nerium oleander* Linn. shows most of its parts and plant products such as flowers, leaves, leaf juice, latex and bark are used for the treatment of microbial and fungal diseases. They are also used for warts and ulcers.Used traditionally by herbalists as a folk remedy for a wide variety of conditions, including dermatitis, abscesses, eczema, psoriasis, sores, warts, corns, ringworm, scabies, herpes, skin cancer, asthma, dysmenorrheal, epilepsy, malaria, abortifacients, heart tonics, leprosy and tumors. (Shanthi R, Lakshmi G, Priyadarshini A. M, Anandaraj L) 2011). The plant has been reported extensively as anticancerous, antimicrobial, anxiolytic and antipsychotic agent**.** (Abhijit Dey (2011).Review of literature till date, regarding *Nerium oleander* Linn. is carried out by chemical abstract, biological abstract, medicinal abstract and other national and international scientific journals. The different parts such as bark, stem, leaves of *Nerium oleander* Linn. have been screened for various photochemical but so far there is no much report regarding the roots of. *Nerium oleander* Linn. Therefore, present investigation is undertaken to find the same in the roots of *Nerium oleander* Linn. hence, this study in essential and justifiable.

MATERIAL AND METHODS

Plant material: Roots of *Nerium oleander* Linn. were collected in the month of December from Dhanavantari, our College Botanical garden. Later they were shade dried until they were free from moisture Then the powered roots were subjected to successive hot-solvent extraction process with the solvents in order of increasing polarity', viz, petroleum ether, alcohol and aqueous. Aqueous extract was performed by cold maceration process. The extracts were subjected to qualitative chemical tests. In present study, the roots of *Nerium oleander*, Linn. were investigated for preliminary phytochemical investigations.

Results are given in Table No-2

Methods: Extraction of plant materials- The air-dried powder of roots of *Nerium oleander* Linn**.** were reduced to fine powder of required particle size and around 200 gm of powder was subjected to successive hot continuous extraction (soxhlet) with petroleum ether (40^0-60^0C), ethyl acetate and finally alcohol. After the effective extraction, the solvents were distilled off. The extract was then concentrated on water bath and finally reduced to dryness. After drying, the respective extracts were weighed and yields were recorded in Table No-1

Preiliminary Phytochemical screening: The condensed extracts were used for preiliminary screening of phytocchemicals and the same were subjected to qualitative chemical investigation.

Screening Procedure: The following procedures were adopted to test for the presence of various chemical constituents in the extracts.

1.Test for Sterols:

a.Salkowski test: When a few drops of conc. sulphuric acid is added to the test solution, shaken and allowed to stand, lower layer turns red indicating the presence of sterols.

b.Liebermann Burchardt test: The test solution treated with few drops of acetic anhydride and mixed well. When conc. sulphuric acid is added from the sides of the test tube, it shows a brown ring at the junction of the two layers and the upper layer turns green.

c.Sulphur test: Sulphur when added into the test solution, it sinks in it.

2. Test for Glycosides:

a.Baljet's test: The test solution treated with sodium picrate gives yellow to orange colour.

b.Keller-Killiani test: The test solution with few drops glacial acetic acid in 2ml of ferric chloride solution and conc. sulphuric acid is added from the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green.

3. Test for Saponins:

a.Foam test: Saponins when mixed with water and

Table 2. Results of Qualitative chemical investigations of Roots of *Nerium oleander* Linn.

S. No.	Name of the Test	Extracts		
		Pet. Ether	Alcohol	Aqueous
I.	Test for Carbohydrates:			
	a.Fehlings Test	-	+	+
	b.Benedicts test	+	+	+
	c.Molischs Test.	-	+	+
	B.Test for Monosaccharide			
	a. Barfoed's test	-	+	+
II	Test for Proteins:			
	a.Biurett test(General Test)	-	-	+
	b. Million's test	-	-	-
III	Test for Steroids:			
	a. Salkowski test:	+	+	-
	b. Liebermann Burchardt test	+	-	-
	c. Liebermann reaction	+	-	-
IV	Saponins			
	a. Foam test:	-	-	-
V	Test for Flavonoids:			
	a. Ferric chloride test	-	-	+
	b.Shinoda test:	-	-	+
	c.Zinc–Hydrochloric acid reduction test	-	-	+
VI	Tests for Alkaloids:			
	a. Mayer's test:	-	-	-
	b.Wagner's test:	-	-	-
	c.Hager's test:	-	-	-
	d.Dragendorff's test:	-	-	-
VII	Test for glycosides			
	a.Baljet test	-	-	-
	b.Killer Killiani test	-	-	-
VIII	Test for Fats and Oils			
	a. Solubility Test.	+	+	-
IX	Test or Tannins and Phenolic compounds			
	a.5%Ferric chloride solution	-	-	-
	b.Lead acetate solution	-	-	-
	c.Gelatin solution	-	-	+
	d.Dilute HNO_3	-	-	-
	e.Dilute potassium permanganate solution	-	-	-

shaken shows the formation of foam which is stable at least for 15 minutes.

4. Test for Carbohydrates:

a.Molisch's test: Test solution with few drops of Molisch's reagent and 2ml of conc. sulphuric acid is added slowly from the sides of the test tube shows a purple ring at the junction of two liquids.

b.Barfoed's test: Test solution treated with Barfoed's reagent on boiling on a water bath shows brick red precipitate.

c.Benedict's test: Test solution treated with Benedict's reagent and boiling on a water bath shows reddish brown precipitate.

5. Tests for Alkaloids:

a.Mayer's test: Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream colored precipitate.

b.Wagner's test: The acidic solution treated with Wagner's reagent (Iodine in potassium iodide) gives brown precipitate.

c.Hager's test: The acidic solution with Hager's reagent (Saturated picric acid solution) gives yellow precipitate.

d.Dragendorff's test: The acidic solution with Dragendorff's reagent (potassium bismuth iodide) shows reddish brown precipitate.

6. Test for Flavonoids:

a.Ferric chloride test: Test solution with few drops of ferric chloride solution shows intense green color.

b.Shinoda test: Test solution with few fragments of magnesium ribbon and conc. hydrochloric acid, shows pink to magenta red color.

c.Zinc – Hydrochloric acid-reduction test: Test solution with zinc dust and few drops of Hill shows magenta red color.

7. Tests for Tannins:

a.,Ferric-chloride test: Test solution with few drops of ferric chloride solution gives dark color.

b.Gelatin test: Test solution treated with gelatin solution gives white precipitate.

c.Dilute iodine solution: Test solution treated with dilute iodine solution gives transient red color.
d.Dilute HNO_3: Test solution treated with Dilute HNO3 gives reddish to yellow color.
e.Dilute potassium permanganate solution: Test solution treated with dilute potassium permanganate solution gives red precipitate.
8. Tests for Proteins:
a. Million's test: Test solution treated with million's reagent and heated on a water bath, protein is stained yellow on warming.
b.*Biurett test:* Test solution treated with 40% sodium hydroxide and dilutes copper sulphate solution gives blue color.
9. Test for Fats:
a.Solubility test: Oils are soluble in ether, benzene and chloroform, but insoluble in 90% ethanol and in water.

RESULTS

The present study carried out on roots of *Nerium oleander* Linn. revealed the presence of medicinally active metabolites. The results of phytochemicals screened are summarized in the Table 3 given above.The percentage yield obtained from the non-polar solvents i.e. petroleum ether (2.87%) was less as compared to the yield obtained from the polar solvents i.e. ethanol and water (5.7% and 6.8%respectively). The percentage yield obtained from the successive extraction using the solvents petroleum ether, ethanol and aqueous extract obtained by maceration are as given in Table 2. The aqueous extract was found to contain carbohydrates, proteins alkaloids, flavonoids and phenols and terpenoids. But alkaloids, saponins, steroids, and glycosides were absent.
Ethanolic extracts of the roots was found to contain steroids and fats. . But tannins, phenolics glycosides, alkaloids and flavonoids were absent. The Pet. Ether extract of the roots showed the presence of carbohydrates, steroids and fats but absences of tannins, phenolics, glycosides, alkaloids saponins, proteinsand flavonoids.

DISSCUSSIONS

The phytochemical investigation studies of the roots of *Nerium oleander* Linn. studied showed the presence of Carbohydrates, proteins, steroids, flavanoids, tannins and phenolic compounds in one or the other extracts. Presence of the above mentioned phytochemicals are of great Value in the pharmaceutical industries. Carbohydrates are the primary dietary source of energy for cells and are called as metabolic fuels. They are the main reserve food material in plants and animals. They are used as raw materials in several industries such as paper, textile etc.Proteins are also of great importance .They help in chemical coordination of body, act as growth hormones, and also help in defense mechanism. It should be noted that sterol compounds are of importance are of interest in pharmacy due to their relationship with sex hormones. Flavanoids, tannins and phenolic also have great importance. Thus the plant *Nerium oleander* Linn. can be seen as a potential source of useful drugs. The presence of various bioactive compounds justifies the use of the roots for various ailments by traditional practitioners. Based on the present account of biochemical compounds found in the roots of the *Nerium* oleander Linn. plant is opined that they can be used for therapeutic purposes. This awareness can be used for the further development of phytomedicines from the roots of this plant. It can also help the biotechnologists to study the types of phytochemicals and their production in the cells and, mass production of these so as to meet the needs of the public and also their genetic control. Further studies are going on, in the plant to identify furthermore use in the field of science.

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