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# DETERMINATION OF ANTIOXIDANT, ANTIBACTERIAL AND ANTICOAGULANT ACTIVITY OF *Artemisia nilagirica* ROOT AND STEM

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## Abstract:

***Artemisia nilagirica*** is a wild perennial hardy plant belongs to Asteraceae family. It has important medicinal values to cure human sickness.In the present study, aqueous, methanol and diethyl ether extract of *Artemisia nilagirica*stem and root was extracted. The phytochemical screening, antioxidant, antibacterial and anticoagulant activity were determined. In phytochemical screening the stem and root of *Artemisia nilagirica*proved the presence of rich bioactive compounds in all the three solvent extracts. The antioxidant potential was determined using DPPH with vitamin C as standard, vitamin C estimation to determine the non enzymic antioxidant potential and reducing power assay which all gave values close to standard ascorbic acid. Antibacterial activity was performed using disc diffusion method against *Staphylococcus aureus, Klebsiella pneumonia* and *Enterococcus faecalis*in the presence of stem and root extract of all 3 solvents. It was found that diethyl ether extract of stem showed maximum activity against *Enterococcus faecalis* at the range of 7mm. Anticoagulant activity was conducted which exhibited that the aqueous root showed the maximum coagulation time as 30mins:10sec by prothrombin test compared to all the other samples indicating the stem and root of *A.nilagirica* is a potential candidate rich in phytochemicals, antioxidant, antibacterial and anticoagulant properties.

**Keywords:***Artemisia nilagirica*, bioactive compounds, antioxidant, antibacterial, anticoagulant, medicinal values.

## I.INTRODUCTION:

Medicinal plants are presently in demand and their acceptance is increasing progressively. Undoubtedly, plants play an important role by providing essential services in ecosystems (Singh, 2002). More than a tenth of the plant species (over 50000 species) are used in pharmaceutical and cosmetic products. However, the distribution of medicinal plants across the world is not uniform and medicinal herbs are mainly collected from the wildlife population (Huang, 2011).

The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, skin, flowers or even the whole plant. The active compounds in most parts of the medicinal plants have direct or indirect therapeutic effects and are used as medicinal agents. In the body of these plants, certain materials are produced and stored that are referred to as active compounds (substances), which have physiological effects on the living organisms (Phillipson, 2001; Verma and Singh, 2008).

In recent years, the humans are showing interest for composition of plants due to bioactive compounds. Medicinal plants are considered as chemical industry because it produces several applications. Traditional medicine has gained a fast development since it does not contain any side effects (Palaniswamy and Padma, 2018). More than 40% of the plants have been reported to be used as medicinal herbs to treat diseases in the west (Sarad*et al.*, 2017, Prakash *et al.*, 2018). Stem, leaf, fruit, flower, root or even whole plant may be used for active compounds since they have both indirect or direct medicinal agents and therapeutic effects. The toxicity and adverse effects of conventional and allopathic medicine are important factors in increasing the value of plants with respect to herbal drugs (Pradeep *et al.*, 2014).

Aromatic and medicinal plants are sources of diverse nutrient and non-nutrient molecules, many of which display antioxidant and antimicrobial properties that can protect the human body against both cellular oxidation reactions and pathogens (Manoharan *et al.*, 2013). The plant derived medicines are relatively safer than synthetic alternatives, offering very good therapeutic benefit and affordable treatment (Aiganesh, 2012).

Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack. The medicinal values of all plants are embedded in their component phytochemical (Biradar*et al.,* 2007).The most important phytochemical of plants are alkaloids, saponins, tannins, glycosides, flavonoids and phenolic components. The detection plays a strategic role in the phytochemical investigation of crude plant extract and is very important in regards to their potential pharmacological effects (Briskin, 2000).

Antioxidants in the dietary supplement help to reduce or prevent the damage of cells or tissues in the human body. Recent studies are paying attention for considering traditional plant as medicines. Medicinal plants are important for natural antioxidant bioactive compound to prevent or neutralize the oxidative damage caused by ROS (Palaniswamy and Padma, 2017; Scalbert*et al.,* 2005).

Medicinal plants exhibit several activities among which the antibacterial properties play a major role. In the modern world, use of medicines as antibiotics cause resistance against clinical and pathogenic bacteria, hence there is a need to develop new drugs from plant sources as they contain many biologically active compounds which contain antibacterial property without producing any side effects (Rao and Ravishankar 2000).

## II. METHODOLOGY:

The present study was carried out using the medicinal plant *Artemisia nilagirica*as the experimental material for phytochemical analysis, antibacterial activity, antioxidant activity and anticoagulant activity.

### 2.1 SAMPLE COLLECTION:

*Artemisia nilagirica*root and stemwere collectedfrom the local areas of Sathyamangalam, Erode district by using sterile bags. Identification of plant sample was authenticated in Botanical Survey of India, Tamil Nadu Agricultural University (TNAU) campus, Coimbatore and the authentication number is as follows: *Artemsianilagirica*(Voucher Number: BSI/SRC/5/23/2021/Tech /203).

### 2.2 SAMPLE PREPARATION:

The samples were washed thoroughly using sterile water and dried and powdered. Then 5gm powder was weighed and mixed with 50 ml of three different solvents like methanol, diethyl ether and aqueous in 500ml separate beaker respectively. The beaker was kept in water bath for 30 minutes for additional solvent to evaporate. Then 2 mL Dimethyl Sulfoxide (DMSO) solution was added to it. After that filtered the extract using Whatman No 1 filter paper and stored the sample.

### 2.3 PHYTOCHEMICAL SCREENING:

Phytochemicals are bioactive compounds present in the plants, formed during the normal metabolic process. The extracts were subjected to the phytochemical screening to detect the presence or absence of various bioactive compounds like alkaloid, tannin, quinone, flavonoid, cardiac glycosides, coumarins, phlobatannin, steroid and phytosteriod, terpenoids, and phenols (Kalpana Devi*et al.,* 2014). The protocol for screening phytochemical components is given below:

#### 2.3.1 Test for Alkaloids:

1.36 g of mercuric chloride dissolved in 60 ml and 5g of potassium iodide were dissolved in 10 ml of distilled water respectively. These two solvents were mixed and diluted to 100 ml using distilled water. To 1ml of crude extract are mixed with a few drops of reagent was added. Formation of white or pare precipitate showed the presence of alkaloids.

#### 2.3.2 Test for Tannins:

To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

#### 2.3.3 Test for Quinones:

To 1 ml of extract, 1 ml of concentrated sulphuric acid was added. Formation of red color indicates the presence of quinones.

#### 2.3.4 Test for Flavonoids:

To 2 ml of plant extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.

#### 2.3.5 Test for Cardiac Glycosides:

To 0.5 ml of extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloridewere added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of the brown ring at the interface indicates the presence of cardiac glycosides.

#### 2.3.6 Test for Coumarins:

To 1 ml of extract, 1 ml of 10% NaOH was added. Formation of yellow colorindicates the presence of coumarins.

#### 2.3.7 Phlobatannins:

To 1 ml of plant extract a few drops of 2% HCl was added the appearance of redcolor precipitate indicates the presence of phlobatannins.

**2.3.8Steroids and Phyto steroid:**

To 1 ml of plant extract equal volume of chloroform is added and subjected with few drops of the concentrated sulphuric acid appearance of brown ring indicates the presence of steroids and appearance of bluishbrown ring indicates the presence of phytosteroids.

**2.3.9 Test for Terpenoids:**

To 0.5 ml of extract, 2 ml of chloroform was added and concentrated sulphuricacid was added carefully. Formation of red-brown color at the interface indicates the presence of terpenoids.

**2.3.10 Test for Phenols:**

To 1 ml of the extract, 2 ml of distilled water followed by a few drops of 10% ferric chloride was added. Formation of blue or green color indicates the presence of phenols.

**2.4ANTIOXIDANT ACTIVITY:**

**2.4.1 DPPH Assay:**

The free radical scavenging activity of the extracts were measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (1,1- diphenyl- 2- picryl-hydrazyl). 4mg of DPPH was allowed to dissolve in 100ml of methanol, which served as a standard. Each extract was taken at a concentration of500µl. These samples were made up with methanol to 1ml and then 5ml of DPPH was added to all the test tubes, incubated for 20min in dark at room temperature. The OD reading at 517nm was taken (Gopal and Subramaniam, 2017). It was calculated using the below formula

$$\% \text{ inhibition} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

**2.4.2 Estimation of vitamin C:**

The method of Irving *et al.*(1951) was followed to estimate the vitamin C content. The stock standard and working standard solution were prepared by 4% of oxalic acid solution (4gm in 100ml of water), the working standard was prepared by taking 10ml of standard and make up to 90ml of water and it was pipetted out in 0.2, 0.4, 0.6, 0.8 and 1ml in boiling tube. The samples ofconcentration 500ul were taken. Volume was made up to 3ml with water. To this 1ml of DNPH (2, 4-Dinitrotphenylhydrazine) was added and 1-2 drops of thiourea solution was added to it. It was kept in water bath for 20 minutes at 40°C for incubation, after which orange red ozone crystals were formed, 7ml of $H_2SO_4$ was added (Saeed *et al.*, 2008). The OD readings at 540nm were taken.

**2.4.3 Ferric Reducing Power Assay:**

3.5 ml of ferrocyanide solution was determined as blank solution. In standard individually added 0.2ml of extract with 3ml of potassium ferrocyanide solution was mixed with the samples. The optical density was measured at670nm min 10, 20, 30, 40 minutes of time interval (Maruthamuthu and Kandhasamy, 2016).

**2.5ANTIBACTERIAL ACTIVITY:**

Antibacterial activity is completely associated with the compounds that provincially kill bacteria and slow down their rate of growth, without being extensively toxic to nearby tissues.

**2.5.1 Disk Diffusion Method**

Antibacterial activity was performed by disc diffusion method against *Saphylococcusaureus, Klebsiella pneumonia* and *Enterococcus faecalis.* The culture was inoculated in nutrient broth and kept in shaker for 24hr. The culture was swabbed in nutrient agar plates and wells were made using sterile cork borer of 11mm. The samples were filtered on Whatman filter paper for disc diffusion method and kept for incubation for 24hrs on the petri plates and the zone of inhibition was observed (Signe and Goran, 1988).

**2.6*IN VITRO* ANTICOAGULANT ACTIVITY:**

**Determination of Prothrombin Time (PT)**

**2.6.1 Collection of blood sample**

The blood sample was obtained from the healthy volunteers from superior cubital vein of right arm of each person and dispensed into the polypropylene container containing 3.2% tri sodium citrate to prevent the process of clotting (9 parts of blood to 1 parts of tri sodium citrate) and centrifuged at 3000rpm for 15 minutes to separate blood cell and plasma. The plasma was separated and used for determination of Prothrombin time test. The Freshly prepared plasma was stored at 4°C until its use.

**2.6.2 Collection of blood and plasma re-calcification**

To a clean test tube 0.2ml of plasma, 0.1ml of aqueous, diethyl ether and methanol of *Artemisia nilagirica* extract was added. 0.3ml of 25mm $CaCl_2$ were added to the above mixture. All the test tubes were shaken and mixed well and incubated for 1minute at 37°C in a water bath. For Negative Control tube, 0.2ml of plasma, 0.1ml of 0.9% Saline water and 0.3ml of 25mm $CaCl_2$ were taken and incubated at 37°C in a water bath. The plant extract of aqueous, methanol and diethyl ether was replaced by saline. 0.01ml of 50mg/ml of EDTA and 0.2ml of plasma was used as positive control tube. 0.3ml of $CaCl_2$ was also put into the

test tube and placed immediately in the water bath pre warmed at 37°c. The clotting time was measured by tilting the tubes at an angle of 45°C every 5second to recognize the presence or absence of coagulumuntil a clot was formed. A stopwatch was started to record the coagulation time in separate concentration of the extracts, control in seconds and stopwatch was stopped as soon as the clot formation began. The activity is expressed in term of clotting time in seconds. Each test was performed three times to obtain three determinants in coagulation time exerted with different concentration of extracts (Ashwin and Usha, 2017).

## III. RESULTS AND DISCUSSION:

### 3.1 PHYTOCHEMICAL ANALYSIS OF *Artemisia nilagirica*

The tests conducted were alkaloid, flavonoid, tannin, quinone, cardiac glycoside, coumarin, phlobatannin, steroid, phytosteroid, terpenoid and phenol and reported in Table 1. The phytochemical screening of the plant showed the presence of flavonoid, tannin, quinone, phlobatannin, phytosteroid, terpenoid and phenol in aqueous, methanol and diethyl ether extracts of both stem and root. However, some of the components were absent in few extracts of stem and root. The results are presented in Table 1.

From the literature it was found that preliminary phytochemical analyses by different solvents like n – hexane, chloroform, ethyl acetate, ethanol and aqueous extracts were done for the plant rhizome of *Acorus calamus Linn*. The test was positive for alkaloid, coumarin, flavonoid, steroid, tannin, glycoside and terpenoid. In this study all the extracts of plant showed the presence for all tests indicating it was good candidate for source of rich phytochemicals (Ajay *et al.,* 2010).

The recent study of phytochemical was done by using the plant *Hibiscus sabdriffa* of roselle seeds. Here, methanolic plant extract was used. The test that are followed for analysis are flavonoid, terpene, oxalate, tannin, phylate, steroid, glycoside, polyphenol, alkaloid, phlobatannin and saponin showed the presence of all major phytochemicals in *Hibiscus sabdriffa*of roselle seeds (Charles *et al.,* 2019).

Phenol plays an important role in cancer prevention and treatment. This compound is present in almost all the plant species making phenol a widely found phytochemical in plant sources (Indhumathy*et al.,* 2014).

**Table 1: Phytochemical analysis of *Artemisia nilagirica***

| PHYTOCHEMICAL TEST | *Artemisia nilagirica* | | | | | |
|---|---|---|---|---|---|---|
| | Aqueous extract | | Methanol extract | | Diethyl ether extract | |
| | Stem | Root | Stem | Root | Stem | Root |
| Alkaloid | - | - | - | - | - | - |
| Flavonoid | + | + | + | + | + | + |
| Tannin | + | + | + | + | + | + |
| Quinone | + | + | + | + | + | + |
| Cardiac glycosides | + | - | - | + | + | + |
| Coumarin | + | + | - | - | - | - |
| Phlobatannin | + | + | + | + | + | + |
| Steroids | - | - | + | + | + | + |
| Phytosteroids | + | + | + | + | + | + |
| Terpenoid | + | + | + | + | + | + |
| Phenol | + | + | + | + | + | + |

### 3.2 ANTIOXIDANT ASSAY OF *Artemisia nilagirica:*

#### 3.2.1 DPPH (2,2-Dipheny-1-picrylhydrazyl) Assay:

DPPH assay was compared with vitamin C (ascorbic acid) as standard and free radical scavenging activity was calculated and noted. For aqueous extract of stem it was 87.8% and for root it was 89.1%, methanol extract of stem exhibited 79.7% and root showed 85.1% and diethyl ether extract of stem presented 78.3% with root almost same as 79.7%. The standard value of ascorbic acid was 81%. These values were compared with ascorbic acid which was treated as the standard. The value of DPPH activity was calculated using the formula below:

$$\text{Scavenging activity\%} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100$$

**Table 2: DPPH assay of *Artemisia nilagirica***

| DPPH ASSAY | | |
|---|---|---|
| % SCAVENGING ACTIVITY | | |
| **SAMPLE** | **STEM** | **ROOT** |
| **Aqueous Extract** | 87.8 | 89.1 |
| **Methanol Extract** | 79.7 | 85.1 |
| **Diethylether Extract** | 78.3 | 79.7 |

The above values were concurrent with other reports which stated that DPPH assay was done by *A. laricinus* with the comparison of ascorbic acid and showed a high activity with $SC_{50}$ from a concentration of 1.25mg/ml. The scavenging activity of aqueous extract of *A. laricinus* leaf showed high activity with $SC_{50}$ <2.5mg/ml. The *A. laricinus* stem extract did not show any scavenging activity when compared to ascorbic acid (Polo-Ma-Abiele and Samson Mashele, 2014).

Another study proved results that were expressed as $IC_{50}$ value to decrease the initial DPPH concentration by 50%. The extracts of alcoholic and aqueous extract were compared with standard ascorbic acid. The alcoholic extract was determined as 1.825 ± 0.122 and in aqueous extract 1.035 ± 0.09 and in ascorbic acid it was 0.207 ± 0.006. It was observed that the inhibition of plant extract towards Free Radical Production increases with increase in concentration of DPPH test (Rasha, 2019).

Palaniswamy and Raghunathan (2017) reported a study on the DPPH activity of *Artemisia pallens, Majorana hortensis* and *Cymbopoganflexuosus.* The leaves were used for the experiment where aqueous, methanol and chloroform were used as solvents. Out of three, the leaves of *Majorana hortensis* showed the maximum scavenging activity compared to others in all the three extracts which was as high as 80%.

### 3.2.2 Reducing power assay:

Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. The assay works on the above principle (Shiva *et al.*, 2005).

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. C ompounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidat ion processes, so that they can act as primary and secondary antioxidants (Chanda and Dave, 2009).

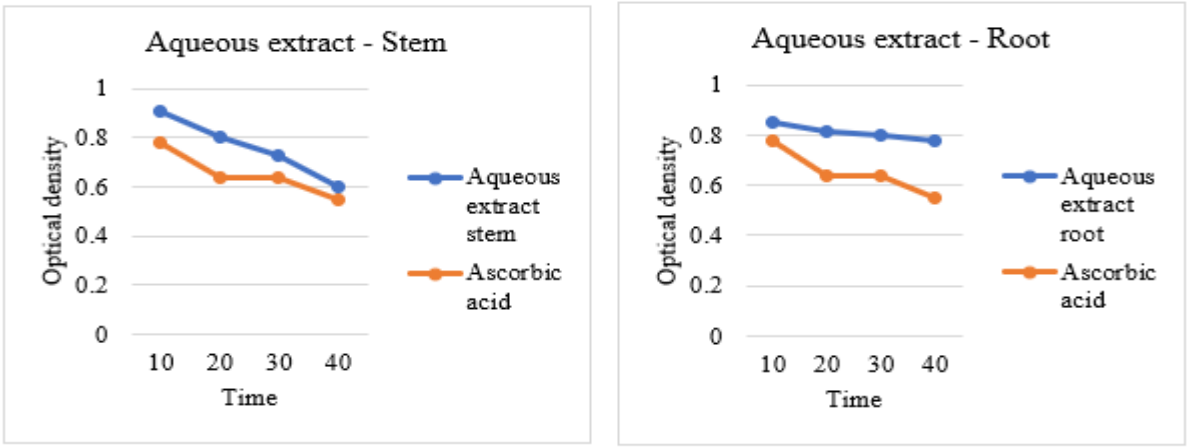
Reducing power assay activity of aqueous, methanol and diethyl ether extract of *Artemisia nilagirica*was done. The results are shown below:

This experiment was carried out by using time function with intervals of 10mins, 20mins, 30mins and 40mins. The samples of stem and root were used in the preparation of 3 solvent extracts namely aqueous, methanol and diethyl ether. In aqueous stem it was noted that with increase in time upto 40 min the reduction assay slowly tapered down indicating that all the ferric ions were converted to ferrous and none were reaming. The grater the absorbance value the greater is the reducing power indicating its potential as an antioxidant. In methanolic extract of the stem, the reducing power was slightly better than the root since it had greater absorption. In the case of diethyl ether, it was same for the stem and root which was better than methanolic extract. As with increasing time, the values for reduction property decreased showing that the antioxidant potential also was becoming lesser as all the ferric ions were used up. The results are presented in Table 3.

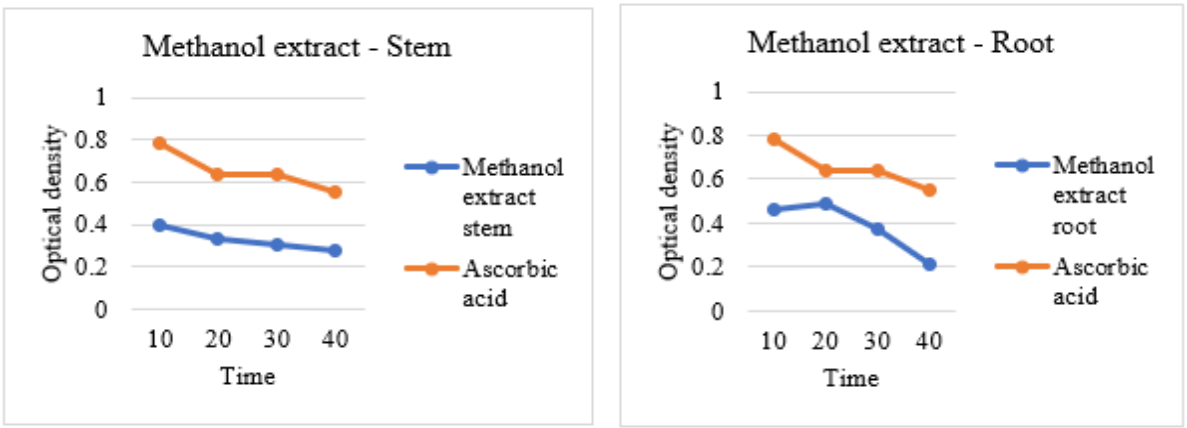
**Table 3: Reducing power assay of *Artemisia nilagirica***

| REDUNCING POWER ASSAY | | | | | | | |
|---|---|---|---|---|---|---|---|
| OD READING AT 670nm AT DIFFERENT TIME INTERVALS | | | | | | | |
| MINUTES | Standard (Ascorbic acid) | AQUEOUS EXTRACT | | METHANOL EXTRACT | | DIETHYL ETHER EXTRACT | |
| | | STEM | ROOT | STEM | ROOT | STEM | ROOT |
| 10 MINS | 0.78 | 0.91 | 0.85 | 0.40 | 0.46 | 0.44 | 0.39 |
| 20 MINS | 0.64 | 0.80 | 0.81 | 0.33 | 0.49 | 0.36 | 0.24 |
| 30 MINS | 0.64 | 0.73 | 0.80 | 0.30 | 0.37 | 0.32 | 0.22 |
| 40 MINS | 0.55 | 0.60 | 0.78 | 0.28 | 0.21 | 0.29 | 0.19 |

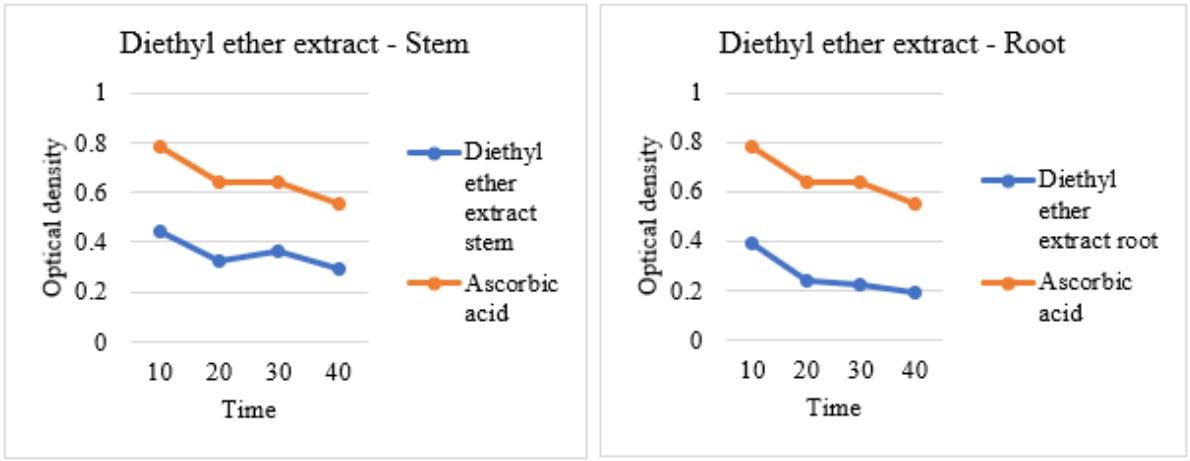
**Graph 1: Reducing power assay for aqueous extract with standard (Ascorbic acid)**




**Graph 2: Reducing power assay for methanol extract with standard (Ascorbic acid)**



**Graph 3: Reducing power assay for Diethyl ether extract with standard (Ascorbic acid)**




The recent study of reducing power was carried out in *Clerodendrumcrytophyllum*by ethyl acetate fraction (EAF) and n – butyl alcohol fraction (BAF). $EC_{50}$ value of reducing power shows 0.203 mg/ml for EAF and 0.216 mg/ml for BAF. While the other sub-fractions exhibited lower reducing power that vary from 0.473 to 1.562 mg/ml. $EC_{50}$ value for gallic acid is 0.045 mg/ml. The results conclude that gallic acid has better reducing ability than *C. crytophyllum*(Jing Zhou *et al.,* 2020).

Manmohan (2011) reported that *Chalcone Semicarbazones* had 4 methyl substituted compounds which showed more reducing power in comparison to the 2 methyl substituted compounds. The substitution with different substituent on the phenyl of the aldehydic and acetophenic group of chalcone moiety plays an important role in reducing potential of the compounds.

In a study on *Punica granatum* fruit peel of aqueous and methanol extract, with ascorbic acid as a standard (0.28 µg/ml), the absorbance of aqueous extract was 0.30 and methanolic extract was 0.26 which was very close to the standard value. It was concluded that aqueous extract exhibited maximum reducing power in comparison with methanol extract and standard ascorbic acid (Sushil Kumar *et al.*,2013). Hence with the above sources, we can infer that our values are similar to the above study and thus it has potential reducing power to be a candidate for antioxidant properties.

### 3.2.3 Estimation of vitamin C (Ascorbic acid) of *Artemisia nilagirica:*

The antioxidant activity is seen in both enzymic and non - enzymic which plays a significant role. The example of non enzymic antioxidant is vitamin C. The vitamin C assay levels were noted to understand the efficacy of aqueous, methanol and diethyl ether extract of stem and root of *Artemisia nilagirica.*

For estimation of vitamin C, ascorbic acid was used as standard with OD reading of 570nm. A small volume of 0.5ml of sample was used for all extracts. The concentration of standard for 0.5ml is 100µg/ml. In all three extracts, the stem values were comparatively lesser in vitamin C content than the root extracts. However, the best activity among the stem was seen in methanolic extract followed by aqueous and then diethyl ether. Nevertheless, all the three root extracts showed better results than stem beginning with the aqueous at the highest followed by methanolic and lastly diethyl ether extract at 0.28 µg/ml, 0.26 µg/ml and0.20 µg/ml respectively. Estimation of vitamin c compared to all extracts is tabulated in Table 4.

The earliest study was conducted with *Moringa oleifera* leaves which is a rich source of vitamin C. The dried leaves of *Moringa* were found to contain 0.07 – 0.14mg/g of vitamin C which was low when compared to fresh leaves of *Moringa*. The dried stem of *Moringa* was found to contain 0.10-0.15mg/g which showed less activity when compare to fresh stem of *Moringa*. The value obtained in this study showed that *Moringa* has provided more activity when compared to citrus fruits and thus proved to be a rich source of antioxidant (Nidhi *et al.,* 2013).

Another study was conducted by *Moringa oliferal*eaf extract using Fenton reaction where concentration from 10 – 100 µl. In this experiment, methanolic extract of *Moringa oliferal*eaf has shown 73.72 ± 0.18 and ascorbic acid shown 83.63 ± 0.73 at concentration of 100 µl. This proved that the leaf extract has antioxidant values close to vitamin C (Ekta *et al.,* 2019).

In a study by Nishanta*et al., (*2016) the concentration of vitamin C was evaluated in lemon, bitter orange, pomelo, sweet orange, grapefruit and citron. The pomelo contained 61.29 mg/100ml of ascorbic acid and 17.4 mg/ml for citron. For lemon it was 34.8mg/100ml, 28.89mg/100ml for bitter orange, 39.80mg/100ml for grape fruit and 25.11mg/100ml for sweet orange which concluded that pomelo has maximum concentration and citron has minimum concentration indicating that it has high antioxidant values with increased vitamin C content.

**Table 4: Estimation of Vitamin C of *Artemisia nilagirica***

| EXPERIMENT | Concentration of Standard Ascorbic Acid (100µg/ml) | AQUEOUS EXTRACT | | METHANOL EXTRACT | | DIETHYL ETHER EXTRACT | |
|---|---|---|---|---|---|---|---|
| | | STEM | ROOT | STEM | ROOT | STEM | ROOT |
| OD values 570 nm | 0.42 | 0.19 | 0.28 | 0.25 | 0.26 | 0.14 | 0.20 |

### 3.3 ANTIMICROBIAL ACTIVITY OF *Artemisia nilagirica:*

The extracts nullified the effect of microbes like *Staphylococcus aureus, Klebsiella pneumoniae* and *Enterococcus faecalis.* The positive control of the activity is Streptomycin used as antibiotic disk and negative control is aqueous, methanol and diethyl ether solvents respectively.

The aqueous extract of the stem and root showed protective activity against *Staphylococcus aureus, Klebsiella pneumoniae* and *Enterococcus faecalis*indicating maximum inhibitory activity of the bacteria *Staphylococcus aureus* when compared to the other 2 organisms. The zone of inhibition exhibited by the root and stem extract was measured to comprehend the level of protection provided against the microbial strains. In this case, the antimicrobial activity of the sample against all the 3 species is very close indicating the protective activity of the sample is almost similar irrespective of the 3 microbes used in the current study. It shows values of 6 mm, 4mm and 5 mm for *S. aureus, K. pneumoniae* and *E. faecalis*. Aqueous extract of root showed minimum inhibitory activity against *Klebsiella pneumoniae* when compared to *Staphylococcus aureus* and *Entercoccus faecalis*.Similarly the effect of methanolic extract and diethyl ether extract of root and stem also showed protective activity as indicated in Table 5 which shows the measure of zone of inhibition. The zone of clearance can be viewed in Figures 1, 2 and 3 against *S. aureus, K. pnemoniae* and *E. fecaelis.*

**Table 5: Antimicrobial activity of *Artemisia nilagirica***

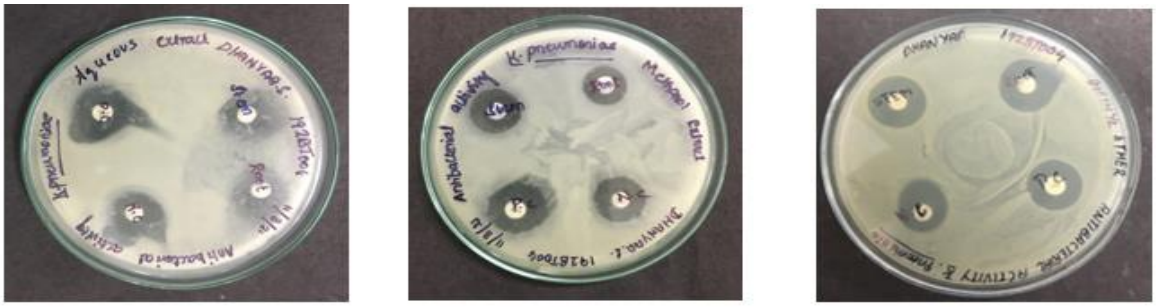
| Species | *Artemisia nilagirica* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aqueous extract | | | | Methanol extract | | | | Diethyl ether extract | | | |
| | Stem | Root | PC | NC | Stem | Root | PC | NC | Stem | Root | PC | NC |
| | Zone of inhibition (mm) | | | | | | | | | | | |
| ***Staphylococcus aureus*** | 6 | 4 | 4 | 2 | 5 | 4 | 5 | 3 | 4 | 3 | 3 | 3 |
| ***Klebsiella pneumoniae*** | 4 | 3 | 4 | 3 | 3 | 2 | 4 | 3 | 4 | 4 | 3 | 2 |
| ***Enterococcus faecalis*** | 5 | 4 | 3 | 2 | 5 | 3 | 3 | 2 | 7 | 4 | 5 | 2 |

PC –Positive Control NC – Negative Control

**Figure 1: Antibacterial activity against *Staphylococcus aureus***

Aqueous extract Methanol extract Diethyl ether extract

**Figure 2: Antibacterial activity against *Klebsiella pneumoniae***



Aqueous extract Methanol extract Diethyl ether extract

**Figure 3: Antibacterial activity against *Enterococcus faecalis***

Aqueous extract Methanol extract Diethyl ether extract

Our study is supported by a report from Reshma and Omar (2014) who studied anti-bacterial activity against medicinal plants by agar cup method. They reported that ethanolic leaf extracts of *Justicia adhatoda* and *Ocimum sanctum* showed antibacterial activity against all pathogenic bacteria. Leaf extract of *O. sanctum* showed largest zone of inhibition (22 mm) against *Shigelladysenteriae* and *Bacillus cereus* whereas smallest zone of inhibition was exhibited against *E. coli* with the root extract of *Acorus calamus*. This shows that if the sample contains antimicrobial activity, the zone of inhibition would be greater or more profound as in this case.

In another study on antimicrobial activity of*Moringa olifera* by aqueous leaf extract in the presence of bacteria namely *S. typhymurium, P. aeruginosa, E. coli* and *B. cereus*, the antibiotic like ciproflaxin, gentamycin, chloroma-penicol, tetracycline and ceftriaxone were used. Ciproflaxin showed highest inhibition against *S. typhymurium*with a zone of inhibition of 27mm indicating the sample to be a good source of antibacterial medicine (Rasha and Fatma, 2019).

### 3.4 ANTICOAGULANT ACTIVITY OF *Artemisia nilagirica:*

Prothrombin test was performed with the extract of aqueous, methanol and diethyl ether of stem and root of *Artemisia nilagirica*. The anticoagulant activity was noted and compared with negative control which was saline and positive control was EDTA (anticoagulant that is commercially available).

It was observed that aqueous and diethyl ether extracts of stem of *Artemisia nilagirica* showed similar coagulating time that is average time compared to other extracts. The methanolic extract of stem showed lowest coagulating time (20mins: 3sec) compared to other two extracts. Aqueous extract of root showed greater coagulating time (30mins:10sec) compared to other extracts. Similarly the root samples also were subjected to prothrombin test and coagulation time was noted as tabulated in Table 6. The time has been calculated in terms of minutes and seconds. It is evident from the results obtained that all the solvent extracts of both stem and root showed almost similar values except the methanolic stem which showed only 20 min 03 sec whereas all the others showed greater time. The results are displayed in Table 6 below.

**Table 6: Anticoagulant activity of *Artemisia nilagirica***

| EXPERIMENT | + CONTROL | — CONTROL | AQUEOUS EXTRACT | | METHANOL EXTRACT | | DIETHYL ETHER EXTRACT | |
|---|---|---|---|---|---|---|---|---|
| | | | STEM | ROOT | STEM | ROOT | STEM | ROOT |
| AMOUNT OF PLASMA | 0.1 | 0.1 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| AMOUNT OF EXTRACT (ml) | 0.5 ml EDTA | 0.5ml saline | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| AMOUNT OF CALCIUM (ml) | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| TIME OF COAGULATION | --- | 10 mins: 5 sec | 27 mins: 44 sec | 30 mins: 10 sec | 20 mins: 03 sec | 22 mins: 54 sec | 27 mins: 30 sec | 24 mins: 45 sec |

An earliest study of anticoagulation activity was from *Nelumbo nuclifera* extracts of methanol, ethanol, ethyl acetate leaf samples. The experiment was carried out by 6 different groups at different concentrations, group 1 positive control, group 2 negative control, group 3,4,5, and 6 as 0.062 g/ml of leaf extract, 0.125 g/ml, 0.25 g/ml, 0.5 g/ml of plant extract. The methanolic extract of leaf demonstrated better anti-coagulant activity at 0.25 g/ml by plant extract compared to all other extracts (Ramya *et al.*, 2017).

Our results were supported by another anticoagulation activity in *Allium sativum* with methanol and aqueous extract at different concentration of 100-500µg/ml. This activity was carried out by prothrombin test. The amount of plasma taken for the study was 0.2ml. The methanol and aqueousextracts of 500µg/ml shows maximum anti-coagulant indicating greater the concentration of the extract better the activity (Vijayaya*et al.*, 2017).

Chandra *et al*. (2016) reported a study with *Cestrum nocturnum* aqueous extract of leaf, where prolonged PT (Prothrombin test), APTT (Activated partial thromboplastin time) and TT (thrombin time) assay were done. It prolonged APTT clotting time from 45 (2mg/ml) to 82.2 (10mg/ml). PT clotting time 20.4 (2mg/ml) to 31.4 (10mg/ml). TT clotting time 9.2 (2mg/ml) to 17.4 (10mg/ml). Heparin prolonged APTT and PT clotting time more than 111. Heparin prolonged TT clotting time more than 20.6 s at a concentration of 1 IU/ml. This information creates evidence that plant extracts with anticoagulant activity show better results based on their concentration.

## IV. CONCLUSION:

It can be concluded that the stem and root of *A.nilagirica* has potential bioactive compounds. It has been proved to possess antioxidant activity, antibacterial activity against *Staphylococcus aureus, Klebsiella pneumoniae* and *Enterococcus faecalis* and has anticoagulant activity also which is supported by literature. Hence, the stem and root of *A.nilagirica* can be considered to possess potential medicinal values when compared with standard ascorbic acid and hence can be used to produce synergistic effect if used along with other medicinal plants too.

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