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Research Article

Preliminary Pharmacognostic and Phytochemical Studies on the Flowers of *nerium oleander* linn.(white cultivar)

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ABSTRACT
Nerium oleander is an evergreen shrub or small tree in the dogbane family Apocyanaceae. 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 flowers of 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* flowers 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 flowers 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 Apocyanaceae. 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 cardiotonic, 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 authentification of plant material

Plant Material – The flowers 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 flowers were taken and studied for their various parameters[4].

Physical appearance

Microscopic studies

Powder studies – Fine powder # 60 of the dried flowers 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. Microscopic cells like parenchymatous cells, vessels, unicellular trichomes, starch grains were observed in powder[5].

Physical evaluation

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

Nature	Colour	Odour	Taste
Coarse powder	White	Faint	Slightly bitter

Table 2: Foreign organic matter

Foreign organic matter	%age
	1.2%

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

Parameter	%age value
Total Ash	5.3
Water soluble ash	2.4
Acid insoluble ash	1.7
Volatile oil content	0.2
Loss on drying	0.3

Table 3: Extractive values

Extract	%age w/w	Colour of extract
Petroleum ether extractive	12.8	Brown
Chloroform soluble extractive	13.2	Yellow
Water extractive	17.6	Yellowish brown
Methanolic extractive	16.0	Yellow

Table 4: Fluorescencence Analysis

50% Sulphuric acid	Dark Brownish
Ammonia Solution	Pale yellow
Ethanol	Pale yellow
Picric Acid	Pale green
50% HCl	Pale green
1N NaOH (Alc.)	Brownish

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].

Ash values[7]

Total ash - The ground drug 2 gm was incinerated in a silica crucible at a temperature not exceeding 450 ^{0}C until free from carbon. It was cooled and weighed to get the total ash content.

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 450^0 C to a constant weight.

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 105^0 C for 6 hours and weighed.

Volatile Oil content- The 50g of the fresh flowers were taken and hydro-distillation was carried out in clavenger apparatus. Volatile oil was obtained.

Extractive values[8]

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 105^0 C to constant weight.

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 105^0 C to constant weight.

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 105^0 C to constant weight.

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 105^0 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.

Fluorescencence Analysis

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

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

Dragendroffs reagent - few drops of dragendroffs reagent were added to each of the extracts and observed for orange yellow ppts.

Mayers reagent- few drops of Mayers reagent were added to each of the extracts and observed for the formation of white or cream ppts.

Test for Protein[10]

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

Xanthoprotic 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

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

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 110^0 C for 5 minutes. Violet spots indicated the presence of proteins/free amino acids.

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	-	-	+	+

Test for Carbohydrates[11]

Molish test- to about 2ml of a alcoholic exract 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.

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

Salkowanski 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.

Libermann - Burchard's test:- the test solution in Chloroform is treated with few drops of acetic anhydride, Conc. $H_2 SO_4$ is added from the side of the test tude. 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 Anthraqinone glycosides

Bontragers test- powderd 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 equiliberated blood (pH 7.40) in added. Haemolysis of red blood cells occurs and can be noticed in the microscope.

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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