RESEARCH ARTICLE

Qualitative and Quantitative Phytochemical Investigation of Leaves of *Narium indicum* Collected from Panjgur, Balochistan

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Abstract: *Nerium Indicum* (commonly called isherk in Balochi) is an ornamental shrub. It belongs to the family Apocynaceae. *Nerium Indicum* plays a vital role in traditional system of medicine in Panjgur. Nearly all parts of the plant including shoots, barks, flowers and leaves are used as traditional medicines in different parts of the world. Plant contains bioactive phytochemicals. The present study investigates the qualitative and quantitative phytochemical analysis of leaves of water extracts and methanolic extracts of *Nerium Indicum* to compare it with already reported phytochemicals. The results of qualitative analysis of phytochemicals of *Nerium Indicum* in the methanol and water extract showed the presence of flavonoids, tannins, Terpenoids, saponins, glycosides, phenols, carbohydrates, alkaloids and steroids. Alkaloids, flavonoids and saponins are also measured quantitatively in leaves of the plant and were found to be rich in these three compounds. The alkaloids content of *Nerium Indicum* was found to be 12.8%, the content of flavonoids was 29.5% and saponins were 24.85% and each gram of dried plant leaves contained 0.128gm of alkaloids, 0.249gm of saponins and 0.295gm of flavonoids.

Keywords: phytochemical constituents, Nerium indicum, Methanol extract, water extract, qualitative and quantitative analysis.

INTRODUCTION

Naturally occurring chemicals, known as phytochemicals are found in fruits, vegetables, whole grains, nuts, beans, seeds, flowers and leaves. The study of these chemicals is called phytochemistry(Suganya, Priya, & Roxy, 2012). Phytochemicals are biologically active chemical compounds, and gives herbs, vegetables and fruits their odor, flavor, and colors. They basically form the plant's immune system and help to protect them from fungus, sun’s ultraviolet rays and many diseases. Recent research demonstrates that they are also largely used to protect the humans against several diseases,(Tiwari, Kumar, Kaur, Kaur, & Kaur, 2011), i.e. saponins and isoflavones potentially decrease the risk of osteoporosis, Beta-carotene reduces risk of cataracts, and d-limonene reduces premenstrual systems. There are more than thousand known phytochemicals which provide definite physiological actions on the human body, the most important bioactive constituents of the plants are flavonoids, saponins, alkaloids, phenolic, terpenoids, tannins, steroids and glycosides that are synthesized by the primary or rather secondary metabolism of living organisms (Yadav & Agarwala, 2011). The Phytochemical screening involved standardized chemical tests to evaluate the presence of several active constituents like Flavonoids, Alkaloids, Saponins, Tannins, Terpenoids, Phenolic, Steroids, Cardiac glycosides and Carbohydrates. (Mir, Sawhney, & Jassal, 2013). *Nerium Indicum* (Common name Isherk in Balochi) belongs to family Apocynaceae and is naturally occurring evergreen shrub or tiny tree and has many other names including Nerium oleander or Narium odorumsoland. It is widely distributed in Iran, India, China and Pakistan (Balochistan). It is a smooth and an erect shrub 1.5-3 meters in height and used as an ornamental shrub(Nagargoje & Saraswati, 2013).

*Nerium Indicum* plays a vital role in traditional medicine. Nearly all parts of the plant

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including shoots, barks, flowers and leaves are used as traditional medicines in different parts of the world (Bhuvaneshwari, Arthy, Anitha, Dhanabalan and Meena, 2007). *Nerium Indicum* is also used as medicinal plant in Chinese folk medicines (Nagargoje & Saraswati, 2013). The leaves of *Nerium Indicum* are most commonly used for the treatment of Nervous system, Hemiplegia, Epilepsy and skin diseases like Leprosy or ringworm infection (Dey & Chaudhuri, 2014). The bark of the plant is used as intermittent fever and cathartic, febrifuge and because of its toxic nature the powerfully resolving roots are used in the form of plasters and is usually applied externally and have been used against corns, warts, cancerous ulcer, carcinoma, ulcering or hard tumors. It has been discovered that consuming high quantity of *Nerium Indicum* becomes poisonous and toxic to the Herbivores (Alobaidi, 2014). The flowers and leaves of the *Nerium Indicum* are also toxic for certain insects and are used to treat malaria, but in Human and Animals, the proliferation disorder can be treated by using the methanolic extract of the plant (Patel, 2011). Its methanolic leaves extract is analyzed for Anti-oxidant activity (Nagargoje & Saraswati, 2013). The juice of leaves of *Nerium Indicum* is used for snake bites (Singhal & Gupta, 2012), Scabies in order to reduce swelling. The hot water extract of seeds and leaves are used for gastrointestinal infections and upper respiratory tract in Kenya. It is used in Tobago and Trinidad for reproductive problems. The ear pain is also treated with this plant water extract in the traditional therapeutic system in Kancheepuram District of Tamil Nadu, India. (Nagargoje and Saraswati, 2013).

The main objective of this research was to investigate the phytochemical composition of the leaves of wild plant, *Nerium indicum*, collected from district Panjgur in May 2015. As this plant is widely used by the local community in traditional system of medicine for different diseases i.e., its leaves and flowers are locally used to treat malaria, leaf decoction has been applied externally to reduce scabies and swelling, roots are locally used for abortion, the pounded bark and leaves of plant used as insecticide and rat poison.

**METHODOLOGY**

**Collection of plant**

*Nerium Indicum* was collected from the farm land of district Panjgur, Balochistan. The botanical identification of the plant was done by the botanist Prof. Dr. Rasool Baksh from University Of Balochistan, Quetta.

**Preparation of Methanolic Extract**

For an extract preparation only leaves of *Nerium Indicum* were collected. These leaves were washed properly with distilled water to remove dust and left to dry under shade. These dried leaves were grind in the grinding Machine (Schleifmaschine Und Zwergmixer grinder and mini blender) to get the fine powder. 60gm of powdered leaves were soaked into 1 liter of methanol for one week and then filtered with the help of filter paper No 1. The solvent was evaporated from the extract by using rotary evaporator with temperature below 50˚C at 75 RPM. This crude methanolic extract was preserved at -20˚C in the freezer and was evaluated for qualitative and quantitative phytochemical analysis.

![Fig.1. Dried leaves and powdered material](image-url)
Qualitative Analysis of Phytochemical Constituents in crude methanolic extract and powdered material of Nerium Indicum Leaves

Qualitative Phytochemical analysis of plant sample and crude methanolic extract was conducted by using standard procedure (M. Amin et al, 2012).

Quantitative Analysis of Phytochemical Constituents in Leaves of Nerium Indicum


Determination of Total Alkaloid
200ml of 10% acetic acid in ethanol was added into 5g of sample powder in a 500mL beaker and covered with Aluminum foil, and allowed to stand for 4 hours. After 4 hours mixture was filtered. The filtrates were concentrated on water bath to one quarter of the original volume. After that few drops of concentrated Ammonium solution was added to the filtrates until the Precipitation was complete. The filter paper was weighed and precipitates were collected on this filter paper after the settlement of the whole solution. The collected precipitates were washed with dilute Ammonium Hydroxide. The residue was collected, dried and weighed. This residue was total Alkaloid contents present in leaves.

Determination of Total Flavonoids
10g of powdered sample was added into a 250ml of conical flask and 100ml of 70% methanol was added into it and were shaken vigorously. After that the mixture was put on hot plate and magnetic stirrer was added in the beaker and was allowed to shake for 3 hours. After shaking it was filtered. The residue was re-extracted with 100mL of 70% Methanol and the whole process was repeated again. After that precipitates were filtered and the both filtrates were concentrated on water bath to one quarter of the original volume. It was then allowed to dryness in open air and then weighed. This was Total flavonoids contents present in leaves.

Determination of Total Saponins
20gm of powdered sample was added in 100cm³ of 20% ethanol in a conical flask. The sample was heated over a water bath for 4 hours with continuous stirring at about 60°C. The mixture was filtered and the residue was re-extracted with another 200ml 20% ethanol. Then both extracts were combined and were heated over water bath at 90°C to reduce into 40ml. After that the concentrate was transferred into a separating funnel and 20ml of petroleum ether, was added and shaken vigorously to form an aqueous layer and organic layer. While the organic layer was discarded and aqueous layer was collected. The same process was repeated twice. After that 60ml of n-butanol was added to the aqueous layer and mixture is shaken vigorously for half hour. The butanol fraction was collected and evaporated in a vacuum rotary evaporator and the residue was collected and weight was measured. This residue was Total Saponin contents in leaves.

RESULTS AND DISCUSSION

Qualitative analysis of Nerium Indicum exhibited positive results for 9 medicinally important phytochemicals such as Flavonoids, Alkaloids, Tannins, Saponins, Terpenoids, Glycosides, Steroids and Phenol compounds all were present in Nerium Indicum leaves. Similarly, methanol extracts showed positive results for tannins, flavonoids, saponins, terpenoids, cardiac glycosides, phenols, alkaloids, carbohydrates and steroids. Table I shows the result of phytochemicals screening of water extract and methanol extract of Nerium indicum leaves.

Quantitative analysis was carried out by using the standard procedure (Chengaiah, Rao, Kumar, Alagusundaram, & Chetty, 2010) shows that 1gm of dried plant leaves contains approximately 0.128gm of alkaloids, 0.295gm of flavonoids, and 0.249gm of saponins. These results evaluate that leaves of plant are rich in flavonoids, saponins, and alkaloids and is a good source of bio-active compounds. Flavonoids have high antioxidant capacity and have protective role in inflammation, atherosclerosis, carcinogenesis and thrombosis. They also have anticancer, anti-
allergic, anti-viral, anti-inflammatory, antioxidant activity.

Alkaloids, one of the most diverse groups of secondary metabolites have role in chemical ecological perspectives like plant-microbial interaction, plant-plant interaction and plant-herbivore interaction. Alkaloids acts at DNA and RNA polymerase level and are carcinogenic and mutagenic due to their properties to bind DNA. Many alkaloids have physiological effect that renders them valuable medicine against various diseases such as cardiac dysfunction, malaria, cancer, diabetics etc. RNA polymerase level (Singh, Singh, and Singh, 2016). Several alkaloids exhibit significant biological activities, such as, the analgesic action of morphine, the anticancer effects of vinblastine and the relieving action of ephedrine for asthma. An isoquinoline alkaloid such as Berberine has a broad range of bioactivities, such as antiulcer, antibacterial, anti-inflammatory, antidiabetes, sedation, expansion of blood vessels, protection of myocardial ischemia-reperfusion injury, inhibition of platelet aggregation, protection of myocardial ischemia-reperfusion injury, and expansion of blood vessels, neuroprotective and neuroprotective effects. It has been used in the treatment of neurasthenia, diarrhea, diabetes, arrhythmia, and so forth(Lu, Bao, Chen, Huang, and Wang, 2012). Saponins are used in veterinary vaccines as adjuvant helping to enhance immune response. Saponins have relationship with sex hormones such as oxytocin (Oxytocin is a sex hormone involved in controlling the onset of labour pain in women and the subsequent release of milk (Savithramma, Rao, and Ankanna). The flowers and leaves of Nerium oleander L. are emetic (causes vomiting), diuretic (promotes perspiration), cardiotonic (tonic effect on the heart), expectorant (cough remedy), diaphoretic (promotes perspiration), sternutatory (substance) as well as treatment of abortifacient (induces the expulsion of embryo), malaria and dysmenorrhea. The roots, bark, leaves, leaf juice or latex, and flowers have been used against cancerous ulcers, ulcerating or hard tumor, carcinoma, warts, corns, etc. The bark is used as intermittent fever, febrifuge and cathartic. The root paste can be applied externally to haemorrhoids (dilation of the blood vessels around the anus), ulcers (open sore) on the penis and chancres (syphilis).Leprosy and skin diseases of scaly nature are treated by using the oil prepared from the root bark. The bark is used as intermittent fever, febrifuge and cathartic. Seeds are alternative, abortifacient and poisonous. They used as pugative in rheumatism and leprosy. This plant is also used as an insecticide and rat poison and the pounded bark and leaves are used as an insecticide (Zibbu & Batra, 2010). Anticancer properties such as emetic cardio tonic and diuretic are also found in leaves and flowers of this plant(Jawarkar et al., 2012). Nerium oleander also have larvacidal activity against larva west Nil vector mosquito culexpiipiens such as ethanolic extract of Nerium oleander could serve as a natural biocide operator against larvae mosquito, particular culexpiipiens and potential larvacidal (El-Akhal, Guemmouh, Ez Zoubi, and El Ouali Lalami, 2015).

This plant is a medicinal as well as it is toxic plant. All parts of Nerium Indicum are toxic, green or dry. It is highly poisonous to pets, birds, humans and livestock because it contains cardiac glycosides, mainly oleandrin. Ingestion causes cardiac arrhythmias, vomiting, nausea, vomiting, hypotension and death(Sridhar, Vinesh and Mani, 2016). The toxicity is due to the two potent cardiac glycosides, oleandrin and nerine, which are very similar to the toxin of Foxglove and can be isolated from all parts of the plant. The toxicity is due to the two potent cardiac glycosides, oleandrin and nerine, which are very similar to the toxin of Foxglove and can be isolated from all parts of the plant (El-Akhal et al., 2015). Some medicinally vital Phytochemicals, Flavonoids, Alkaloids and Saponins were also analyzed quantitatively.
Fig 2. Flavonoids test of (A) water extract and methanol extract.

Fig 3. Tannins test of (A) water extract and (B) methanol extract.

Fig 4. Saponins test of (A) water extract and (B) methanol extract.

Fig 5. Terpenoids test of (A) water extract and (B) methanol extract.

Fig 6. Glycosides test of (A) water extract and (B) methanol extract.

Fig 7. Phenolic test of (A) water extract and (B) methanol extract.

Fig 8. Dragendorff’s test of (A) water extract and (B) methanol extract.

Fig 9. Picric acid test; (A) water extract and (B) methanol extract.
Fig. 10. Fehling’s test of (A) water extract and (B) methanol extract. Fig. 11. Molisch’s test of (A) water extract and (B) methanol extract.

Fig. 12. Steroid test of (A) water extract and (B) methanol extract.

### Table:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of Phytochemical</th>
<th>Quantity (gm/gm of dried plant leaves)</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>0.295</td>
<td>29.5%</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>0.128</td>
<td>12.8%</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>0.249</td>
<td>24.85%</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The photochemical screening of *Nerium Indicum* and literature survey indicates that this plant is rich in bioactive constituents. This plant can be used further for the isolation of novel compounds that can be used as remedy for antimicrobial activity, anticancer, mollisicidal, antioxidant, antiulcer, analgesic, ant diabetic and immunomodulating activities. Most importantly this plant can be used for the isolation of natural pesticides which can be a good replacement of synthetic toxic pesticides.
Table. I. Results of qualitative analysis of photochemical in powdered leaves material and methanol extract of *Nerium Indicum* leaves.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Phytochemicals</th>
<th>Screening Tests</th>
<th>Observations</th>
<th>Powdered leaves material</th>
<th>Methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>3ml of defatted filtrate + 4ml of 1% potassium hydroxide</td>
<td>Dark yellow color observed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>0.5 gm extract was boiled in 20ml of distilled water, filtered + 0.1% FeCl₃</td>
<td>Brownish green or a blue black coloration observed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>0.5gm of extract was dissolved in boiling water in a test tube, was cooled and shaken vigorously</td>
<td>Froth formation was observed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Terpenoids</td>
<td>5 ml of extract of each + 2 ml CHCl₃ + 3ml of H₂SO₄</td>
<td>An interface with a reddish brown coloration formed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Glycosides</td>
<td>0.5 ml of plant extract + 2 ml of glacial acetic acid + few drops of 5% ferric chloride + 1 ml of conc. sulfuric acid</td>
<td>Formation of brown ring at the interface was observed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Phenols</td>
<td>Plant extract + few drops of neutral FeCl₃ solution</td>
<td>Intense color developed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Alkaloids</td>
<td>Dragendorff’s reagent 2ml of plant extract + few drops of Dragendorff’s reagent</td>
<td>Yellow precipitate appeared</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayer’s reagent</td>
<td>1ml filtrate + few drops of Mayer’s reagent.</td>
<td>white precipitates appeared</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Picric acid solution</td>
<td>2ml of plant extract + few drops of Wagner’s reagent</td>
<td>Red precipitates are formed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Carbohydrates</td>
<td>Fehling’s reagent 5ml Fehling’s solution A and B + aqueous solution of plant extract + heat</td>
<td>Reddish brown precipitates were formed</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Molisch’s reagent</td>
<td>Water solution of extract + 10% alcoholic alpha naphthol + 2 ml sulfuric acid</td>
<td>Appearance of bluish violet zone</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Steroids</td>
<td>5 ml of extract of each + 2 ml CHCl₃ + 3ml of H₂SO₄</td>
<td>An interface with a reddish brown coloration formed</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(+) indicates the presence of phytochemicals.
ACKNOWLEDGMENT

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