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# PHYTOCHEMICAL AND CYTOTOXIC SCREENING OF JUSTACIA ADHATODA AND NARIUM OLEANDER LEAVES EXTRACT

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## ABSTRACT

Natural plant based therapeutics are getting more attention as a source of novel and safe drug therapeutic option as compared to synthetic drugs.

Plants contain various secondary metabolites called phytochemicals, which have effect on several bioactivities and are important source of antioxidant and antimicrobial agents.

This study is conducted to evaluate the phytochemical potential and cytotoxic screening of two indigenous medicinal plants Narium oleander and Justaciaadhatoda. These plants have shown high levels of phytochemical with 23mg/g saponins in both plants leave extracts. The concentration of flavonoids and phenolic are found slightly higher in Justacia adhatoda leaves extract as 21mg/g and 18 mg/g, respectively. The cytotoxic level in both plants has shown

higher. This reveals the medicinal importance of plants as source of secondary metabolite, which could potentially be downstream for industrial purposes.
Key words: Phytochemicals, Cytotoxic Screening, Medicinal Plants

## INTRODUCTION

In recent years, an increasing number of individuals have opted for alternatives that are derived from plants due to worries over the safety of synthetic products (Zhishen et al., 1999). Researchers from all over the world are concentrating their efforts on plants that have the potential to be used as medicine. This is due to the fact that these plants contain a diverse range of naturally occurring compounds that can be used in the production of drugs to treat a wide variety of illnesses (Krishnaraju, 2005). Scientists have been researching phytochemicals, which are secondary metabolites found in plants and have heretofore undiscovered bioactivities, in an effort to find naturally occurring medicinal properties that may be used as a source for the creation of new medications. Because these plants exhibit bioactivities that show promise against microbial infections, they have been investigated for possible application in the development of new classes of antibiotics (Parekh and Chanda, 2007). According to the World Health Organization, more than 80 percent of the world's population solely depends on herbal medicines in order to satisfy their fundamental needs in terms of health care. In addition, thirty percent of all pharmaceutical preparations are derived from active components derived from plants (Khattak, 2012).

A collection of naturally occurring substances known for their antioxidant properties is referred to as polyphenolics. Flavonoids are included in this group. In addition to being present in fruits, vegetables, and beverages, they may also be found in a broad range of ornamental and medicinal plants. Flavonoids inhibit the oxidation of low-density lipoproteins, which results in a reduction in thrombotic activity (LDL). Examples of naturally occurring flavonoids include apigenin, quinine, chamaecrin, and kaempferol, as well as myricetin, luteolin, and myricetin (Hertog et al., 1993).

Flavonoids are potent anti-oxidants that carry out their activities in a biochemical manner during the severe oxidative process that takes place during digestion (Jovanovic et al., 1994). Because flavonoids are able to inhibit lipid peroxidation, chelate redox-active metals, and slow down other biochemical processes in the body, research suggests that they may lessen the risk of cardiovascular disease. Flavonoids are the name given to polyphenolic compounds that may be found in food as glycosides and polymers (Heim et al., 2002).

Many different biochemicals, such as alkaloids (vesicinone, vesinol, and vesicine), vitamins (vitamin C, B-Carotenes), essential oil (butane), non crystallinic steroid (vasakin), and fatty acid combination, have been hypothesised to be responsible for the significant medical benefits that plants provide (Das et al., 2005).

N. oleander (Linn.), more commonly referred to as nerium oleander, is a species of plant that is generally thought to have medicinal chemicals that are effective in

the treatment of skin diseases, edoema, leprosy, and eye disorders. The phrase, which is more often referred to as "Kaner," has been in use for a considerable amount of time. Numerous constituents of this plant have been hypothesised to possess cardiotonic and antimicrobial properties (Siddiqui et al., 1985). The shrub with the many flowers may be seen growing close to rivers and streams. Despite having a broad variety of beneficial properties, it has been shown that this plant may be rather hazardous to the health of both people and animals. When consumed, you run the risk of experiencing dizziness, nausea, depression, fever, bloody diarrhoea, a slow or irregular heartbeat, tingling or numbness in your limbs, and even loss of consciousness. It's possible that the outcome will be heart failure. In recent scientific investigations, it has been used for both the production of tumours and the elimination of germs (Adome et al., 2003).

The plant leaves of *J.adhatoda* suggested the existence of adhalanine, quercitin, alkaloids, pyrroquizoline alkaloids, 2-4-dihydroxychaleone 4-glucoside, and kaemppherol. *J.adhatoda* is also a rich source of phytochemicals (Ahmad et al., 2009). (Ahmad et al., 2009). The chemopreventive action of *J.adhatoda* has been reported, in addition to the hepatoprotective effect it exerts on rats whose livers have been damaged by D-galactosamine (Bhattacharyya et al., 2005). It has also been discovered that a dose of 800 milligrammes per kilogramme of body weight of *J.adhatoda* leaf extract may successfully restore glutathione levels and lipid peroxidation in the liver. This finding was made possible by the fact that *J.adhatoda* leaf extract contains anti-oxidant properties. It also involves in the regulation of acid and alkaline phosphatases in mice testes after exposure to

irradiation. Reduction factor of radiation dose was 1.43 (Das et al., 2005). This study is conducted to evaluate the secondary metabolites quantification and cytotoxic levels of Narium oleander and Justacia adhatoda leaves extract to unveil their potential to be used in novel drug therapeutic options.

## MATERIALS AND METHODS

### Sampling

*Justacia adhatoda*(JA) and *Nerium oleander*(NO)were collected from Rawalpindi hills. Botany department of PMAS-AAUR taxonomist did taxonomic identification.

### Samples processing

The plants were oven dried at $60^0$C for overnight and grounded into fine powder using electric grinder. To remove larger particles samples were sieved through 80 meshes sieve and stored at $4^0$C for processing.

### Extracts Preparation

10 grams of powdered sample was mixed in methanol,chloroform and water in 1:10,incubatedat$24^0$C for 1 day. Then centrifuged at13000 rpm for 20 min, filtered and dried at $37^0$C.

### Proximate analysis

For biochemical analysis, standard methods of AOAC (AOAC, 1990), was used to estimate moisture, crude protein, carbohydrates, fiber, and ash content.

**Moisture and dry matter estimation**

A plant sample weighing 5 grammes was dehydrated in an oven at a temperature of 1050 degrees Celsius for 13 hours. In order to determine whether or not there was a significant weight change, samples were weighed both before and after therapy. The following method was used to calculate the amount of moisture that was present:

Moisture content % $= W_2 – W_3 / W_1 \times 100$

Dry matter of leaves sample was estimated according to calculations

% Dry matter $= W_3 - W_1 /$sample weight $\times 100$

Whereas, $W_1$ is Weight of basin, $W_2$ and $W_3$ are Weight of basin + sample before and after drying, respectively

**Crude Protein estimation**

The protein content of the sample was estimated using factor of 6.5, by the kjeldhal method.

Protein content % $= (V1 - V2)\ N\ /1000\ W \times 14 \times 6.25 \times 100$

Whereas, V1 is sample titer, V2 is blank titer, V3 is normality of standardized $H_2SO_4$ and W is sample weight

**Ash content determination**

Total 5 g of samples taken in glass crucible were previously weighed to have a constant weight and placed in furnace at $540^0$C for 14 hrs. The crucible that contained the ash was given another careful weighing once it had cooled to ambient temperature.

Ash % = X – Y/Z× 100

The weight of the crucible with the sample is denoted by X, the weight of the crucible with the ash is denoted by Y, and the weight of the sample is denoted by Z.

**Determination of Crude Fat**

3 mins In a solution consisting of chloroform and methanol, each sample was combined with 10 grammes of the test material (1:2). A second round of sample homogenization and centrifugation was performed using methanol as the solvent. Chloroform may be replaced with water as a substitute (2:1:8). The supernatants were cooled down inside of a separating funnel before being diluted with ten times their volume in water. After separating the supernatant from the underlying layer of chloroform in each sample, the supernatants were washed twice with very diluted chloroform solutions. After that, we evaporated the extracts until they were completely dry, and then, in order to get an exact sample weight, we deducted the final weight from the initial weight.

**Carbohydrate Content**

According to Khalifa's report, the total carbohydrate content of each plant sample was calculated by subtracting 100 from the sum of the sample's crude protein, crude fat, crude fibre, ash, and moisture-free base. This allowed the total carbohydrate content to be measured (1996).

**Carbohydrate Content** = 100 – (% residual moisture + % protein + % ether extract + ash).

## QUANTITATIVE DETERMINATION OF PHYTOCHEMICALS

### Determination of phenols

For the purpose of determining the total phenolic content of each plant extract, the Folin-Ciocalteu method was used (Harnafi and Amrani, 2008). This test is based on the hypothesis that the presence of phenols would result in the formation of a blue reaction product after the reduction of an alkaline phosphotungstate-phosphomolibdate complex. One millilitre of plant extract was added to a mixture that consisted of 250 millilitres of folin-ciocalteu reagent and 20% of 1.25 millilitres of sodium carbonate. After incubating the sample in the dark for half an hour, the spectrophotometer was used to determine the absorbance of the sample. The absorbance was measured in comparison to a blank sample at a wavelength of 725 nm (1ml of plant sample each replaced by 1 ml of solvent used in extract prepration). Comparing each plant extract to a gallic acid calibration curve allowed for the determination of the total polyphenol content of the extracts. The results were given as the equivalent in milligrammes of gallic acid per gramme of plant material. The plant material was measured in grammes.

### Estimation of alkaloids

Before being put through the fiteration process, five grammes of each plant were mixed with an ethanol solution containing 10% acetic acid and then agitated for

five hours. After being filtered, the liquid was transferred to a heated plate where it stayed there until it had lost three-quarters of its initial volume via evaporation. Take it easy for now. The different alkaloids that were found in the plant samples precipitated when a concentrated solution of NH3OH was added to the mixture. The precipitates were filtered and washed with a solution that contained 1% NH3OH. The precipitates were reweighed after being dried in an oven at 600 degrees Celsius for forty minutes. This was done to check that the weight of the precipitates was consistent. The relative concentration of alkaloids in each sample was determined by comparing the weight of that sample to the weight of the other samples. The mean was calculated based on the results of three independent measurements of each sample (Adeniyi et al., 2009).

**Estimation of saponins**

The amount of saponin found in a number of different plant samples was determined by using the methodology proposed by Kuar and Arora (2009). Following the sampling, twenty grammes of each plant were mixed with two hundred millilitres of ethanol at a concentration of twenty percent. A temperature of about 560 degrees Celsius was maintained during the 4.5 hours that the solution was heated in a water bath while being continuously stirred. The resulting mixture was filtered, and then it was extracted a second time using 200 millilitres of ethanol at a concentration of twenty percent. After being subjected to heat treatment at a temperature of about 900 degrees Celsius, the extracts were reduced to a final volume of 40 ml. After the concentrations were moved into their own separate funnel, 20 ml of diethyl ether was added to each example's

mixture, and then the mixture was stirred. After that, 60 ml of another solvent, n-butanol, was added to each solution, and the mixtures were washed twice with 10 ml of aqueous solution that contained 5% sodium chloride. Finally, the aqueous layer was collected, and the ether layer was discarded. Each solution was separated into two layers. The aqueous layer was collected, and the ether layer was discarded. By heating the solution in a water bath, the remaining portion of the solution was able to be evaporated. After the sample had been dried to a constant weight, the percentage of saponin that it had contained could be estimated.

**Estimation of flavonoids**

AlCl3 calorimetric test was used to calculate the total amount of flavonoids in each plant material (Marinova et al., 2005). Extracts were made using methanol as the solvent from 5 g of plant material for each sample. Two millilitres of distilled water with five percent sodium nitrite was added to a half millilitre aliquot (0.15 ml). After waiting 5 minutes, 15 ml of 10% NH3Cl solution was added and left to sit for 6 minutes before 1 ml of 1 M NaOH solution and 2 ml of dist. H2O were added to bring the level up to 5 ml. The solution was mixed thoroughly and absorbance was obtained at 510 nm. In order to standardise the amount, we converted 1 gramme of plant material to milligrammes of quercetin, and we ran the experiment three times to get an average number.

**CYTOTOXIC BRINE SHRIMP ASSAY**

Using the procedure described by Meyer et al.,, sea salt was dissolved in deionized water at a concentration of around 28 g/L. (1992). The Artemia salina (shrimp) eggs were hatched in saline solution divided into parts of a tray. Larvae were placed to the dark part of the tray and then transferred to the well-lit side using a micropipette following an overnight incubation period. Each reaction mixture (10, 100, and 1000 g/ml) was put in vials, and the solvents were evaporated, before being placed in test tubes for the bioassay. As a positive control, 2 millilitres of sea salt was employed. About 2 millilitres of sea salt solution was used to redissolve the remnants. At least ten shrimp were divided among five millilitre (ml) vials, each of which was placed in a 24-hour incubator set at a temperature of 25 to 28 degrees Celsius. Abbot's method was used to determine the percentage of shrimp that died during incubation.

## RESULTS

### PROXIMATE ANALYSIS OF PLANT SAMPLES

By performing proximate analysis on *J.adhatoda* and Nerium oleander leaves, we were able to identify the levels of dry matter, moisture, crude protein, carbohydrates, crude fat, and ash content. The amount of carbohydrates found in N. oleander was the largest (59 g/100 g), while the amount found in *J.adhatoda* was the lowest (57 g/100 g). *J.adhatoda* had a crude fat content of 6.75 grammes per one hundred grammes, but N. oleander only had 3.9 grammes per one hundred grammes of dry weight. It was discovered that the crude fat content of *J.adhatoda* leaves is higher than that of N. oleander leaves. [Citation needed] We observed that *J.adhatoda* had a greater concentration of dry matter (90.5 0.05 g/100g) when compared to N. oleander, which had a concentration of dry matter that was 89.7

0.05 g/100g. A thorough investigation into the raw materials of these excellent medicinal plants made it feasible to conduct a phytochemical screening and a qualitative assessment of the plants' potential.

**QUALITATIVE AND QUANTITATIVE DETERMINATION OF PHYTOCHEMICALS FROM PLANT SAMPLES**

By doing a qualitative examination, it was discovered that both plant extracts included secondary metabolites such as alkaloids, flavonoids, saponins, cardiac glycosides, anthraquinone glycosides, and triterpenoids (fig. 2). It's possible that the solvents in which particular phytochemicals were discovered are responsible for the bioactivity of certain phytochemicals, such as the antibacterial and antioxidant characteristics.

According to the results of the quantitative research, both *J.adhatoda* (3.74 0.29% w/w) and N. oleander (3.24 0.25% w/w) contain trace levels of the alkaloid oleandrin. Flavonoids are able to shield cells from the damaging effects of oxidative stress thanks to their ability to chelate metal ions. Flavonoids are also water-soluble antioxidants and free radical scavengers. The levels of flavonoid found in *J.adhatoda* (21.90 0.2% w/w) and N. oleander (20.90 0.25% w/w) are much higher than those of alkaloid. When samples of leaves were analysed to determine the presence of saponins, it was found that the leaves of *J.adhatoda* contained (23.0 0.30% w/w) of saponins, while the leaves of N. oleander contained (23.20 0.31% w/w) of saponins. Both of these results were based on the analysis of leaf samples. The presence of these phytochemicals in both plants

results in an increase in the plants' antimicrobial, antioxidant, and anticancer activities, among other beneficial effects.

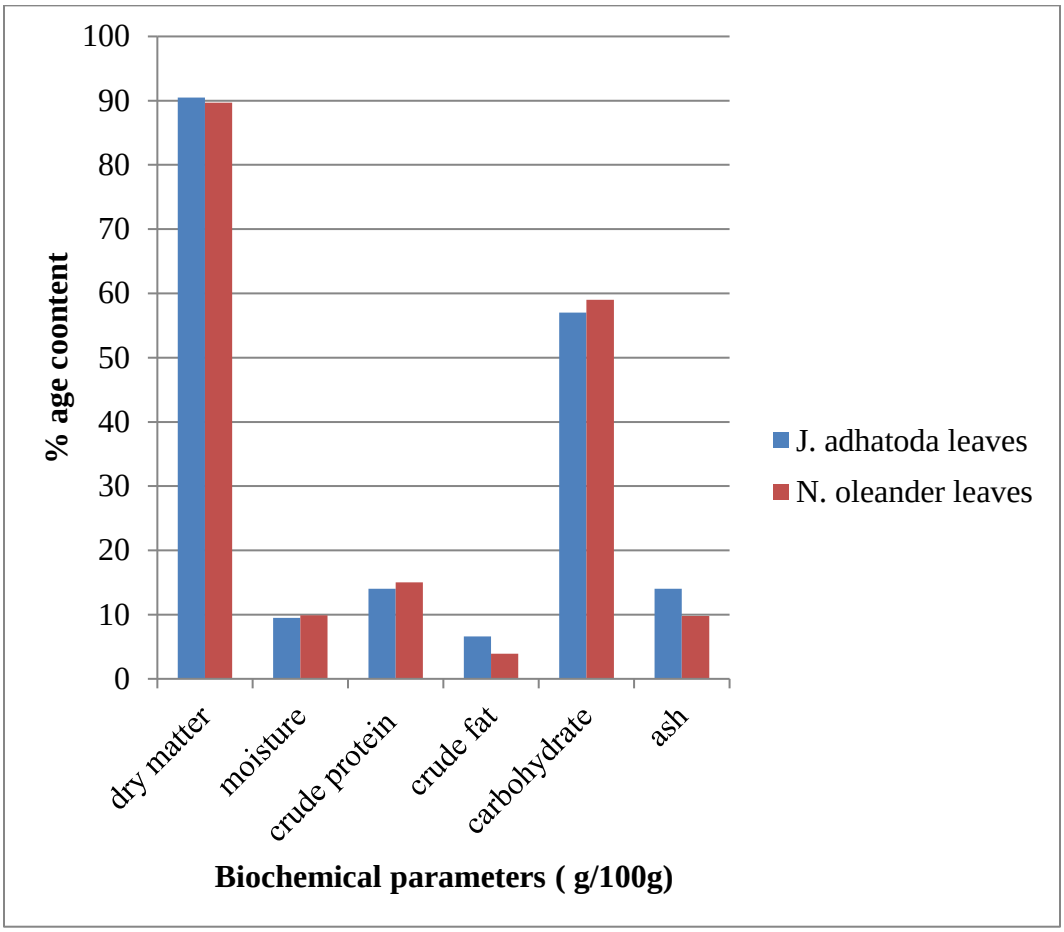


**Figure 1: Proximate Analysis of *Justaciaadhatoda* and *Nerium oleander* leaves**

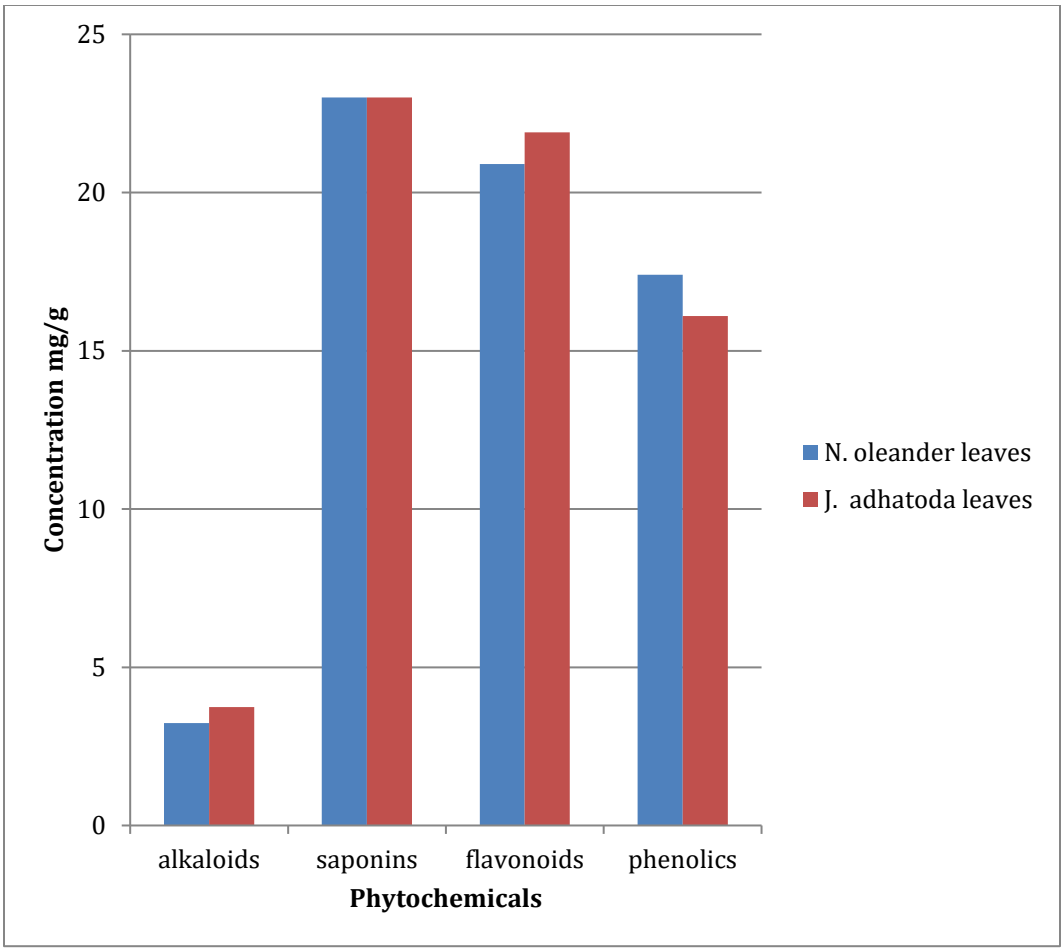


**Figure 2: Quantitative analysis of phytochemicals of *N. oleander* and *J. adhatoda* leaves extract.**

**CYTOTOXIC SECREENING OF PLANT SAMPLES**

The cytotoxicity of plant extracts was evaluated using a bioassay conducted on brine shrimp, which provided helpful preliminary data for selecting plant extracts. Tables 1 and 2 provide the findings of studies conducted in controlled environments to evaluate the cytotoxic influence of plant extracts and various fractions of *N. oleander* and *J.adhatoda* leaves on the growth of brine shrimp. These evaluations were carried out using plant materials. In the course of this experiment, we discovered that the capacity of the brine shrimp to remain alive

was inversely proportional to the concentration of each percentage. The number known as the LD50 was determined to be the concentration at which fifty percent of the shrimp in the population under study perished. According to the findings of this inquiry, the LD50 value for an extract of leaves from *N. oleander* and *J.adhatoda* is highest for methanol, followed by water, then n-hexane, and finally chloroform. This ranking indicates that methanol is the most dangerous of the four solvents that were investigated. At a concentration of 1000 mg/L, almost all of the samples exhibited unsafe effects. (Table 1, 2).

**Table 1: Cytotoxic screening (Brine shrimp lethality assay; % death) of various solvent fractions of methanolic extract of *J. adhatoda* leaves**

| *J. adhatoda* Leaves Extracts | **Cytotoxic screening (Concentration mg/L)** | | | |
|---|---|---|---|---|
| | 10 mg/L | 100 mg/L | 1000mg/L | $LD_{50}$ mg/L |
| Methanol Extract | 19.25±2.85 | 29.0±2.53 | 48.01±2.39 | >1000 |
| n-hexane extract | 27.10±3.60 | 37.99±4.49 | 56.99±4.59 | 743 |
| Chloroform Extract | 41.93±2.22 | 55.13±2.54 | 71.33±1.23 | 68 |
| Residual Aqueous Fraction | 32.22±1.88 | 50.12±1.26 | 64.77±1.66 | 100 |
| Positive Control | 54.7±1.62 | 78.77±2.89 | 89.43±2.75 | <10 |

Each value in the table is represented as mean ± SD (n=3), ($P < 0.05$)
Positive control; Saline sea salt.

**Table 4.7. Cytotoxic screening (Brine shrimp lethality assay , % death) of various solvent fractions of methanolic extract of *N. oleander*leaves**

| N. oleander Leaves Extracts | **Cytotoxic screening (Concentration mg/L)** | | | |
|---|---|---|---|---|
| | 10 mg/L | 100 mg/L | 1000mg/L | $LD_{50}$ mg/L |
| Methanol Extract | 18.75±2.75 | 28.9±2.63 | 47.77±2.39 | >1000 |
| n-hexane extract | 26.90±3.50 | 38.34±4.59 | 57.20±4.59 | 740 |
| Chloroform Extract | 42.33±2.12 | 54.33±2.64 | 70.33±1.23 | 66 |
| Residual Aqueous Fraction | 31.22±1.98 | 49.43±1.16 | 64.97±1.66 | 100 |
| Positive Control | 54.7±1.62 | 78.77±2.89 | 89.43±2.75 | <10 |

Each value in the table is represented as mean ± SD (n=3), ($P < 0.05$).

Positive control; Saline sea salt.

**DISCUSSION**

Many different biochemicals, such as alkaloids (vesicinone, vesinol, and vesicine), vitamins (vitamin C, B-Carotenes), essential oil (butane), non crystallinic steroid (vasakin), and fatty acid combination, have been hypothesised to be responsible for the significant medical benefits that plants provide (Das et al., 2005). The selected medicinal plants in this study have shown high potential of accumulating carbohydrates, saponins, flavonoids and polyphenols, which depicts their role as antimicrobial and antioxidant agent. The cytotoxic screening of plants has shown their potential toxicity levels. Narium oleander leaves have shown comparably high toxin levels as compared to second plant. In previous literature, these plants are shown to have hepatoprotective role on liver damage. In addition to its hepatoprotective effect on liver damage caused by D-galactosamine in rats, the chemopreventive activity of J.adhatoda has also been identified. This activity helps prevent cancer from developing (Bhattacharyya et al., 2005). These plants could be potential source of extracting various biochemical of medicinal importance for human welfare.

**CONCLUSION**

The plant based secondary metabolites known as phytochemiicalswiht previously un-noticed bioactivities, have been investigated to undercover all natural beneficial properties as a drug developmental source. Therefore, plants with adequate bioactivities against microbial infections have been screened to develop novel therapeutic drugs. In this study Narium oleander and Justaciaadhatoda have been screend for their phytochemicals and cytotoxic levels.The cytotoxic level in both plants has been found high with n-hexane extracts. Both plants have shown

higher levels of saponins, flavonoids, and polyphenols, which depicts their importance as a rich source of secondary metabolites of therapeutic interest.

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