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**EXPLORING ANTICANCER POTENTIAL
OF VANDA ROXBURGHII, DENDROBIUM
MONILIFORME & PHALAENOPSIS
AMABILI FOR HERBAL DRUG
DEVELOPMENT**

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Exploring Anticancer potential of Vanda Roxburghi, Dendrobium moniliforme & Phalaenopsis amabilis for herbal drug development

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Abstract:

Cancer is a huge worldwide health challenge, demanding the development of new therapeutic techniques. Orchids, which are recognized for their various chemical components and therapeutic characteristics, have recently received attention for their possible anticancer effects. When coupled with herbal medication formulations, synergistic effects may improve anticancer qualities. This research explores the potential anticancer properties of three distinct orchid species. The study aimed to investigate the phytochemical composition of the orchid and assess their impact on cancer cells to determine their efficiency as natural anticancer agents.

Keywords: Cancer, Orchid, Herbal medication, Anticancer

1. Introduction:

Orchids are the most diverse angiosperm family around the world, with an estimated >25,000 species. They exhibit a vast range of epiphytic and terrestrial growth types and have effectively colonized nearly every ecosystem on Earth with the exception of hot deserts and frigid Antarctica, they are almost universally distributed, but their variety is highest in tropical and subtropical areas [1]. The main reason orchids are planted is due of their exotic beauty and long flowering period, which makes them valuable as cut flowers and ornamentals [2]. Furthermore, Orchid have not only captured the attention of horticulturists and flower enthusiasts but also that of scientists and researchers. The family Orchidaceae is widely employed, either directly in traditional medicine or indirectly in the manufacturing of modern pharmaceuticals [3]. Appropriate use of herbal medicines can effectively and safely cure a wide range of illnesses [4]. Research on medicinal plants, particularly orchids, has advanced, leading to the discovery of several active metabolites that have demonstrated promise in the treatment of chronic illnesses [5]. The cytotoxic Properties like polysaccharides, alkaloids, phenols, flavonoids, phenanthrenes, stilbenes, and numerous more phytochemicals with proven medicinal qualities can be found in abundance in orchids. The anti-inflammatory, antioxidant, antimicrobial, neuroprotective, anti-cancer, anti-diabetic, anti-aging, immunomodulatory, and hepatoprotective properties that these bioactive chemicals primarily impart. properties of orchid extracts are ascribed to the existence of several bioactive chemicals. These substances have the ability to stop the growth of cancer cells and cause them to undergo apoptosis, which suggests that anticancer medications could be developed using them [6]. Therefore, these plants have the potential to pave the way for the development of novel plant based medications [7]. According to the World Health Organization (WHO) "Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems", up to 80% of the world's population rely on herbal medicines as a primary source of treatment [8]. Herbal medications play an important

role in cancer prevention and therapy, and they are widely available and cost effective. Pharmaceutical research in advanced countries such as the United States, Germany, France, Japan, and China has significantly increased the quality of herbal medications used to treat cancer. Herbs can help with detoxification and prevent cancer [9]. Cancer is a chronic disease that is a threat to mankind. It's important to know that the cancer is not only a single disease but also a bunch of hundred diseases. Cancer is an abnormal proliferation of cells in our bodies that can cause death. It damages regular cells and causes imbalance in the body. This is a major health issue in both underdeveloped and industrialized countries. Identifying the exact etiology of cancer can be challenging [10]. Smoking causes 90% of lung cancer [11]. Insulin-like growth factors and their binding proteins promote cancer cell proliferation [12]. Physical inactivity, obesity, and diet contribute to 30-35% of cancer mortality [13]. Radon gas and medical imaging are sources of ionizing radiation, which is not a significant mutagen. When radiation mixes with other cancer causing chemicals, such as radon and tobacco smoke, its potency increases. Prolonged exposure to UV rays from the sun can cause skin cancer [14]. Hormones like estrogen progesterone's, IGF has also played a major role in women's breast cancer. Ionizing radiation, which comes from medical imaging examinations or radiation therapy, is also a known risk factor [15]. Cancer is a major global health concern and the top cause of mortality worldwide [16]. In 2020, 19.3 million new cancer cases and nearly 10 million cancer deaths were reported globally. The most common were female breast tumors (11.7%), lung cancer (11.4%), and colorectal cancer (10.0%). Furthermore, by 2040, the number of cancer cases detected each year is predicted to grow by around 47% [9]. In 2020, there were approximately 10.0 million cancer-related fatalities (9.9 million excluding non-melanoma skin cancer) and 19.3 million predicted new cases of cancer globally. Globally, female breast cancer (2.26 million cases), lung (2.21), and prostate cancer (1.41) were the most often diagnosed malignancies; lung (1.79 million deaths), liver (830000), and stomach cancers (769000) were the most frequently caused cancer-related deaths [9]. There has been significant advancement in cancer detection and therapy. At the turn of the twentieth century, few cancer patients lived. By the 1930s, one out of every five people survived five years after being diagnosed. In the 1940s, this proportion reached one in four, in the 1960s one in three, and by 1990, it was about 50% [17]. Various therapies are available for the cancer treatment but they have several limitations such as kidney damage, gastrointestinal disorder, etc. But plant derived compounds possessing anti-cancerous activities have received huge amount of scientific attention. They play vital role in the cancer prevention and treatment. Pharmaceutical research has been done in several countries [18]. Orchids are one of the useful medicinal plants, used since antiquity, however there is very little advance work related to experimental research done with orchid and their oral use in therapeutic aims for local and tribal usage.

The present study aims to evaluate the anticancer potentiality of *Vanda Roxburghii*, *Dendrobium moniliforme*, *Phalaenopsis amabilis*. These species should be highlighted in this thesis because they have shown noteworthy anticancer properties. In this study we uncover their hidden pharmacological potential, and the way for future research and application in the field of herbal medicine and oncology.

2. Methodology:

Plant material was collected from Sanjay nursery in Mohali Chandigarh to Rapture biotech private limited mohali. Parts of plants were separated and cut into small pieces and then preserved in incubator for 3 days in Rapture biotech private limited mohali. After dried crush dried parts of plant in mortar pestle and make a fine powder.

Preparation of methanolic extract :

Plant extract was prepared by using dried parts of plant i.e Stem, Roots, Flower and leaves in Methanol [22]. 3g of dried plant powder in 150 ml of Methanol. Methanol is employed as a solvent due to its low boiling point and favorable characteristics for secondary plant chemicals [23]. Medicinal plant extraction involves isolating potent compounds like alkaloids, flavonoids, terpenoids, Sterols, saponins, phenols, Flavonoids, Tannis , Fixed oil, Fats by standard Tests Glucosinolates and glycosides from less active components, utilizing suitable solvents and established extraction techniques [24].

Qualitative Tests for Phytochemical Screening:

SNo	Test	Procedure	Observation (Indicating positive Test)	Reference
Detection of alkaloids				
1	Dragendroff's test	To a few ml of filtrate add 1-2 drops of dragendroff's reagent was added along with the side of test tube.	A reddish brown precipitates	25
2	Mayer's test	To a few ml of filtrate add 1-2 drops of Mayer's reagent along with the sides of test tube.	A creamy white or yellow precipitate	26
3	Wagner's test	To a few ml of filtrate add 1-2 drops of Wagner's reagent along with the side of test tube.	A reddish-brown precipitate	26
4	Iodine Test	To a 3ml of filtrate add few drops iodine solution. Along with the side of test tube.	A blue color which disappears on boiling and reappears on cooling shows positive result	27
5	Boucharde's test	To a 6 ml of plant extract evaporated completely + 6 ml of ethanol (@60°C) + Few drops of Boucharde's reagent (dilute iodine solution).	A reddish-brown color	28

6	Tannic acid test	To a few ml extract add 10% of Tannic acid solution along with the side of test tubes. A buff color precipitates	A buff color precipitates	29
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Detection of Terpenoids

1		Take 2ml of chloroform in a test tube + 5 ml plant extract (evaporate on water bath) + 3ml of conc. H ₂ SO ₄ (boiled on water bath).	A grey color solution	30
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Test for phenolic compound

1	Iodine test	To 1 ml of extract few drops of dil. Iodine solution is added along with the sides of test tube.	A transient red color	31
2	Ferric chloride test	Take a Few ml of extract aqueous solution add few drops of ferric chloride sol. Along with the sides of test tube.	A dark green or bluish black color	32
3	Gelatin test	Plant extract is dissolved in 5 ml distilled water + 1 ml gelatin solution + 10% Nacl along with the sides of test tube.	A white precipitate	26
4	Lead acetate test	Plant extract is dissolved in 5ml DH2O+3 ml of 10% lead acetate solution along with the sides of test tube.	A white precipitates	26
5	Ellagic acid test	Plant extract aqueous solution + 5% glacial acetic acid + 5% sodium nitrite solution along with the sides of test tube	. A muddy solution with Niger brown precipitates	33
6	Potassium dichromate test	To a few ml of plant extract add few drop of potassium dichromate solution along with the sides of test tube.	A dark color shows the positive result	34

Test for flavonoids

1	Alkaline Reagent	1mL plant extract + 2mL of 2% Noah solution + few drops dil. HCl along with the side of test tube.	an intense yellow color, becomes colorless on addition of diluted acid	35
2	LeadAcetate test	1mL plant extract + few drops of 10% lead acetate solution in a test tube.	A yellow precipitate	26
3	Shibata's reaction/ Cyanidin test	1gm Aq. extract + dissolved in 1-2 mL 50% methanol by heating + metal magnesium + 5-6 drops of conc. HCl.	A red color {flavonols}, orange color {flavones}	36

4	Ferric Chloride test	Extract aqueous solution + few drops 10% ferric chloride solution	A green precipitate	35
5	Zinc hydrochloride reduction test	Few ml plant extract + pinch of zinc dust + conc. HCl along the side of test tube	Magenta color	37
6	Ammonia test	Filtrate + 5mL dil. Ammonia solution + conc. H2SO4	A yellow color	38
7	Con H2SO4 test	Plant extract + conc. H2SO4	A orange color	39

Test for cardiac glycosides				
1	Keller-killani test			27
Test for Tannis				
1	Gelatin test	Plant extract is dissolved in 5mL distilled water + 1% gelatin solution + 10% NaCl	A white precipitate	32
2	Braymers test	: 1mL filtrated + 3mL distilled water + 3 drops 10% Ferric chloride solution	Blue-green color	27
3	10% Noah test	0.4mL plant extract + 4mL 10% Noah + shaken well	Formation of emulsion	27
4	Lead Sub acetate test	1mL filtrate + 3 drops of lead sub acetate solution.	A creamy gelatinous precipitate	40
Test for fixed oil and fats				
1	Spot test/ Stain test	Little quantity of plant extract is pressed in between filter paper oil stain	Stain show positive result	41
2	Saponification test	Extract + few drops of 0.5N alcoholic KOH + A drop of phenolphthalein [Heat for 2 hrs.]	A transparent appearance of oil & resins	41
Tests for Saponins				
1	Foam test	0.5 gm plant extract + 2ml water shake well if foam is present after 10 minutes	if foam is present after 10 minutes	41
Test for Hae molysis				
1	Haemolysis test	Drop of fresh blood on glass slide + plant extract.	if zone of hemolysis is present	40

3. Quantitative phytochemical investigation

3.1 Thin layer chromatography (TLC):

One of the most simple, cost effective and easy chromatographic method for separating mixtures is thin layer chromatography (TLC). M. Tswett made the discovery of chromatography in 1906 by using a sheet of glass, plastic, or aluminum foil coated with a thin layer of an adsorbent substance—typically cellulose, silica gel, or aluminum oxide—thin layer chromatography is carried out (blotter paper). The stationary phase is this layer of adsorbent. A solvent or solvent combination (referred to as the mobile phase) is dragged up the plate by capillary action following the application of the sample on it. Separation is accomplished because various analytes ascend the TLC plate at varying speeds. Allowed the solvent to run around 10-15 cm on silica plate. Plates were removed and examined visually after that R_f was calculated by following formula [42].

RF = Distance traveled by solvent from origin line X 100

Distance traveled by solute from origin line

3.2 Determination of the DPPH radical scavenging test

This technique makes use of a readily available, stable free radical that is soluble in methanol (DPPH⁺, 2,2-diphenyl-1-picrylhydrazyl) and is commercially available. When DPPH is reduced by an antioxidant agent, its absorption peak at 515 nm disappears. A newly made DPPH solution (0.25g/L in methanol) containing 1.5 mL was mixed with the extract. Twenty minutes after the reaction was initiated, at 515 nm, absorbance was measured. By comparing it to the control, the percentage suppression of the DPPH radical was computed [43].

3.3 Estimation of Total flavonoid content

Due to the presence and arrangement of free OH groups, flavonoids are secondary metabolites that exhibit strong antioxidant properties [44]. Flavonoid content overall (TFC). The extracts' total flavonoid content (TFC) was determined using a modified colorimetric technique. In a 10-milliliter volumetric flask, 4 milliliters of deionized water were added to 1 milliliter aliquots of suitably diluted samples or standard solutions of quercetin (0–500 mg/L, 0.3 cc of 5% NaNO₂ was introduced to the flask at zero time. 0.3 ml of 10% AlCl₃ was added after 5 minutes. After adding 2 ml of 1 M NaOH to the mixture after 6 minutes, 2.4 ml of ddH₂O was added right away. The mixture's absorbance was measured at 510 nm in comparison to a blank. The amount of total flavonoids was represented as mg of extract-to-quercetin equivalents [45].

4. Determination of antibacterial activity of the extracts

Using the disk diffusion method, the plant extract's antimicrobial activity was ascertained. To achieve a final concentration of 10 mg/disc, the plant extract leftovers (50 mg) were again diluted in 2.5 mL of ethanol and put onto sterile filter paper discs. To achieve 10⁵ CFU mL⁻¹ of media, 10 mL of Mueller-Hilton agar medium was added to sterile Petri plates, which were then followed by 15 mL of seeded medium that had been previously infected with bacterial suspension (100 mL of medium/1 mL of 10⁷ CFU). The Mueller Hilton agar plates were covered with sterile filter paper discs that were loaded with a plant extract concentration of 10 mg/ml. A 5µg dose of gentamycin served as the positive control. The plates were refrigerated to allow the plant extracts to diffuse, and they were then incubated for twenty-four hours at 35°C. Inhibition zones' existence [46].

4.1 Determination of mic

The minimum inhibitory concentration (MIC) is the antimicrobial agent concentration that, following a 24-hour incubation period, stops microbiological growth. Using the broth dilution approach, the most potent plant extracts with substantial antibacterial activity were modified to estimate their minimum inhibitory concentration (MIC) and assess their effectiveness in containing bacterial strains that cause food-borne illnesses. In this investigation, the serial broth dilution method was utilized to determine the minimum inhibitory concentration (MIC) [47]

A two-fold serial dilution was then performed to achieve 1000, 500, 250, 125, 62.5, and 31.2 µg mL⁻¹ concentrations of stock solutions. The initial concentration of 2000 µg mL⁻¹ was prepared by putting 0.6 g of each plant extract in 300 mL of sterile nutritional broth. After adding 1 mL of standardized inoculums of every test organism to each extract nutrient broth mixture, the mixture was incubated at 37 °C. MIC is the lowest medication concentration at which no growth is apparent,

as indicated by the absence of turbidity in the tube. By using both visual inspection and an optical density (OD) measurement at 630 nm, growth was found [48].

5. Dinitro salicylic acid (DNSA)

The approach is based on detecting the presence of free carbonyl C=O groups in reducing sugars. In an alkaline media, the ketonic and aldehyde functional groups of fructose and glucose are first oxidized by 3, 5-Dinitrosalicylic acid (yellow) to 3-amino-5-nitrosalicylic acid (orange-red) [49]. Stock solutions of monosaccharides were produced in distilled water on different concentration. Mean data was used to find a peak in the visible wavelength range of 400-800 nm. A 3 mL reaction mixture was made from the extract stock solution, and the volume was equalized to 3 mL with distilled water. DNSA reagent (2 mL) was added and incubated in a water bath at 80°C to 85°C for 15 minutes. The reaction mixture was cooled, and its absorbance [50].

6. MTT assay:

Among viability assays that rely on live cells converting substrate to chromogenic product, the MTT assay remains one of the most versatile and popular. The MTT assay involves the conversion of the water-soluble yellow dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to an insoluble purple formazan by mitochondrial reductase. Formazan is subsequently solubilized and its concentration evaluated using optical density at 570 nm. The result is a sensitive assay with high linearity up to about 106 cells/well. As with the alamarBlue assay, minor changes in metabolic activity can result in substantial changes in MTT, allowing for the detection of cell stress in the absence of direct cell death. The technique has been standardized to detect adherent or nonadherent cells cultured in numerous wells. The procedure employs a typical 96-well plate. This can, however, be scaled up to accommodate a different plate format. Place 500-10,000 cells per well on a 96-well plate. The assay exhibits good linearity up to 106 cells [51].

7. Result and discussion:

Table 1: Photochemical analysis of Vanda Roxburghii

Tests for Alkaloid	Leaves	Stem
Dragendroff's test	+ve	+ve
Mayer's test	+ve	+ve
Wagner's test	-ve	-ve
Iodine Test	-ve	-ve
Boucharde's test	+ve	+ve
Tannic acid test	+ve	+ve
Terpenoids test	-ve	-ve
Test for phenolic compound		
Iodine test	+ve	+ve
Ferric chloride test	+ve	+ve
Gelatin test	+ve	+ve
Lead acetate test	+ve	+ve
Ellagic acid test	+ve	+ve
Potassium dichromate test	+ve	+ve

Test for flavonoids		
Alkaline Reagent	+ve	+ve
Lead Acetate test	-ve	-ve
Shibata's reaction/ Cyanidin test	+ve	-ve
Ferric Chloride test	+ve	+ve
Zinc hydrochloride reduction test	+ve	-ve
Ammonia test	+ve	+ve
Con H2SO4 test	+ve	+ve
Test for cardiac glycosides		
Keller-killani test	+ve	-ve
Test for Tannis		
Gelatin test	+ve	+ve
Braymers test	+ve	+ve
10% NaOH test	+ve	+ve
Lead Sub acetate test	+ve	+ve
Test for fixed oil and fats		
Spot test/ Stain test	-ve	-ve
Saponification test	-ve	-ve
Tests for Saponins		
Foam test	-ve	-ve
Test for Haemolysis		
Haemolysis test	+ve	-ve

Table 2: Photochemical analysis of *Dendrobium moniliforme*

Tests for Alkaloid	Flower	Leaves	Stem	Root
Dragendorff's test	-ve	-ve	-ve	-ve
Mayer's test	+ve	+ve	+ve	+ve
Wagner's test	-ve	-ve	-ve	-ve
Iodine Test	-ve	-ve	-ve	-ve
Boucharde's test	+ve	+ve	+ve	+ve
Tannic acid test	+ve	+ve	+ve	+ve
Terpenoids test	-ve	-ve	-ve	+ve
Test for phenolic compound				
Iodine test	+ve	+ve	+ve	+ve
Ferric chloride test	-ve	-ve	+ve	+ve
Gelatin test	+ve	+ve	+ve	+ve

Lead acetate test	+ve	-ve	-ve	+ve
Ellagic acid test	-ve	+ve	-ve	+ve
Potassium dichromate test	+ve	+ve	+ve	+ve
Test for flavonoids				
Alkaline Reagent	+ve	-ve	+ve	+ve
Lead Acetate test	-ve	+ve	+ve	+ve
Shibata's reaction/ Cyanidin test	+ve	+ve	-ve	-ve
Ferric Chloride test	-ve	+ve	-ve	-ve
Zinc hydrochloride reduction test	+ve	-ve	-ve	-ve
Ammonia test	-ve	+ve	+ve	+ve
Con H2SO4 test	+ve	-ve	+ve	-ve
Test for cardiac glycosides				
Keller-killani test	+ve	+ve	+ve	+ve
Test for Tannis				
Gelatin test	-ve	-ve	-ve	-ve
Braymers test	-ve	-ve	-ve	-ve
10% NaOH test	-ve	-ve	-ve	-ve
Lead Sub acetate test	+ve	-ve	-ve	-ve
Test for fixed oil and fats				
Spot test/ Stain test	-ve	+ve	-ve	-ve
Saponification test	-ve	-ve	-ve	-ve
Tests for Saponins				
Foam test	-ve	-ve	-ve	-ve
Test for Haemolysis				
Haemolysis test	-ve	+ve	-ve	-ve

8. Thin layer chromatography

Thin Layer Chromatography (TLC) was used to determine the chemical content of *Vanda Roxburghii*, *Dendrobium moniliforme*, and *Phalaenopsis amabilis*. The separation was carried out using a silica gel plate and a mobile phase of methanol, and when the development took place under UV light. The following results were observed for each sample:

Sample	Solvent	Rf value	Pictures
Vanda Roxburghii			
Stem	Methanol	1.22	
Leaves	Methanol	1.09	


Dendrobium moniliform			
Stem	Methanol	1	
Leave	Methnol	1.2	
Root	Methanol	1.1	
Flower	Methanol	1.1	
Phalaenopsis amabili			
Stem	Methanol	1.33	
Leave	Methanol	1.71	
Root	Methanol	1.71	
Flower	Methanol	1.33	

Table 4: Thin layer Chromatography

9. Antioxidant activity test for plant extract:

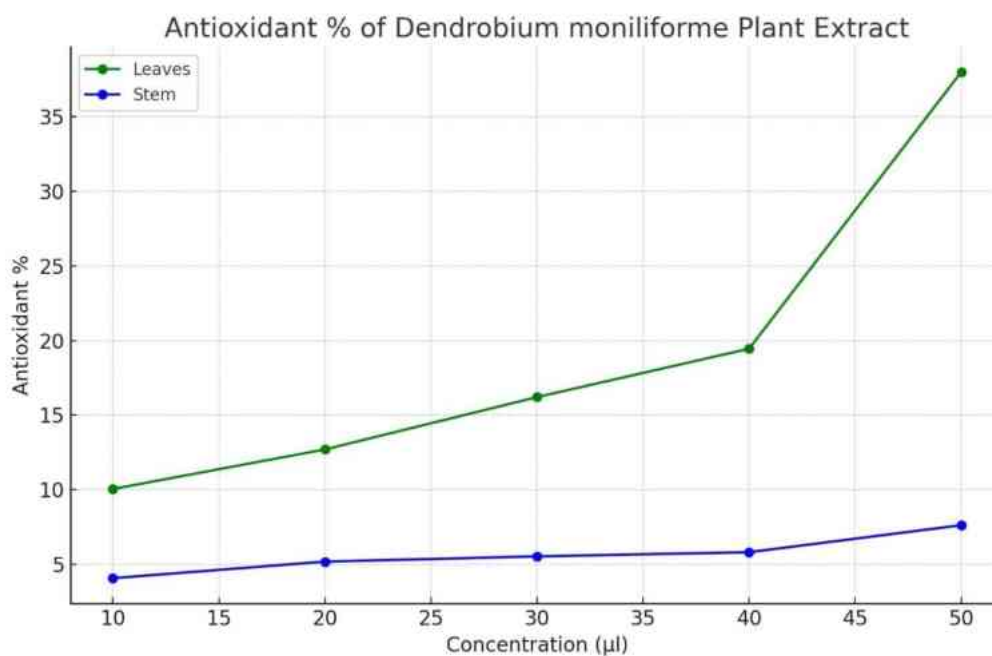
The DPPH model provides a method for evaluating antioxidant activity of a compound for plant extract in a relatively shorter time as compared to other methods. The disappearance of DPPH radical is directly proportional to the amount of antioxidant present in the reaction mixture [52]. The proton radical scavenging action is known to be one of the important mechanisms for measuring antioxidant activity. This assay determines the scavenging of stable radical species DPPH by antioxidant compounds present in plant extracts [53]. The results of DPPH radical scavenging activity for the methanolic extract of *Vanda Roxburghii*, *Dendrobium moniliform* & *Phalaenopsis amabili* are depicted as follows in table.

Test tubes	Plant extract	DPPH	30 Minutes incubation In dark	OD at 517 nm	
				Leaves	Stem
Control	0 μ l	3000		2.873	2.873-
T1	10 μ l	2990		2.584	2.756
T2	20 μ l	2980		2.508	2.724
T3	30 μ l	2970		2.407	2.714
T4	40 μ l	2960		2.314	2.706
T5	50 μ l	2950		1.781	2.654

Table 5: DPPH radical scavenging activity of vanda Roxburghii

Test tube	Conc. In μl	Antioxidant % of plant extract	
		Leaves	Stem
T1	10 μl	10.05	4.07
T2	20 μl	12.70	5.18
T3	30 μl	16.21	5.53
T4	40 μl	19.45	5.81
T5	50 μl	38.00	7.62

Table 6: Antioxidant percentage



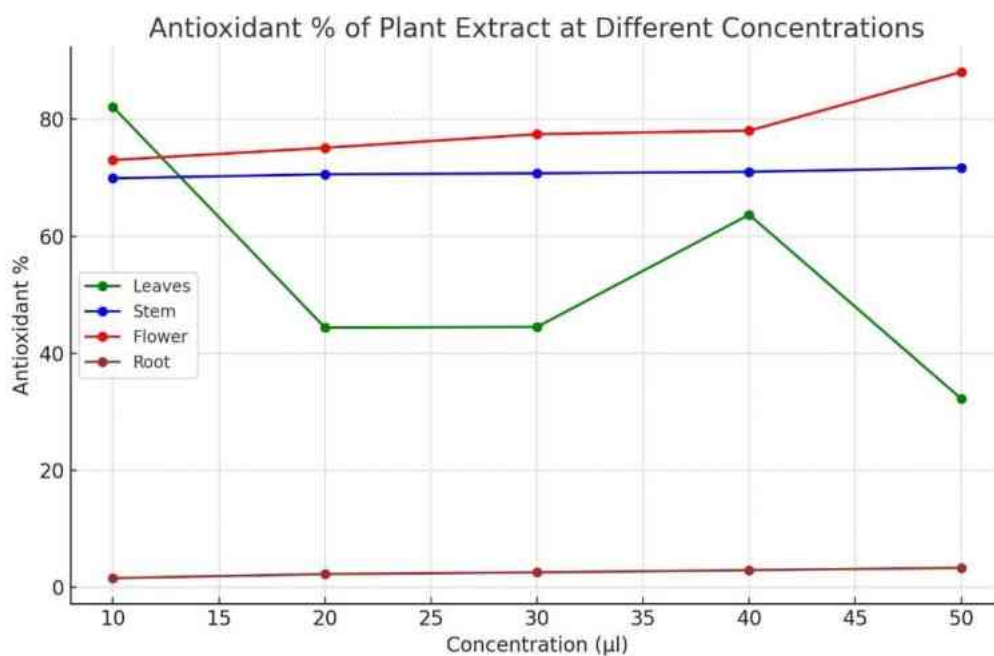
Test tubes	Plant Extract	DPPH	30 Minutes Incubation In dark	OD at 517 nm			
				Leaves	Stem	Flower	Root
Control	0 μl	3000 μl		2.829	2.829	2.829	2.829
T1	10 μl	2990 μl		0.506	0.851	0.763	2.784
T2	20 μl	2980 μl		0.461	0.832	0.704	2.764

T3	30 μ l	2970 μ l	0.460	0.827	0.638	2.756
T4	40 μ l	2960 μ l	0.301	0.820	0.621	2.745
T5	50 μ l	2950 μ l	0.241	0.798	0.338	2.734

Table 7: DPPH radical scavenging activity of *Dendrobium moniliforme*

Test tube	Conc. in μ l	Antioxidant % of plant extract			
		Leaves	Stem	Flower	Root
T1	10 μ l	82.11	69.91	73.02	1.59
T2	20 μ l	44.39	70.59	75.11	2.29
T3	30 μ l	44.51	70.76	77.44	2.58
T4	40 μ l	63.69	71.01	78.04	2.96
T5	50 μ l	32.24	71.70	88.05	3.35

Table 8: Antioxidant percentage

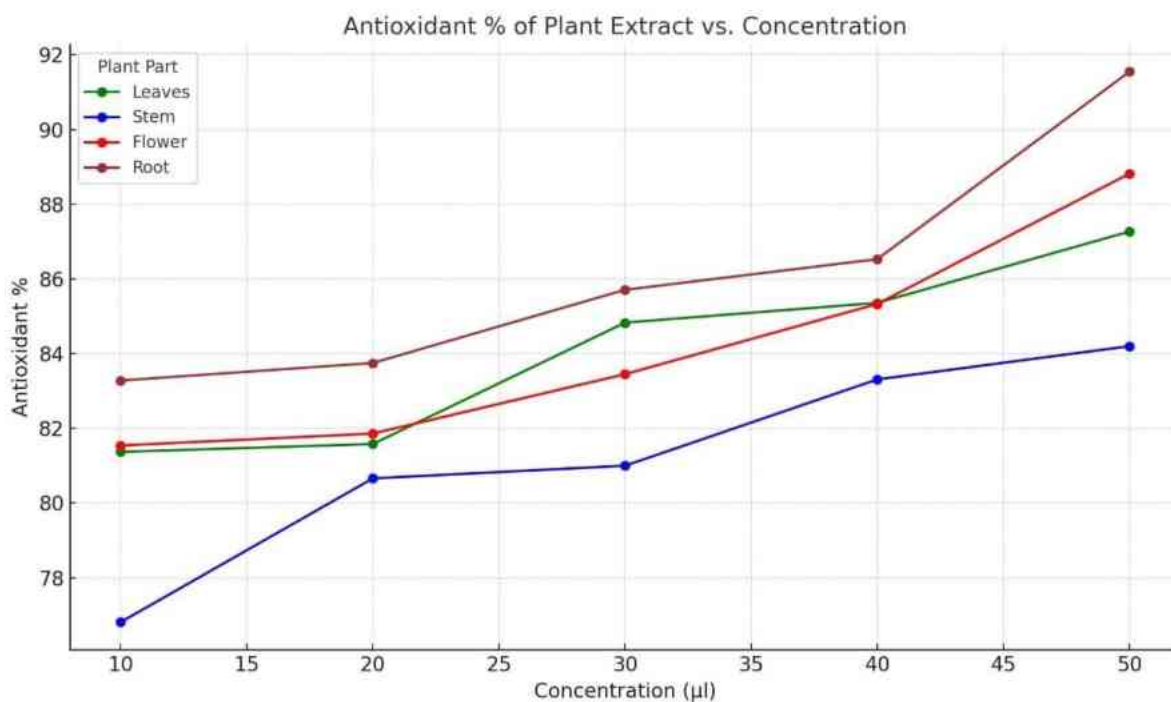


Test tubes	Plant extract	DPPH	Incubation In dark	OD at 517 nm			
				Leaves	Stem	Flower	Root
Control	0 µl	3000µl	30 Minutes	2.829	2.829	2.829	2.829
T1	10 µl	2990 µl		0.527	0.656	0.522	0.473
T2	20 µl	2980 µl		0.521	0.547	0.513	0.460
T3	30 µl	2970 µl		0.429	0.530	0.468	0.404
T4	40 µl	2960 µl		0.414	0.472	0.415	0.381
T5	50 µl	2950 µl		0.360	0.452	0.316	0.239

Table 9: DPPH radical scavenging activity of *Phalaenopsis amabilis*

Test tube	Conc. In µl	Antioxidant % plant extract			
		Leaves	Stem	Flower	Root
T1	10 µl	81.37	76.81	81.54	83.28
T2	20 µl	81.58	80.66	81.86	83.75
T3	30 µl	84.83	81	83.45	85.71
T4	40 µl	85.36	83.31	85.33	86.53
T5	50 µl	87.27	84.2	88.82	91.55

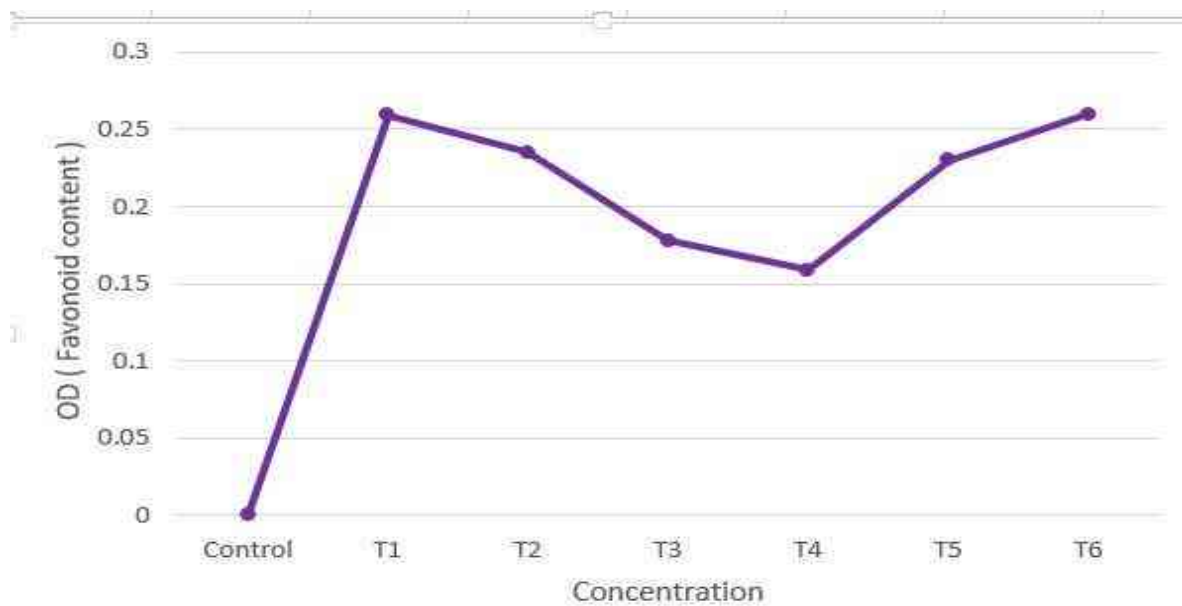
Table 10: Antioxidant percentage



Total flavonoid content:

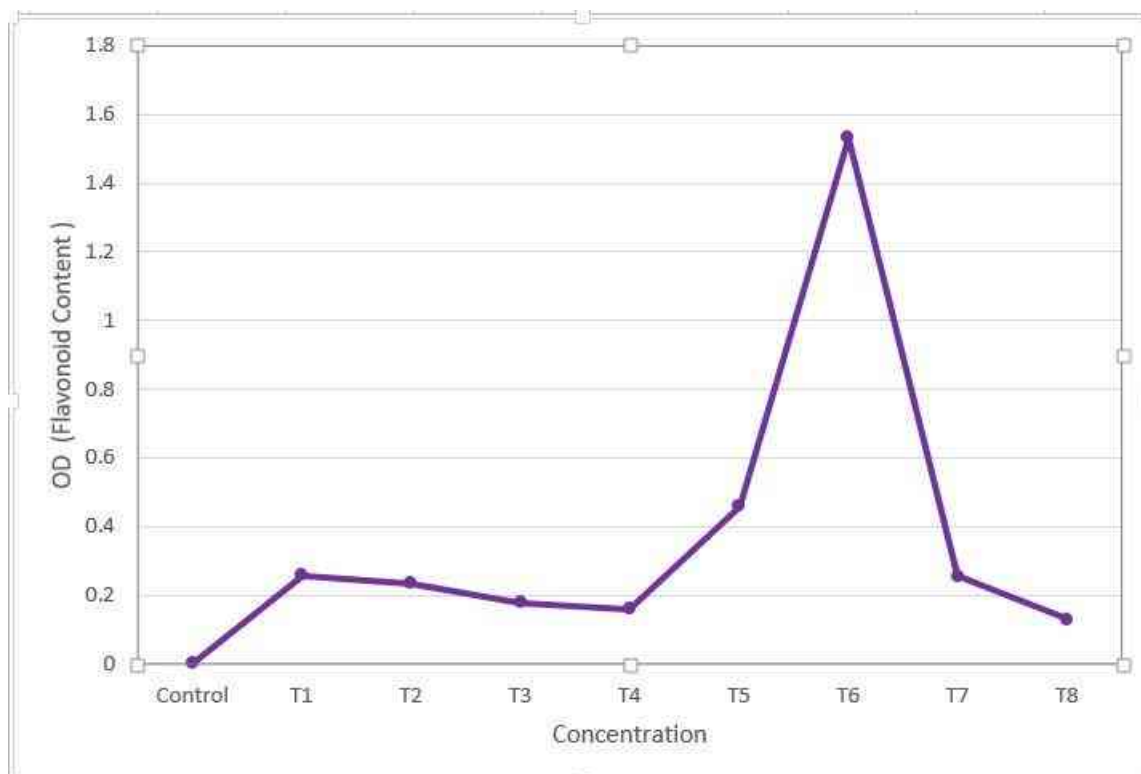
Test tube	Quercetin solution	DH2O	NaNo2	Incubate 5 minutes	NaOH	OD
Control	Sample 1ml				-	0
T1	200 µl	7.2 ml	300 µl		2 ml	0.259
T2	400 µl	7 ml	300 µl		2ml	0.235
T3	600 µl	6.8 ml	300 µl		2ml	0.178
T4	800 µl	6.6 ml	300 µl		2 ml	0.159
Plant extract						
T5	Leaves 500 µl	6.9ml	300 µl		2ml	0.230
T6	Stem 500 µl	6.9ml	300 µl		2ml	0.260

Table 11: Flavonoid content of vanda Roxburghii



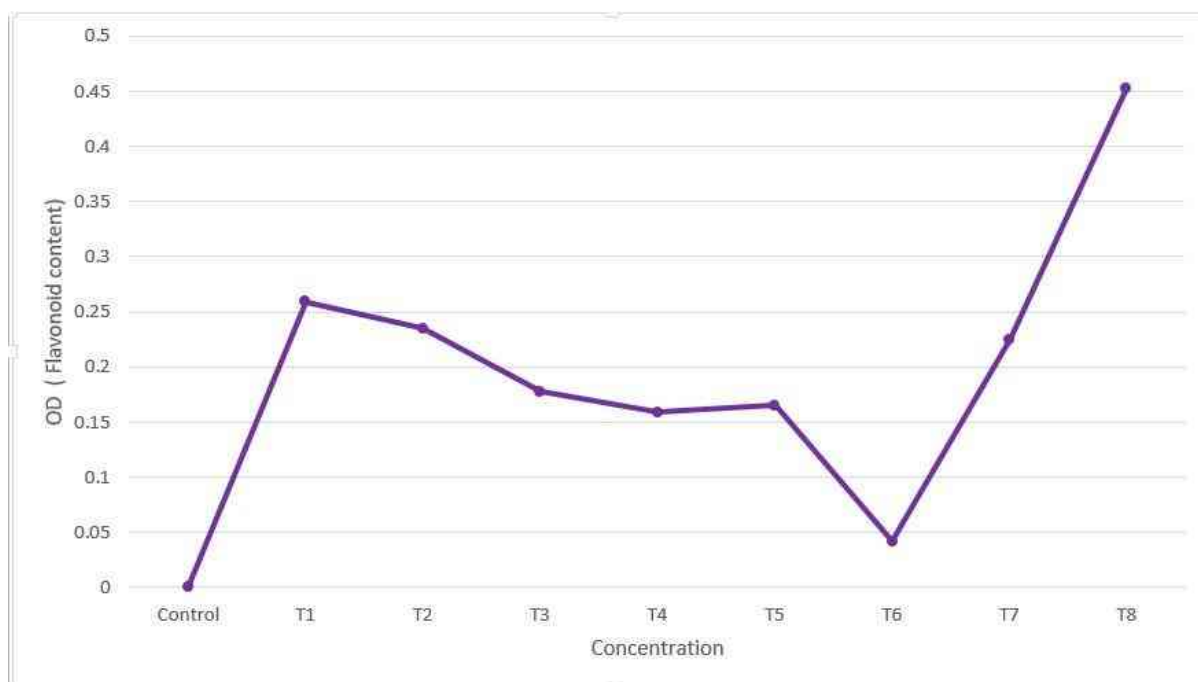
Test tube	Quercetin Solution	DH2O	NaNo2	Incubate 5 Minutes	NaOH	OD	
Control	Sample 1 ml						0
T1	200 µl	7.4 ml	300 µl			2 ml	0.259
T2	400 µl	7.2 ml	300 µl			2 ml	0.235
T3	600 µl	7 ml	300 µl			2 ml	0.178
T4	800 µl	6.8 ml	300 µl			2 ml	0.159
Plant extract							
T5	Leaves 500 µl	6.9 ml	300 µl			2 ml	0.458
T6	Stem 500 µl	6.9ml	300 µl			2 ml	1.533
T7	Root 500 µl	6.9ml	300 µl			2 ml	0.256
T8	Flower 500 µl	6.9ml	300 µl		2 ml	0.130	

Table 12: Flavonoid content of Dendrobium moniliforme



Test tube	Quercetin Solution	DH2O	NaNo2	Incubate 5 Minutes	NaOH	OD	
Control	Sample 1 ml					0	
T1	200 µl	7.4 ml	300 µl			2 ml	0.259
T2	400 µl	7.2 ml	300 µl			2 ml	0.235
T3	600 µl	7 ml	300 µl			2 ml	0.178
T4	800 µl	6.8 ml	300 µl			2 ml	0.159
Plant extract							
T5	Leaves 500 µl	6.9 ml	300 µl			2 ml	0.165
T6	Stem 500 µl	6.9ml	300 µl			2 ml	0.042
T7	Root 500 µl	6.9ml	300 µl			2 ml	0.225
T8	Flower 500 µl	6.9 ml	300 µl		2 ml	0.453	

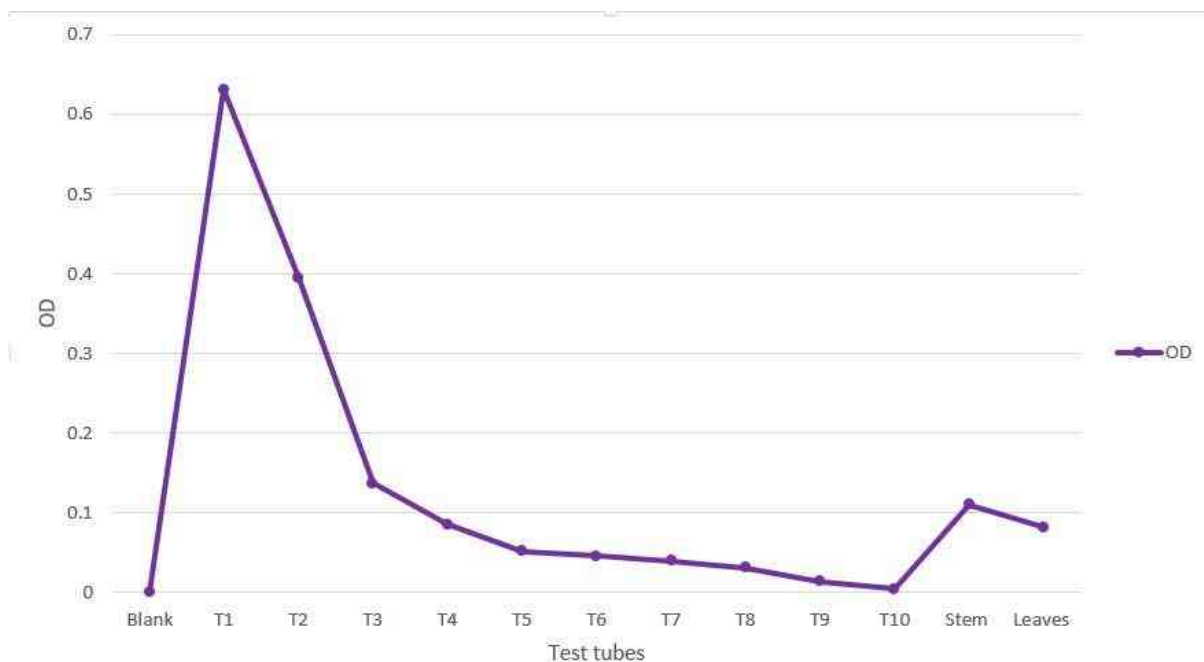
Table 13: Flavonoid content of Phalaenopsis amabilis



Dinitro salicylic acid (DNSA)

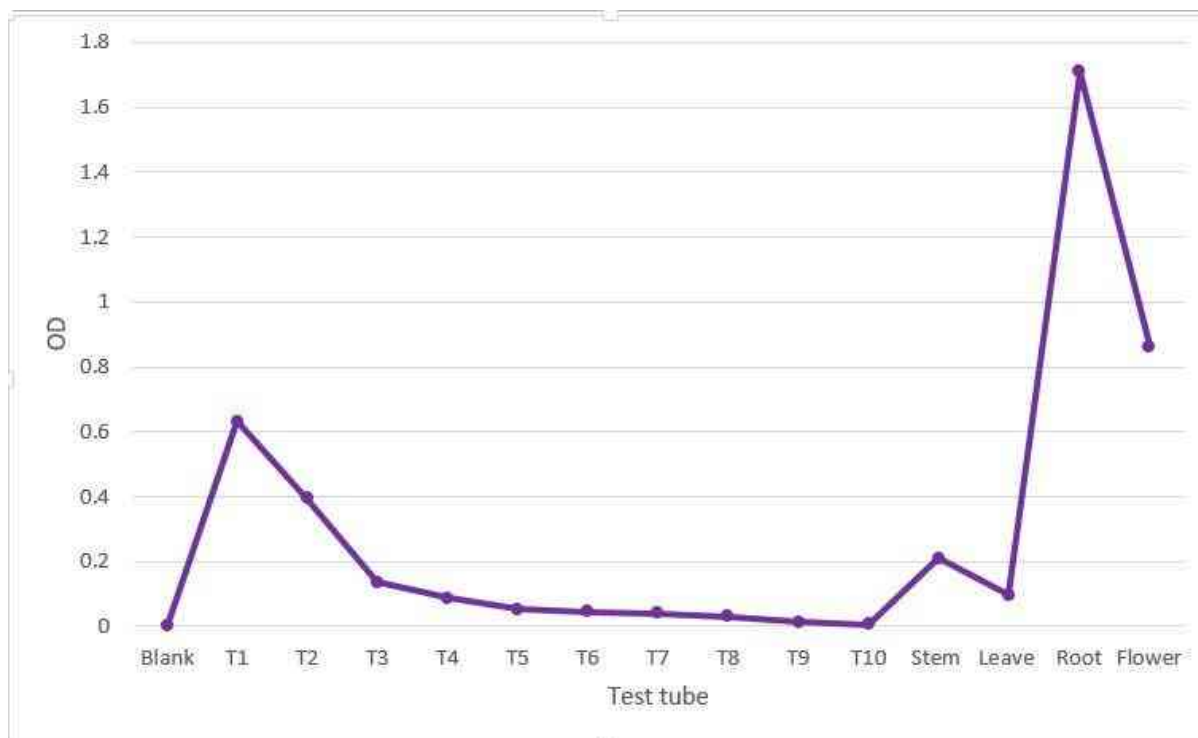
Test tube	D – Glucose	DH2O	DNSA	Water bath For 15 minutes and then let it cool at room temperature.	OD
Blank	0	3 ml	0		0
T1	0.3 ml	2.7 ml	2 ml		0.631
T2	0.6 ml	2.4 ml	2 ml		0.395
T3	0.9 ml	2.1 ml	2 ml		0.137
T4	1.2 ml	1.8 ml	2 ml		0.086
T5	1.5 ml	1.5 ml	2 ml		0.052
T6	1.8 ml	1.2 ml	2 ml		0.046
T7	2.1 ml	0.9 ml	2 ml		0.040
T8	2.4 ml	0.6 ml	2 ml		0.031
T9	2.7 ml	0.3 ml	2 ml		0.014
T10	3.0 ml	0 ml	2 ml		0.005
Plant Extract					
Stem	100 µl	2.9 ml	2 ml	0.111	
Leaves	100 µl	2.9 ml	2 ml	0.082	

Table 14: Vanda Roxburghii



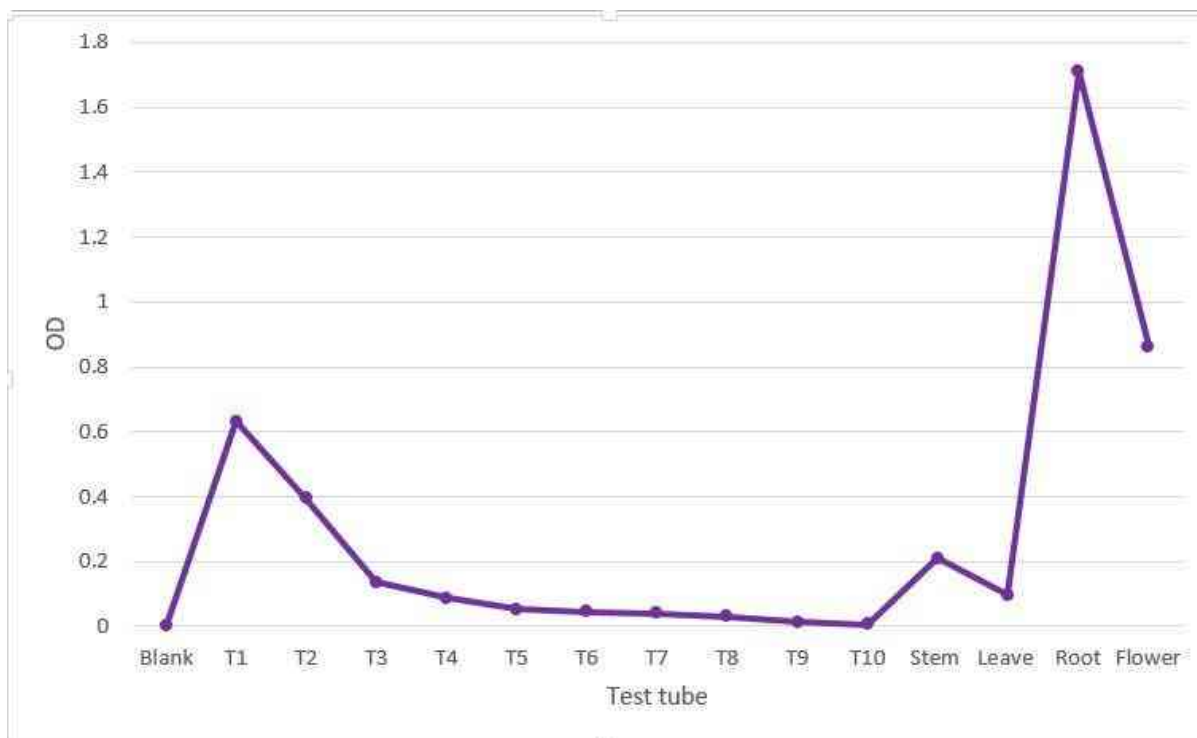
Test tube	D – Glucose	DH2O	DNSA	Water bath for 15 Minutes And then Let it cool at room temperature	OD
Blank	0	3 ml	0		0
T1	0.3 ml	2.7 ml	2 ml		0.631
T2	0.6 ml	2.4 ml	2 ml		0.395
T3	0.9 ml	2.1 ml	2 ml		0.137
T4	1.2 ml	1.8 ml	2 ml		0.086
T5	1.5 ml	1.5 ml	2 ml		0.052
T6	1.8 ml	1.2 ml	2 ml		0.046
T7	2.1 ml	0.9 ml	2 ml		0.040
T8	2.4 ml	0.6 ml	2 ml		0.031
T9	2.7 ml	0.3 ml	2 ml		0.014
T10	3.0 ml	0 ml	2 ml		0.005
Plant Extract					
Stem	100 µl	2.9 ml	2 ml		0.210
Leave	100 µl	2.9 ml	2 ml		0.096
Root	100 µl	2.9 ml	2 ml		1.710
Flower	100 µl	2.9 ml	2 ml		0.862

Table 15: Dendrobium moniliforme



Test tube	D – Glucose	DH2O	DNSA	Water bath for 15 Minutes And then Let it cool at room temperature	OD
Blank	0	3 ml	0		0
T1	0.3 ml	2.7 ml	2 ml		0.631
T2	0.6 ml	2.4 ml	2 ml		0.395
T3	0.9 ml	2.1 ml	2 ml		0.137
T4	1.2 ml	1.8 ml	2 ml		0.086
T5	1.5 ml	1.5 ml	2 ml		0.052
T6	1.8 ml	1.2 ml	2 ml		0.046
T7	2.1 ml	0.9 ml	2 ml		0.040
T8	2.4 ml	0.6 ml	2 ml		0.031
T9	2.7 ml	0.3 ml	2 ml		0.014
T10	3.0 ml	0 ml	2 ml		0.005
Plant Extract					
Stem	100 µl	2.9 ml	2 ml		0.034
Leave	100 µl	2.9 ml	2 ml		0.016
Root	100 µl	2.9 ml	2 ml		0.001
Flower	100 µl	2.9 ml	2 ml		0,024

Table 16: Phalaenopsis amabilis

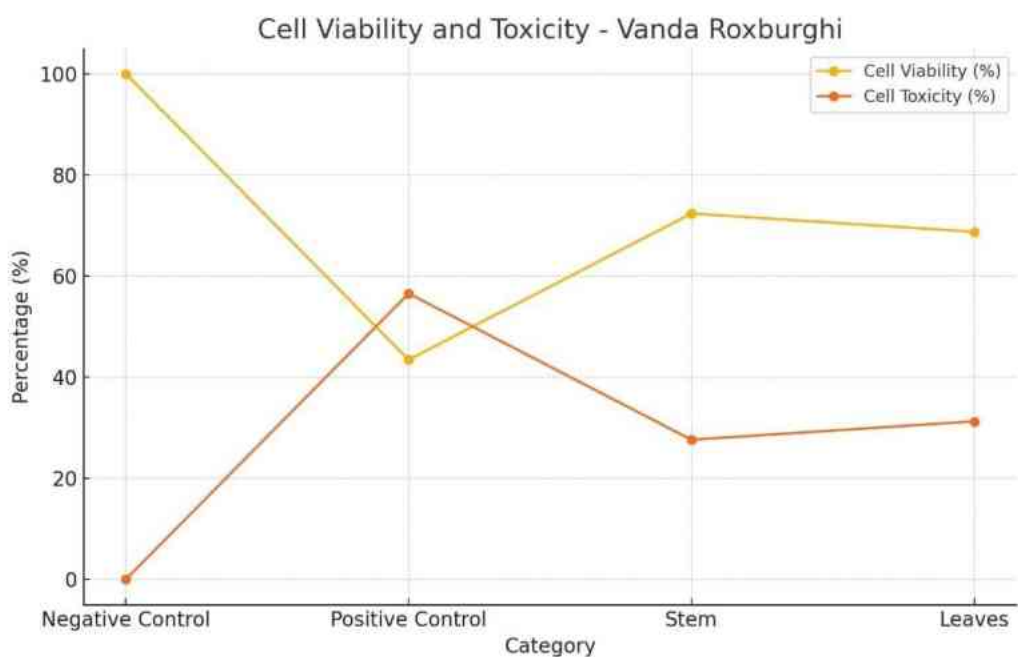


10. MTT Assay:

In our Study, we utilized HeLa cells to perform the MTT assays on different parts of the plant to assess their cytotoxicity and viability, and we are pleased to announce that the roots showed extraordinarily results. Given their powerful bioactive components, which contribute to their cytotoxic effects on HeLa cells, we feel these roots have great promise for the creation of a herbal medication. By utilizing the roots' medicinal characteristics, we want to develop a natural medicine that not only targets dangerous cells but also promotes overall health and well-being.

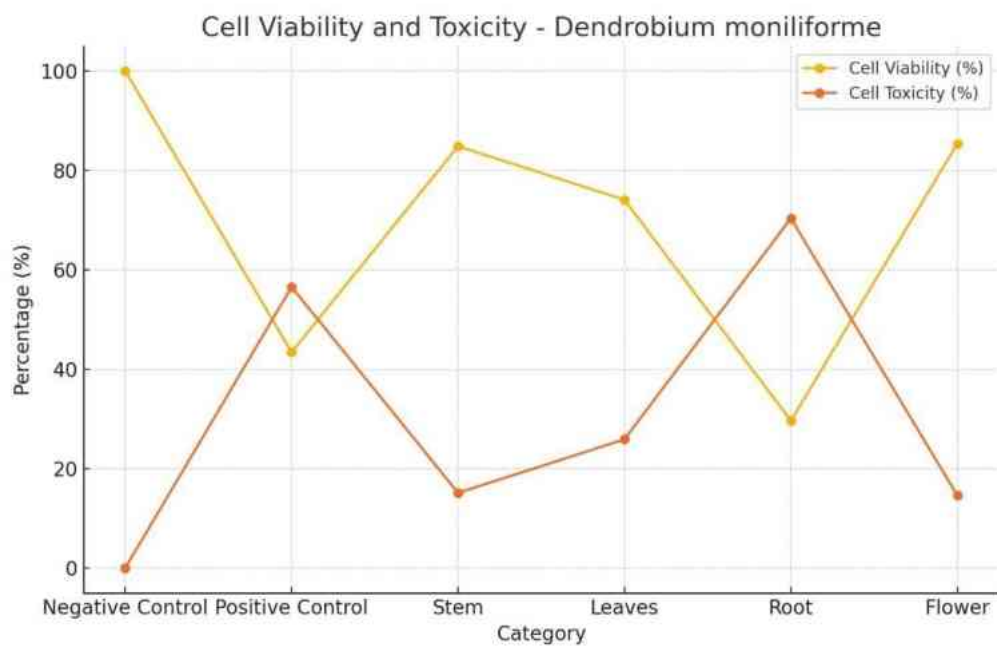
	OD	Cell viability	Cell toxicity
Negative control	0.992	100%	0%
Positive Control	0.431	43.448%	56.552%
Stem	0.718	72.379%	27.621%
Leaves	0.682	68.75%	31.25%

Table 17: Vanda Roxburghi



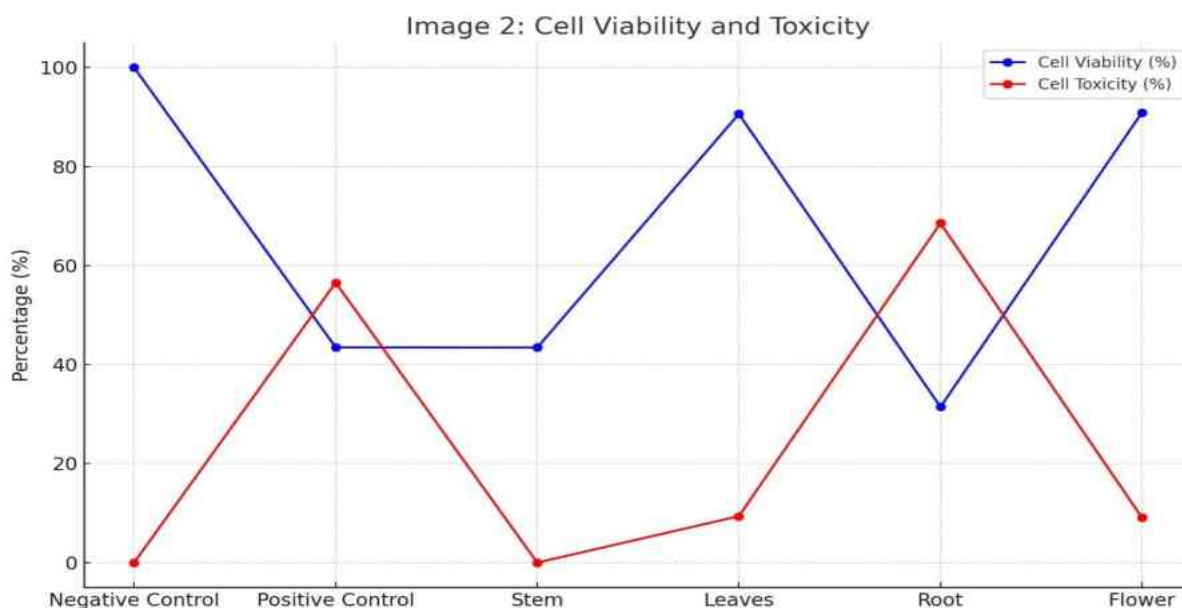
	OD	Cell viability %	Cell toxicity %
Negative control	0.992	100%	0%
Positive Control	0.431	43.448%	56.552%
Stem	0.842	84.879%	15.121%
Leaves	0.735	74.093%	25.902%
Root	0.294	29.637%	70.363%
Flower	0.874	85.383%	14.617%

Table 18: *Dendrobium moniliforme*



	OD	Cell viability %	Cell toxicity%
Negative control	0.992	100%	0%
Positive Control	0.431	43.448%	56.552%
Stem	0.596	43.448%	56.552%
Leaves	0.899	90.625%	9.375%
Root	0.312	31.452%	68.548%
Flower	0.902	90.927%	9.073%

Table19: Phalaenopsis amabilis



11. Conclusion:

In this study we have three different species of orchids that is *Vanda Roxnburghii*, *Dendrobium moniliforme* & *Phalaenopsis amabili* we found very promising results regarding their potential as a source of herbal drug formulation . Through both qualitative and quantitative phytochemical analysis, we found a rich array of bioactive compounds present in the roots of *Dendrobium moniliforme* & *Phalaenopsis amabili* . The MTT assay results further confirm the biological activity of these extracts specially in the roots of *Dendrobium moniliforme* & *Phalaenopsis amabili*. The successful MTT experiment results indicate that the extracts have cytotoxic effects on HELA cell line, implying that they could be used to treat cancer cells. This support the idea that the roots can serve as a valuable source for developing a potent herbal drug.

Overall, the formulation made from these plants' roots has strong biological activity in addition to a rich phytochemical profile. This makes the herbal medication a strong contender for additional study and advancement, which could result in successful herbal medicine therapies. In order to confirm the formulation's effectiveness and safety in human beings, future research should concentrate on improving it and carrying out clinical trials.

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