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Preliminary pharmacognostic and phytochemical studies on *Nerium oleander* Linn. (White cultivar)

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Abstract

Nerium oleander is an evergreen shrub or small tree in the dogbane family Apocynaceae. It is commonly known as oleander but has many other names like *Nerium indicum* Mill. and *Nerium odorum* Soland. The present study was carried out for pharmacognostic evaluation, physical evaluation, and phytochemical evaluation according to standard procedures. The leaves of *Nerium oleander* were collected, shade dried and extraction was done by simple maceration method. The preliminary morphological studies, macroscopic as well as microscopic evaluation, physical evaluation and preliminary phytochemical screening for the presence of alkaloids, flavonoids, carbohydrates, glycosides, tannins, terpenoids, phenolics steroids and saponins for petroleum ether, chloroform, methanol and aqueous extracts of *Nerium oleander* leaves were carried out. The physical evaluation was carried out for the determination of petroleum ether soluble extractive value, methanol soluble extractive value, water soluble extractive value; ash value includes total ash, acid insoluble ash and water soluble ash, moisture content, volatile oil content for the leaves of *Nerium oleander*.

Keywords: *Nerium oleander*, Phytochemical, Pharmacognostic, ash values, extractive values

Introduction

Nerium oleander is an evergreen shrub or small tree in the dogbane family Apocynaceae. It is known as oleander from its superficial resemblance to the unrelated plant *Olive olea* but has many other names like *Nerium indicum* Mill. and *Nerium odorum* Soland. The white and red flowered variety is equated with *Nerium indicum* [1].

N. oleander is distributed in Mediterranean region and subtropical Asia, is indigenous to India-Pakistan subcontinent. Distributed in the Himalayas from Nepal westwards to Kashmir up to 1950m, extending to Baluchistan, Afghanistan and found throughout India in gardens. The white and red flowered variety is equated with *Nerium indicum*.

Leaves, roots, root bark is used to treat various ailments. Charka prescribed the leaves of white flowered variety externally in chronic and obstinate skin diseases of serious nature including leprosy. Sushruta used karavira in medicinal paste for application in alopecia. Root powdered with water was applied to alleviate venereal diseases. The powder of leaves was used as a snuff for treating epilepsy. All parts of plant especially roots were known to be highly poisonous when taken internally. Tincture of flowers exhibited cardiotoxic, root CNS-active and spasmolytic activity. Externally, root exhibited healing properties for haemorrhoids and ulcers. Oil of root bark gave good results in leprosy. In Homoeopathy, tincture of *Nerium oleander* (red laurel) leaves is used in diseases of nervous system, hemiplegia and paralytic conditions under strict medical supervision [2].

Experimental Section

Materials & Methods

Collection and authentication of plant material

Plant Material – The leaves of *Nerium oleander* were collected from the local regions of Naya Nangal in August 2013. They were identified by sending it to Director, NISCAIR New Delhi. The collected parts were dried under shade at room temperature and powdered to coarse material in grind mill. The powder was passed through 40# mesh particle size and stored in airtight container at room temperature [3].

Macroscopic studies

The leaves were taken and studied for their various parameters. The leaves were 13 cm to 17 cm in length & 2 cm to 3 cm in width, color was green with glossy appearance. The leaves

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were broken. Leaves were simple, arranged in whorls, subsessile, exstipulate, linear lanceolate with unicostate reticulate venation, odour was faint. The taste was slightly bitter [4].

Table 1: Physical Test of Crude drug *Nerium oleander*

Nature	Colour	Odour	Taste
Coarse powder	Green	Faint	Slightly bitter

Microscopic studies

T.S. of Leaf was cut with the help of a sharp blade. The section was cleared with chloral hydrate solution and stained with phloroglucinol and HCl then mounted in glycerin which showed the epidermal layer with cuticle and numerous unicellular trichomes on upper and lower side. Epidermal cells were wavy. Lower surface shows pink colour with Phloroglucinol -HCl in midrib region.



Powder studies – Fine powder # 60 of the dried leaves was taken for the observation of powder microscopic characters. The powder was treated separately with glycerine, phloroglucinol HCl, Iodine, and Potassium iodide solution and was observed under compound microscope. Following microscopic cells were observed -Epidermal quadrangular cells with cuticle, collenchymatous cells, unicellular trichomes, starch grains [5].

Physical evaluation

Determination of foreign organic matter

The parts of organs or organs other than those normal in the definition and description of the drug are defined as foreign organic matter [6].

Table 2: Foreign organic matter

Foreign organic matter	%age
	1.8

Ash values [7]

a) Total ash - The ground drug 2 gm was incinerated in a silica crucible at a temperature not exceeding 450 °C until free from carbon. It was cooled and weighed to get the total ash content.

b) Acid insoluble Ash- Ash was boiled with 25ml dil. HCl(6N) for 5 minutes. The insoluble matter was collected on an ashless filter paper, washed with hot water and ignited at a temperature not exceeding 450° C to a constant weight.

c) Water soluble ash – the total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was washed with hot water, ignited and weighed. The %age of water soluble ash was calculated with the reference to the air dried drug.

Loss on Drying- The drug sample (10g) was placed in a tared evaporating dish. The dish was placed in hot air oven at 105° C for 6 hours and weighed.

Volatile Oil content- The 50g of the fresh leaves were taken and hydro-distillation was carried out in clavenger apparatus. No volatile oil was obtained.

Table 2: Ash values, loss on drying and volatile oil

Parameter	%age value
Total Ash	4.95
Water soluble ash	2.25
Acid insoluble ash	2.7
Volatile oil content	0
Loss on drying	5

Extractive values [8]

a) Petroleum ether extractive - The air dried coarse powder (5g) was macerated in Petroleum ether (100ml) in a closed flask for 24 hours shaking frequently during 6 Hours and allowed to stand for 24 hours. It was filtered and filtrate was evaporated to dryness in a tared flat bottom dish and dried at 105° C to constant weight.

b) Chloroform Extractives - The air dried coarse powder (5g) was macerated with 100ml chloroform in a closed flask for 24 hours shaking frequently during 6 Hours and allowed to stand for 24 hours. It was filtered and filtrate was evaporated to dryness in a tared flat bottom dish and dried at 105 °C to constant weight.

c) Methanolic extractive - The air dried powder was macerated with 100ml of methyl alcohol in a closed flask for 24 hours shaking frequently during 6 Hours and allowed to stand for 24 hours. It was filtered and filtrate was evaporated to dryness in a tared flat bottom dish and dried at 105 °C to constant weight.

d) Water Extractives - The air dried powder was macerated with 100ml of distilled water in a closed flask for 24 hours shaking frequently during 6 Hours and allowed to stand for 24 hours. It was filtered and filtrate was evaporated to dryness in a tared flat bottom dish and dried at 105° C to constant weight.

Determination of pH - 1 gm of accurately weighed dry powder was dissolved in water and filtered. pH of filtrate was determined with a standard glass electrode.

Table 3: Extractive values

Extract	%age w/w	Colour of extract
Petroleum ether extractive	2.5	green
Chloroform soluble extractive	17.6	greenish black
Water extractive	11	Greenish black
Methanolic extractive	14.4	Greenish Black

Fluorescence Analysis

Many crude drugs show fluorescence when the sample is exposed to ultraviolet radiation. When powder of *Nerium* was exposed to UV radiations following colors were observed.

Table 4: Fluorescence Analysis

Conc. Sulphuric acid	Brownish black
Glacial acetic acid	Green
5% ferric chloride	---
NH ₃ Solution	Green
Acetic acid +FeCl ₃ +Conc. H ₂ SO ₄	Yellowish brown
Picric Acid	Yellowish green
1N HCl	---
10% NaOH +few drops of 10% Lead acetate	Pale green
10% NaOH+CuSO ₄	Blue ppt
1 N NaOH	---
Iodine	Dark yellowish brown

Phytochemical Screening ^[9]

Test for Alkaloids: the extract residues were taken in 5 ml of 1.5 N HCl and was filtered and the filtrate then tested with following reagents

a) Dragendorffs reagent – few drops of dragendorffs reagent were added to each of the extracts and observed for orange yellow ppts.

b) Mayers reagent – few drops of Mayer's reagent were added to each of the extracts and observed for the formation of white or cream ppts.

Test for Proteins ^[10]

a) Millions Test- to few ml of alcoholic extract 5 ml distilled water was added and filtered. To 2 ml of filtrate 5-6 drops of millions reagent were added. A red ppt is formed

b) Xanthoproteic test – to 2ml of the extract few drops of nitric acid were added by the sides of test tube. Presence of yellow colour showed the presence of proteins and free amino acids

c) Biuret test – to the ammoniated alkaline filtrate of the extract, 2-3 drops of 0.02% copper sulphate solution were added. Presence of red/violet colour indicated the presence of proteins and free amino acids.

d) Ninhydrin test – to the extract lead acetate solution was added to ppt. tannins. The filtrate was spotted in a paper chromatogram sprayed with Ninhydrin reagent and dried at 110^o C for 5 minutes. Violet spots indicated the presence of proteins/free amino acids.

Test for Carbohydrates ^[11]

a) Molish test – to about 2ml of a alcoholic extract few drops of α - naphthol (20% in ethyl alcohol) was added. Then about 1 ml of Conc. Sulphuric acid was added along the sides of the test tube. Reddish violet ring at the junction of two layers separated in the presence of carbohydrates

b) Fehlings solution test – the extract was heated with dilute HCl to hydrolyse polysaccharides. The reaction mixture is neutralized by adding NaOH solution and then Fehlings solution 1 and 2 were added. A red ppt. formed in cases of reducing sugars /carbohydrates

Test for Steroids

a) Salkowski test:- when few drops of conc. sulphuric acid is added to the test solution in chloroform, shaken and allowed to stand, produces red color in the chloroform layer.

b) Libermann – Burchard's test:- the test solution in chloroform is treated with few drops of acetic anhydride, Conc. H₂ So₄ is added from the side of the test tube. It shows a brown ring at the junction of the two layers and the upper layer turns green.

Test for Glycosides ^[12]

The extract is tested for free sugars. After complete removal of sugar, the extract in hydrolysed with dilute mineral acid and then tested for the glycone and aglycone moieties.

Test for Cardiac Glycosides

- Liberman – Burchard's test
- Keller- Kiliani test
- Raymonds test
- Baljet test

Test for Cyanogenetic glycosides

To one gram of powdered drug moistened previously in a test tube suspend a piece of sodium picrate paper above the drug by trapping the top edge between the cork and the tube wall. Allow standing for 30 minutes. The evolution of hydrocyanic and turns paper brick red (sodium isopurperate)

Test for Anthraquinone glycosides

a) Bontragers test:- powdered drug is boiled with dilute sulphuric acid and filtered. The filtrate is gently shaken with organic solvents, Separate the organic layer, to that add ammonia solution. Pink color appears.

Test for Flavonoids:-

- Shinoda test (Mg -Hcl reduction test) To the alcoholic solution add few fragments of magnesium ribbon, add HCl acid dropwise, pink to red crimson red color appears after few minutes.
- Zn- HCl reduction test:- to the test solution add a mixture of Zinc dust and Conc HCl gives a red color.
- Ferric chloride test:- to the test solution with Ferric chloride bluish green to black color in produced.

Test for Phenols and Tannins

- With gelatin solution** –treat the test solution with 1% gelatin solution containing sodium chloride white ppt. appears
- With ferric chloride solution** –treat the solution with few drops of freshly prepared neutral ferric chloride solution separately, bluish black colour appears
- Lead acetate test** –to the test solution add few drops of 10% lead acetate, yellow ppt appears
- Alcoholic HCl acid test** –to the test solution gently add alcoholic hydrochloric acid, red colour appears

Test for Saponins

- Froth test** – Dilute aqueous extracts with distilled water separately to 20 ml and shake in a graduated cylinder for 15 minutes formation of 1 cm layer of foam which is stable for 15 minutes take place
- Haemolysis test** -sample is dissolved in physiological salt solution. To this 4% buffered equilibrated blood (ph 7.40) in added. Haemolysis of red blood cells occurs and can be noticed in the microscope.

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10% NaOH+CuSO ₄	Blue ppt
1 N NaOH	---
Iodine	Dark yellowish brown

Table 5: Phytochemical Screening

Test	Pet ether extract	Chloroform extract	Methanolic extract	Aqueous extract
Test for Steroids	+	+	-	-
Test for steroidal glycosides	-	-	+	+
Test for tannins and phenols	-	-	+	+
Test for Flavonoids	-	-	-	-
Test for Carbohydrates	-	-	+	+
Test for cardiac Glycosides	+	+	+	+
Test for Triterpenoids	+	+	-	-
Test for Alkaloids	+	+	+	+
Test for Proteins	-	-	-	-
Test for Saponins	-	-	-	-

Conclusion

In the present study ash values, extractive values were determined and phytochemical screening for various chemical constituents was carried out. The phytochemical screening of various extracts showed the presence of alkaloids, steroidal glycosides, carbohydrates, tannins and carbohydrates etc. It should be noted that steroidal compounds are of important interest in Pharmacy and so the extracts can be further studied for various pharmacological activities and provide future for the research in this field

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