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## PHYTOCHEMICAL EVALUATION OF A MEDICINAL ORCHID: *NERVILIA PLICATA* (ANDREWS) SCHLTR.

<sup>1</sup>Renjini Haridas, <sup>1</sup>Thangapandian V and <sup>2</sup>Binu Thomas\*

<sup>1</sup>PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore- 641 029.  
Tamil Nadu, India.

<sup>2</sup>PG Department of Botany, Deva Matha College, Kuravilangad, Kottayam-686 633, Kerala, India.

### ABSTRACT

The present study on phytochemical constituents of a medicinal orchid such as *Nervilia plicata* (Andrews) Schltr. reveals that, it is one of the valuable orchid with diverse medico-potentiality. More over this study also highlights the necessity of conservation and sustainable utilization such medicinal plants for future generation.

**Keywords:** Phytochemical evaluation, Medicinal Orchid, *Nervilia plicata*.

### INTRODUCTION

India, with its great biodiversity, has a tremendous potential and advantages in the emerging field of herbal medicines. Medicinal plants as a group comprise approximately 7500 and include representatives of about 17,000 species of higher flowering plants [1]. Around 70% of Indian medicinal plants are found in the tropical zone such as Western and Eastern Ghats. The Western Ghats region of Kerala is great emporium and a treasure of ethnobotanical wealth. There are numerous drugs have entered the international pharmacopoeia via the study of ethno pharmacology and traditional medicine [2].

Ethnomedicine is the mother of all modern drugs and recently the importance of the traditional knowledge based medicines are being utilized throughout the world [3]. Such medicinal plants become the base for the development of a medicine, a natural blueprint for the development of new drugs [4]. The phytochemical constituents of plants, is desirable, not only for the discovery of therapeutic agents, but also a new sources of such economic materials as tannins, oils, gums, flavonoids, saponins, essential oils. These are the precursors for the synthesis of complex chemical substances [5].

The plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains and other metabolites which are rich in antioxidant activity [6]. Most of the medicinal and edible plants having rich antioxidants. Antioxidants may help the body to protect itself against various types of oxidative damage caused by reactive

oxygen species, which are linked to a variety of diseases including cancer, diabetes, shock, arthritis and acceleration of the ageing process. They may act by decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radicals, binding metal ion catalysts, decomposing primary products to non-radical compounds and chain-breaking to prevent continued hydrogen abstraction from substrates [7].

### METHODOLOGY

#### Plant selection

*Nervilia plicata* (Andrews) Schltr. (Orchidaceae), an orchid and it needs hot, humid conditions with a dry winter rest when the deciduous leaf falls, the new leaf appears in the early spring and the flowers a few months later, after the leaf has matured and fallen away (Fig.1).

The phytochemical analysis on *Nervilia plicata* was carried out during this study. There are about four different solvent extracts of plant was individually performed using different qualitative tests for alkaloids, flavonoids, glycosides, steroides, terpenoids, cardiac glycosides, saponin, tannins, resins, phenol, reducing sugar and triterpenoids.

#### Preparation of plant extracts

Dried powder form of bulb and leaf of the plant was extracted with Petroleum ether, Chloroform, Ethyl acetate and Ethanol. Solvents at 60°C for 8 hrs. Using soxtron apparatus. The stored filtrate was used for the various phytochemical and biological studies and balanced

solvent extract was then stored in airtight containers at 4°C for further use.

### Qualitative analysis

All qualitative tests were done to find out the presence of the active phytochemical constituents [8,9,10]. (Table- 1).

### Total phenol contents [11]

The phenolic compounds are oxidized to phenolates by the Folin ciocalteau reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue molybdenum-tungstate complex. The colour development is measured at 650 nm against a reagent blank.

About 3.9 ml of distilled water and 0.5 ml of Folin ciocalteau reagent were added to 0.1ml of methanolic extract of *N. plicata* in a tube and incubated at room temperature for 3 minutes after which 2ml of 20 % sodium carbonate was added to this and kept at boiling water bath for 1 min. Phenols react with phosphomolybdic acid in the Folin ciocalteau reagent in alkaline medium and produce blue coloured complex (Molybdenum blue) that can be estimated colorimetrically at 650nm. A calibration curve was constructed with different concentrations of Gallic acid and the results were expressed as mg of Gallic acid (GAE) equivalents/g of extract.

### Total flavanoid contents [12]

The sample was mixed with a reagent containing  $AlCl_3$  and  $NaNO_2$  and pink coloured flavanoid aluminium complex was formed in alkaline medium and the coloured complex can be estimated calorimetrically at 510 nm against a reagent blank.

An aliquot (1ml) of extract and standard solution of Rutin (100 ml/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To this 0.3ml of 5%  $NaNO_2$  were added. After 5 minutes, 0.3ml 10%  $AlCl_3$  was added. Then after 1 minute 2ml of 1M NaOH was added and total volume was made upto 10ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510nm. The value of optical density was used to calculate the total flavanoid content present in the sample. The flavanoid content was expressed as milligram of Rutin (RE) equivalents/g extract.

### Diphenyl-1-picryl hydrazyl (DPPH) method [13]

Various concentrations of sample (4ml) were mixed with 1ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2mM. The mixture was shaken vigorously and left to stand for 30 min, and the absorbance was measured at 517 nm. The percentage inhibition was calculated according to the formula:

$$(A_0 - A_1) / A_0 \times 100$$

Where  $A_0$  was the absorbance of the control and  $A_1$  was the absorbance of the sample.

### ABTS radical cation scavenging activity [14]

ABTS decolourization assay involves the generation of the  $ABTS^+$  chromophore by the oxidation of ABTS with ammonium per sulphate. It is applicable for both hydrophilic and lipophilic compounds. The scavenging activity of the plant extracts on ABTS radical cation were measured at 734 nm.

The reaction was initiated by the addition of 1ml of diluted  $ABTS^+$  to 10 $\mu$ l of different concentration of methanolic extract of sample and 10 $\mu$ l of methanol as control. The absorbance was read at 734 nm and the percentage inhibition was calculated by the following equation.

$$I = (A_0 - A_1) / A_0 \times 100$$

Where  $A_0$  is the absorbance of control,  $A_1$  is the absorbance of test compound.

### Metal chelating activity [15]

Iron (II) chelating activity was measured by the inhibition of the formation of iron (II) ferrozine complex after preincubation of the samples. The  $Fe^{2+}$  was monitored by measuring the formation of ferrous iron-ferrozine complex against methanol blanks at 562 nm.

The reaction mixture contained 1ml of various concentrations of the extract, 0.1ml of 2mm ferric chloride and 3.7ml of methanol. The control contained all the reaction reagents except sample. The reaction was initiated by the addition of 0.2ml of 5mM ferrozine. After 10 mins at room temperature the absorbance of the mixture was determined at 562 nm against blank. A lower absorbance of the reaction mixture was indicated a higher  $Fe^{2+}$  chelating ability. The capacity to chelate the ferrous ion was calculated by

$$\% \text{ of chelation} = (A_0 - A_1) / A_0 \times 100$$

Where  $A_0$  was the absorbance of the control, and  $A_1$  of the mixture containing the extract or the absorbance of a standard solution.  $IC_{50}$  value ( $\mu$ g extract/ml) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. EDTA was used as standard in the present experiment.

### Inhibition Concentration ( $IC_{50}$ ) [16]

The discoloration of sample was plotted against the sample concentration in order to calculate the  $IC_{50}$  value. It is defined as the amount of sample necessary to decrease the absorbance of the radical by 50%.

## RESULTS AND DISCUSSION

### Phytochemical screening of *Nervilia plicata*

Phytochemicals are organic chemicals that are produced by plants. They may be nutritive or non-nutritive in nature. These can be regarded as naturally occurring non-nutritive chemicals of plant origin.

In the present study the phytochemicals present in *Nervilia plicata* is illustrated in (Table - 2). This plant is highly medicinal and endangered plant belongs to the family

orchidaceae. Bulb of this plant is used to cure the urinary diseases by *Katunayaka* tribe. The qualitative phytochemical analyses were made using leaves and bulb extract of *Nervilia plicata*. The various solvent systems like petroleum ether, chloroform, ethyl acetate and ethanol were employed to extract the various phytochemical constituents in shade dried plant parts. The qualitative test of extracts confirmed in the presence of Alkaloid, Phenol, Tannin, Glycoside and Steroids. The results of the present study showed that the plant *Nervilia plicata* is rich in alkaloids, flavonoids, triterpenoids and steroids. The presence of these secondary metabolites may vary with solvents. This might be due to various degrees of solubility of different solvents for different phytoconstituents.

#### Antioxidant and ABTS<sup>+</sup> Scavenging Activity

Antioxidant activity is a very important parameter used to characterize different plant materials. This activity is related with compounds capable of protecting a biological system against the potential harmful effects of oxidative processes. Antioxidants have received increased attention in the last years from nutritionists and medical researchers for their potential activities in the prevention of several degenerative diseases [17]. In recent years, several works have been published on antioxidant activities of medicinal plants.

ABTS assay measures the relative antioxidant activity to scavenge the radical ABTS<sup>+</sup> and it is an excellent tool for determining the act of hydrogen donating antioxidant and chain breaking antioxidants [18]. The methanol tuber extracts inhibited ABTS<sup>+</sup> radicals were greater than petroleum ether and hexane extracts. Factors like stereo selectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extract to react and quench different radicals [19]. Maximum percentage of inhibition of ABTS<sup>+</sup> radical was observed in bulb and leaf extract of *N. plicata*.

This may be due to the presence of high phenol content of the plant (Fig. 2).

#### DPPH Scavenging Activity

DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which react with suitable reducing agent. In the present study leaf and bulb extract of *N. plicata* exhibited rapid scavenging activity. Therefore the ethanol bulb extract showed weak antioxidant activity. Maximum inhibition of DPPH radical was observed in leaf and bulb extracts of *N. plicata* (Fig. 3).

#### Phenol and Flavanoids

The total phenol and flavanoids contents in the bulb and leaf extract of *N. plicata* in various solvent systems such as petroleum ether, chloroform, ethyl acetate and ethanol were used for the study. The results of the present study also highlights the medico-potentiality of this orchid (Table: 3 & 4).

#### Ferric reducing power

The antioxidant activities of natural components may have a reciprocal correlation with their reducing powers [20]. The reducing power increases as the extract concentration gets increased. This indicates some compounds in *N. plicata* is acting both as electron donors and electron acceptors. They could react with free radicals to convert them into more stable products and to terminate radical chain reactions (Fig. 4).

Methanol leaf extracts showed highest inhibition activity and lower IC<sub>50</sub> value. It may be due to the presence of high phenolic and flavonoid content in these extracts. Earlier workers also discovered that there was a strong relationship between phenol content and antioxidant activity [21]. In *N. plicata* the maximum inhibition was found in the extract of leaf and bulb.

**Table 1. List of phytochemical constituents with their respective identifying characters**

S.No.	Phytocompounds	Name of the tests	Combinations of solutions	Results to be observed
1	Alkaloids	Mayer's test	2ml of extract + few drops of 1% HCl → Take 1ml of this mixture + 6 drops of Mayer's reagent added	Yellow-creamiest precipitate
2	Flavonoids	Ferric chloride test	1ml of extract + two drops of freshly prepared ferric chloride solution	Appearance of green, blue or violet colors
3	Terpenoids	Salkowski test	5ml of extract + 2ml of chloroform + 3ml of concentrated sulphuric acid	Appearance of reddish brown color layer
4	Cardiac Glycosides	Keller-Killani test	5ml of extract + 2ml of glacial acetic acid containing one drop of ferric chloride solution + 1ml of concentrated sulphuric acid was added	Appearance of brown ring
5	Phenol	Ferric chloride test	1ml of extract was added with 1ml of alcohol and then few drops of neutral ferric chloride	Appearance of blue or green color

			solution	
6	<b>Sterols</b>	Liberman-Burchard's test	1ml of extract + 1ml of chloroform + few drops of concentrated sulphuric acid	Appearance of blue color layer
7	<b>Saponin</b>	Foam test	1ml of extract + 5ml distilled water and shaken well	Development of stable foam
8	<b>Tannins</b>	Braemer's test	5ml of extract + 1ml of 5% ferric chloride	Appearance of dark green or deep blue color
9	<b>Resin</b>	Sulphuric acid test	2ml of extract + 5-10ml acetic anhydride dissolved by gentle heating, cooled + 0.5ml of H <sub>2</sub> SO <sub>4</sub>	Appearance of purple color was rapidly changing in to violet color
10	<b>Glycosides</b>	Sodium hydroxide test	2ml of extracts dissolved in 1ml of water and then aqueous sodium hydroxide solution was added.	Formation of yellow color
11	<b>Triterpenoids</b>	H <sub>2</sub> SO <sub>4</sub> test	2ml of extracts +con. H <sub>2</sub> SO <sub>4</sub>	Formation of reddish brown color
12	<b>Reducing sugar</b>	Fehling's test	5ml of extract boiled with few drops of Fehling's solutions A & B for 2 minutes.	Formation of orange red precipitate.

Table 2. The Results of Phytochemical screening of *Nervilia plicata*

Secondary Metabolites	Petroleum ether		Chloroform		Ethyle acetate		Ethanol	
	Leaves	Bulb	Leaves	Bulb	Leaves	Bulb	Leaves	Bulb
<b>Alkaloid</b>	-	+	+	+	-	-	-	+
<b>Flavonoid</b>	+	+	-	+	+	+	+	+
<b>Phenol</b>	+	+	+	+	-	+	-	+
<b>Tannin</b>	+	+	+	+	+	+	+	+
<b>Glycoside</b>	+	+	-	+	-	+	-	+
<b>Saponin</b>	-	-	-	-	-	-	-	-
<b>Resin</b>	-	-	-	-	-	-	-	-
<b>Steroids</b>	+	+	-	+	+	-	-	+
<b>Terpanoids</b>	+	+	-	+	+	+	+	-
<b>Cardiac glycosides</b>	+	-	-	-	-	+	-	-
<b>Triterpanoids</b>	-	-	-	-	-	+	-	+
<b>Reducing sugar</b>	-	-	-	-	-	-	-	-

Table 3. Total Phenol content in Bulb and Leaf extract of *N. plicata*

S. No	Plant parts used	Solvents	Total Phenol
1.	Leaf	Petroleum ether	1.46±0.01
		Chloroform	1.6±0.22
		Ethyl acetate	1.16±0.02
		Ethanol	1.85±0.03
2.	Bulb	Petroleum ether	5.33±0.38
		Chloroform	5.45 ±0.02
		Ethyl acetate	2.33±0.05
		Ethanol	2.81±0.10

Table 4. Total Flavanoids content in bulb and leaf extract of *N. plicata*

S. No	Plant parts used	Solvents	Total flavonoid
1.	Bulb	Petroleum ether	33.22±0.694
		Chloroform	23.86±12.782
		Ethyle acetate	18.34±0.249
		Ethanol	26.11±0.105

2.	Leaf	Petroleum ether	23.63±0.174
		Chloroform	49.82±0.174
		Ethyle acetate	38.76±0.138
		Ethanol	26.00±0.138

Fig. 1. Habit and Flower of *Nervilia plicata* (Andrews) Schltr. (Orchidaceae).



Fig. 3. DPPH antioxidant activity of different extract of *N. plicata*

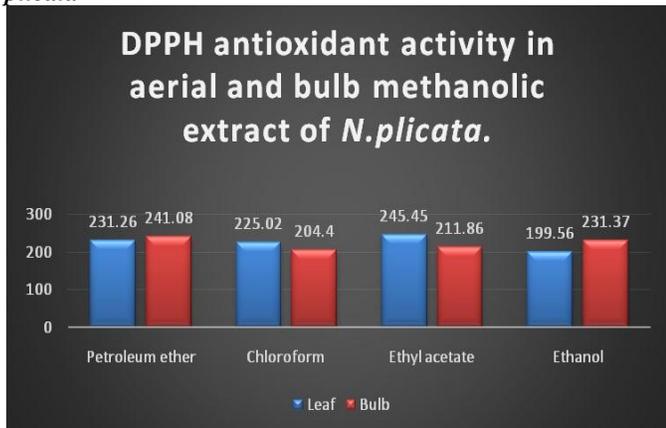


Fig. 2. ABTS<sup>+</sup> radical scavenging activity in *N. plicata*

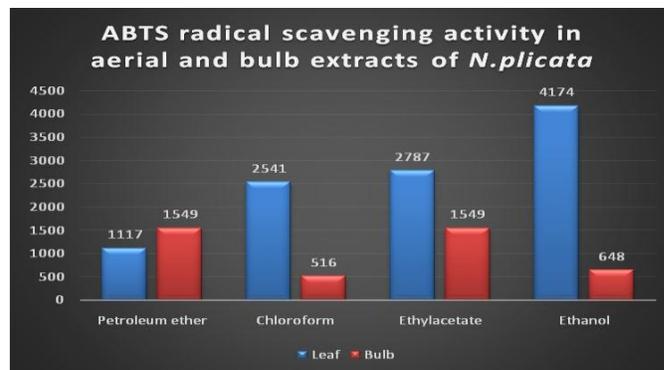
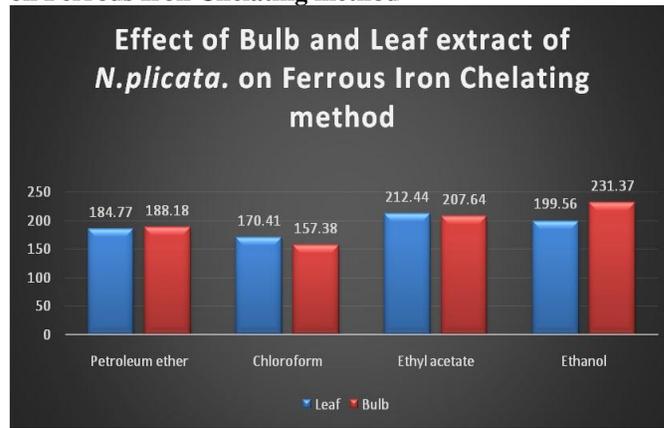


Fig. 4. Effect of Bulb and Leaf extract of *Nervilia plicata* on Ferrous Iron Chelating method



**CONCLUSION**

The present study carried out the potential biological ability of the selected plant *Nervilia plicata* the phytochemical constituents like alkaloids, flavonoids, tannins, phenols, triterpenoids, glycosides, cardiac glycosides and steroids were found to be present. Antioxidant assays ABTS, DPPH and Ferric reducing power. The results are proved that plant *N. plicata* is highly medicinal and effective for the treatment of various ailments. More over this study also strengthens the basic folkloric and traditional usage of *N. plicata*. A more comprehensive research is needed to isolate the essential

compounds in this medicinal plant. More over this study also highlights the importance of conservation and sustainable utilization of such potential medicinal herbs to future generation.

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