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Dpph Assay of Selected Ornamentals of Apocyanaceae

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ABSTRACT

Medicinal plants are considered as an important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the biological and phytochemical screening of plant extracts from traditional preparations used in popular medicine. Plants are beneficial and endless sources for healing various infections because of their ability to synthesize secondary metabolites like alkaloids, quinines, flavones, tannins, phenols *etc*. Various ornamental plants belonging to family Apocyanaceae are known for their pharmacological, ethno botanical and ecological benefits. The experiment performed on the leaves of these ornamental plants beginning with the quest to find the secondary phytoconstituents later, antioxidant properties of theses methanolic extracts were studied. Except for saponins all the other phytochemicals were found to be present in all the white colored flower bearing ornamentals as compared to the leaves of the plants bearing colored flowers. The methanolic extracts of leaves of varieties of *Vinca*, *Thevetia* and *Nerium* showed significant variations in their results for antioxidant potentials that probably would be due to presence or absence of saponins.

KEYWORDS: Apocyanaceae, Selected ornamental varieties, Phytochemical Screening, DPPH Assay.

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INTRODUCTION

Plants are indicators of 'Life'. They are the ultimate necessity of every living organism-"Sole Refuge"; a solution to all our day to day problems beginning from sunrise to sunset. Man uses plants in different ways according to his needs, particularly as food and medicine. Hence we have different systems of medical practices like, Ayurveda, Siddha, Unani and Homeopathy in India. Each plant produce, stores and uses many chemical constituents in it that combine with each other resulting to a stable constant growth of itself and a source of potential bio-active compounds to those organisms depending on them. Every bio-active compound present in a plant is an antidote to a particular ailment i.e., clinical effectiveness. All such information about a particular plant species is an expected natural product helpful for the preparation of cost effective herbal formulations. Plants being the richest bio-resource of drugs used in and as traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Extraction of the bioactive plant constituents has always been a challenging task for the researchers. Plant-derived compounds introduce potential sources for new antioxidant, antibiotics and anticancer agents. Herbal medicine is used widely in the world, since better cultural acceptability, better compatibility with the human body and fewer side effects. Oxidative stress depicts the existence of compounds known as free radicals and ROS (reactive oxygen species) that are formed during normal physiological condition but they become deleterious when it is not getting eliminated by the endogenous system.

All plants contain important compounds i.e., secondary metabolites which include steroids, alkaloids, tannins and phenol compounds that are synthesized and assembled in all parts or specific parts of the plant[25]. Research in finding a natural antioxidant from the plant source is therefore important as plants are potential source of immense chemicals for the treatment of number of ailment such as cancer, aging, arthritis, Parkinson syndrome, ischemia, toxin induced reactions, alcoholism, liver injury etc[15]. Oxidative stress will result in an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. Oxygen derived free radicals like superoxides anions, hydroxyl radicals, hydrogen peroxide, etc. are cytotoxic and give rise to tissue injuries. When mechanism of antioxidants protection becomes imbalanced in human body, antioxidant supplements are used to minimize oxidative damage. Natural antioxidants have potential to increase property of plasma and thus reduce risk to diseases like cancer heart diseases and stroke. Medicinal plants seem to have desired advantage as compared to synthetic antioxidants which induce carcinogenic effects when ingested [28].

Ornamental plants are grown for decorative purposes in gardens and landscape design. It is only because of their aesthetic value like flowers, leaves, scent, fruit, stem or bark features; their

purpose is for the enjoyment of gardener and the person who visits that particular place. The term ornamental plant is used in the marketing of plants used to enhance a given location for horticultural trading. Family Apocyanaceae has a group of such members which are rich in alkaloids and glycosides. They can be valuable source for control of many insect pests, plant fibers, rubber and can be also used in preparations of various medicines. Plant belonging to this family can be tree, shrub or vines with latex or watery juice. It has approximately 5000 species and classified in five subfamilies consisting of 415 genera. It includes most of the well-known tropical ornamental plants (*Oleander, Frangipani, Allamanda, Mandevilla*). The present experiment studies the antioxidant potentials of leaves of selected plant species that have ornamental as well as medicinal properties that can be enhanced and extensively studied.

MATERIALS AND METHODS

Plant Material:

Selected Ornamental species:-The leaves of selected ornamental Apocyanaceae species for the experiment were:-

Selected Genus:

(a)Scientific Name: *Vinca major* (Alba variety, Rosea variety)

(b)Scientific name:-*Thevetia neriifolia*, Juss (White variety, Yellow Variety, Orange Variety)

(c)Scientific Name: *Nerium oleader*. L. (Armin, Mrs. John Adriance, Turner's Katherine Childer's, Magaly)

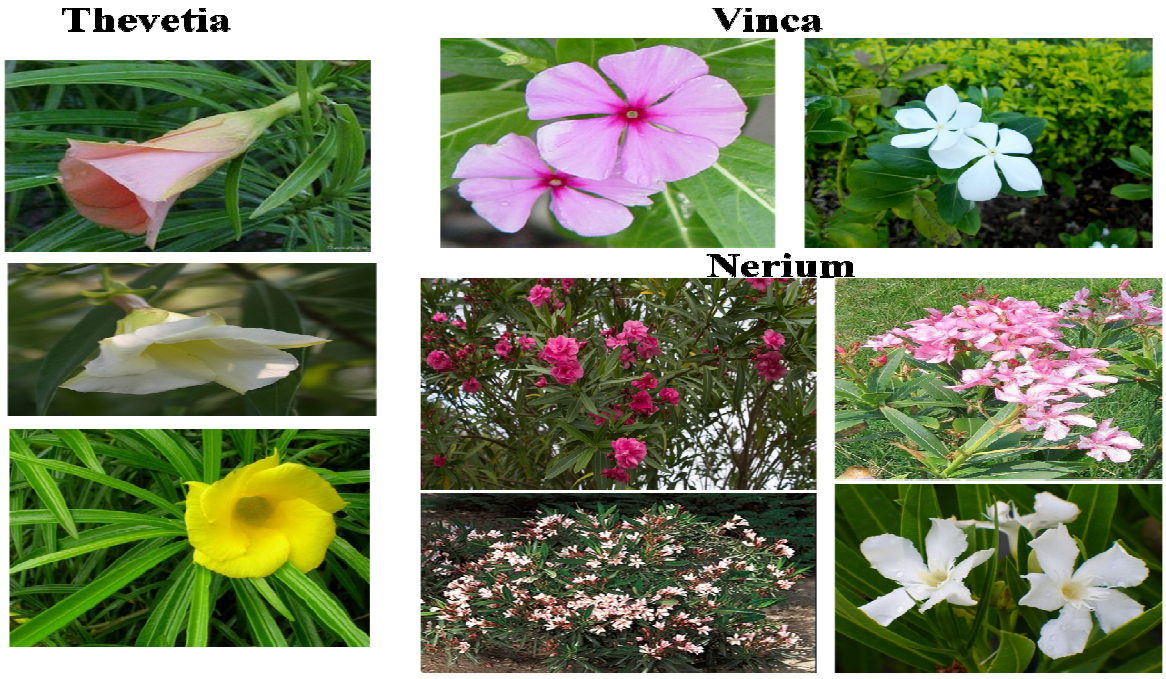

Fig. 1: Selected Apocyanaceae ornamental plants.

Methods:

Collection and Drying of selected plant part:

The leaves of the selected ornamental Apocyanaceae species were collected washed, air dried under open shade, powdered and then stored in paper bags at room temperature .These plant powder were used for extract preparation.

Extract Preparation:

The leaves were dried at room temperature for 15 days. The dried leaves were ground into fine powder. 10gm of dried leaf powder was extracted using 100ml of organic solvent (Methanol) for 24 hours and then filtered using Whatmann filter paper No. 1. The extracted solution, (filtrate) was kept overnight to obtain dry extract while the residue after filteration was discarded. The methanol extracts of leaves were stored in a cool and dry place after transferring them into vials.

Phytochemical Screening:

Preliminary Screening of prepared extracts were done according to standard procedures adopted by Harborne JB, 1998. These extracts were tested for the presence of secondary metabolites like alkaloids, flavonoids, steroids, saponins, tannins, glycosides and phenols.

[I] Alkaloids

6mg of extracts were taken separately and dissolved in 6ml of methanol. These were tested with Mayer's, Wagner's and Dragendroff's Reagent.

Mayer's Test: 1 ml extract was taken and 2ml of Mayer's reagent added into it. A yellowish white precipitate obtained which showed positive test for alkaloids. [Mayer's Reagent: 0.355gm mercuric chloride was dissolved in 60ml distil water and 5gm postassium iodide was dissolved in 20ml distil water so that the final volume came to 100ml using Distil water.]

Wagner's Test: To 1 ml extract was added 2 ml of Wagner's reagent A brown flocculent precipitate obtained which showed the test to be positive for alkaloids. [Wagner's Reagent: 1.27gm of iodine and 2gm of potassium iodide were dissolved in 5ml distilled water and solution was diluted to 100ml.]

Dragendroff's Test: 1ml of extract and 2ml Dragendroff's reagent was added the formation of orange precipitates indicated the presence of alkaloids. [The reagent can be prepared by mixing Solution A and Solution B in proportion of 1:1 (v/v). Solution A: 1.7gm bismuth nitrate and 20gm tartaric acid was dissolved in 80ml distil water; Solution B: 16gm Potassium iodide was dissolved in 40ml distil water.]

[II] Flavonoids:

Alkaline Reagent Test: Few drops of 1N Sodium hydroxide solution (NaOH) and 1 ml 1% Hydrochloric acid (HCl) was added to 1 ml extract the color change in the solution from yellow to colorless indicated the presence of flavonoids.

Zinc Hydrochloride Reduction Test: 1ml of plant extract was taken and into it 2mg Zinc (Zn) dust was added alongwith a few drops of 95% Conc. Hydrochloric acid (HCl) where the formation of red color precipitates indicated presence of flavonoids.

Ferric chloride test: 1ml of 10% Ferric chloride ($FeCl_3$) solution was taken and into it was added 1ml extract were wooly brownish precipitates indicated the presence of flavonoids.

[III] Phenols:

Ferric Chloride Test: To 2ml of plant extract was added 5 ml Distil water along with a few drops of 3% Ferric Chloride solution where, as the solution turned its color blue – green in color indicated the presence of phenols.

Lead Acetate Test: To 1ml of extract was added to 3 ml 10% Lead acetate solution the Bulky White precipitate formed showed the presence of phenols.

Potassium Dichromate Test: To 1ml extract was added Potassium dichromate solution precipitation was seen showing the presence of tannins and phenolic compounds.

Alkaline Reagent Test – To 1ml extract was added 1N Sodium hydroxide (NaOH) solution the formation of yellowish red precipitates showed the presence of phenols.

[IV] Tannins:

Ferric chloride test- To 1ml extract was added 10 ml distil water the solution was boiled for 5 to 10 minutes at 100^{o}C. As the solution cooled down into it was added 0.1% Ferric chloride solution and the formation of blue - black coloration showed the presence of Tannins.

Lead acetate Test – To 1 ml extract was added 1 ml Lead acetate solution the formation of white precipitates showed the presence of Tannins.

Potassium dichromate test –To 1ml extract was added1ml Potassium dichromate solution formation of precipitates indicated the presence of tannins and phenolics.

[V] Saponins:

Frothing Test –To 1ml extract was added 5 ml distil water and shaken vigourously till froth formed remains persistant then into it was added 3 drops olive oil and then shaken till the solution formed an emulsion.

[VI] Steroids:

Libermann - Buchard Test – To 1ml of extract was added few drops Acetic anhydride. The solution was boiled at 100^{o}C and then allowed to cool down. Into it was added few drops of 94% Conc. Sulphuric acid along the sides of test tube the formation of Brown ring indicated the presence of steroids.

To 1ml extract was added 2 ml Acetic anhydride solution alongwith was added 2 ml 94% Conc. Sulphuric acid the change in color from violet to blue or green coloration showed the presence of Steriods.

Libermann - sterol Test – To 1ml extract was added 1 ml glacial acetic acid along with a few drop of 94% Conc. Sulphuric acid. The coloration of the solution will keep changing from red, violet, blue to green.

[VII] Cardiac Glycosides:

Keller - Killiani Test – To 1ml extract was added 2ml distil water along with 0.5ml Glacial acetic acid and a few drops of 1% ferric chloride solution. Into this solution was added with few drops of 94% Conc. Sulphuric acid along the wall of the test tube the formation of brown ring-cardenolides, Violet ring below brown ring or green ring below brown ring formation indicated the presence of cardiac glycosides.

DPPH ASSAY

This is a popular and common method used to check the presence of free radical scavenging activity of a given plant material. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a dark crystalline powder having stable free radical molecules. The application of DPPH is important for Antioxidant Assay. The procedure involves the use of L-Ascorbic acid was used as standard (**Fig.2**). The antioxidant activity is given terms of L-Ascorbic acid equivalent (mg/ml extracted compound). The DPPH solution (0.004% w/v) was prepared in 95% Methanol.

Procedure: The dried extracts were brought into dilution of (1:1). The experiment was done using two replicates and all estimates were repeated thrice. The average values were calculated and based on it the standard deviation and standard errors were also calculated The concentration series for extracts was 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml such that the final volume in each test tube is 1ml. Add into it 2ml each DPPH solution. Allow the DPPH molecules in the solution to react with the phytochemicals in the plant extract, after keeping the test tubes to incubate in a dark chamber for 30-40 minutes. After the period of incubation the purple color of DPPH

solution will change to colorless if the free radical of DPPH gets scavenged by the compounds present in the plant extracts. The standard graph for Ascorbic acid is given in **Figure 2**.The capability to scavenge the DPPH free radical was calculated using the following equation: -

% Radical Scavenging Activity = (**Abs** control – **Abs** sample) × 100%

Abs control

where,

Abs control = Absorbance of DPPH solution

Abs sample = Absorbance of plant extracts

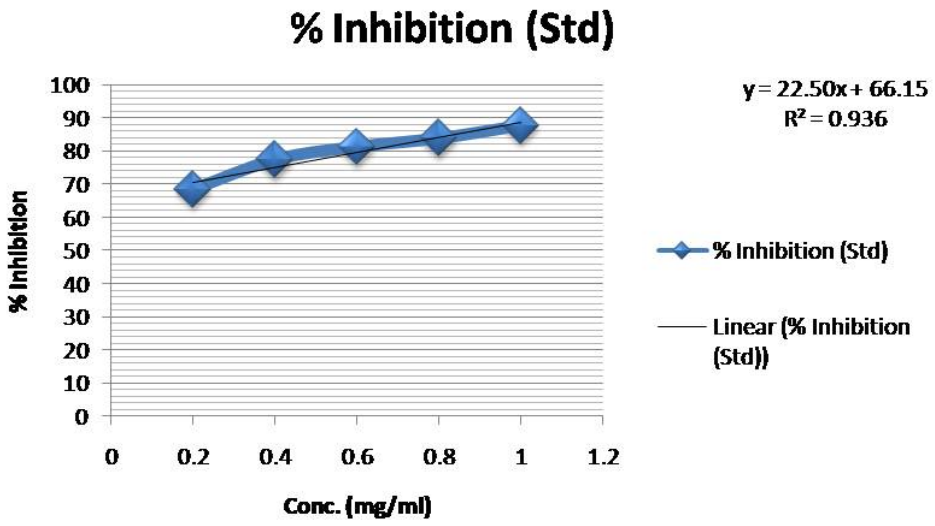

Fig. 2: Standard graph for L- Ascorbic acid (Standard).

RESULT and DISCUSSION

Phytochemical Screening

The screening of the methanol extracts of leaves of two varieties of Genus *Vinca* namely *Vinca alba* and *Vinca rosea*. The phytochemical screening has been performed to detect the secondary metabolites in both the extracts. Alkaloids tannins, flavonoids, phenols, steroids, glycosides are present in both the extract. Saponins were found to be present in *V. alba* while absent in *V. rosea*. Such variation has not yet been reported in any of the reviewed literature done till date **(Table 1).** The presence of alkaloids, phenols, tannins, steroids and saponins detected in the extracts of *Vinca* were also found in the work done by Punia S *et al.*, 2014; Kabesh *et al.*, 2015, Malar Retna A and Ethalsha, 2013.

The phytochemical screening of *Thevetia* varieties for the secondary metabolites showed the presence of flavonoids, phenols, cardiac glycosides, tannins, alkaloids and steroids while the white variety leaves extracts showed the presence of saponins **(Table 2)**. This supports the work done by Nesy and Lizzy, 2014; Ayoola GA *et al.*, 2008; Rahman Nazneen *et al.*, 2014; Mahmood *et al.*, 2014; K. Buvaneswari *et al.*, 2011 where the extracts showed the presence of alkaloids, saponins, flavonoids, glycosides, tannins, anthraquinones, coumarins, oils-fats and terpenoids and phenolic compounds; while the presence of saponins differ from the experimental result.

The phytochemical screening were performed in four varieties of *Nerium* leaf extracts which are Mrs. John Adriance (Dwarf flowers), Armin (Double flowers), Turner's Katherine Childers (White flowers) and Magaly(Light pink Flowers). Secondary metabolites like alkaloids, Flavonoids, Phenols, Tannins, Saponins, Steroids, Glycosides were tested in the leaf extracts. Six of the secondary metabolites i.e., alkaloids, flavonoids, phenols, tannins, steroids and cardiac glycosides were found to be present in all the methanol leaf extracts; while saponins were only present in the Turner's Katherine Childers (White flowers). Such an observation regarding the presence of saponins in white variety leaves has not yet been reported in scientific reviews done in prior to the experiment; while the presence of most of the secondary metabolites in Genus Nerium have already been reported (**Table 3**).

According to the work done by Suganya RS *et al.*, 2012 and Nag Sudipa *et al.*, 2013 *Nerium* extracts showed the presence of alkaloids, flavonoids, terpenoids, cardiac glycosides, tannins, saponins, reducing sugars, starch, amino acids and lignin. In addition to the work done by Suganya RS *et al.*, 2012 and Nag Sudipa *et al.*, 2013 experiments showed that the extracts of *Nerium* leaf also showed the presence of reducing sugars, starch, amino acids and lignin while results of Nag Sudipa *et al.*, 2013 differ with the experimental results with failing to show the presence of saponins, phenols, cardiac glycosides and steroids.

It can be said based on the phytochemical screening results the presence of saponins in leaves of white flower bearing plants has an indication that these plants are more toxic as compared to that of the colored variety leaves as saponins are toxic phytoconstituents. Saponins are glycosides with a sugar moiety along with triterpenes or steroids aglycone. They can be classified into three categories i.e., triterpenes, steroids and steroid alklaoids. Hence, in the white varieties have glycosides, saponins and steroids hence the level of toxicity is more than other varieties for the three genus considered for the study.

Table 1:- Phytochemical Screening of Genus *Vinca* rosea and *Vinca alba* leaf methanol extracts. (where P: Present and A: Absent).

Sr. No.	Secondary Metabolites	Test	Results	
			V. rosea	***V. alba***
1	Alkaloids	Mayer's Test	P	P
		Dragendroff's Test	P	P
		Wagner's Test	P	P
2	Flavonoids	Alkaline Reagent Test	P	P
		Zinc Hydrochloride Reduction Test	P	P
		Ferric chloride test	P	P
3	Phenols	Ferric Chloride Test	P	P
		Lead Acetate Test	P	P
		Potassium Dichromate Test	P	P
4	Tannins	Ferric chloride Test	P	P
		Lead acetate Test	P	P
		Potassium Dichromate Test	P	P
5	Saponins	Frothing Test	A	P
6	Steroids	Libermann - Buchard Test	P	P
		Acetic anhydride Test	P	P
		Libermann - sterol Test	P	P
7	Glycosides	Keller - Killiani Test	P	P

Table 2:- Phytochemical Screening of *Thevetia nerifolia* varieties (orange, white and yellow) methanol leaf extracts. (where P: Present and A: Absent).

Sr. No.	Phytochemical	Test	Results		
			Orange	White	Yellow
1	Alkaloids	Mayer's Test	P	P	P
		Dragendroff Test	P	P	P
		Wagner's Test	P	P	P
2	Flavonoids	Alkaline Reagent Test	P	P	P
		Zinc Hydrochloride Reduction Test	P	P	P
		Ferric chloride test	P	P	P
3	Phenols	Ferric Chloride Test	P	P	P
		Lead Acetate Test	P	P	P
		Potassium Dichromate Test	P	P	P
4	Tannins	Ferric chloride Test	P	P	P
		Lead acetate Test	P	P	P
		Potassium Dichromate Test	P	P	P
5	Saponins	Frothing Test	A	P	A
6	Steroids	Libermann - Buchard Test	P	P	P
		Acetic anhydride Test	P	P	P
		Libermann - sterol Test	P	P	P
7	Glycosides	Keller - Killiani Test	P	P	P

Table 3:- Phytochemical Screening of *Nerium indicum* varieties leaves methanol extracts. (where P: Present and A: Absent).

Sr. No.	Phytochemical	Test	Results			
			Mrs. John Adriance	Armin	Turner's Katherine Childer's	Magaly
1	Alkaloids	Mayer's Test	P	P	P	P
		Dragendroff's Test	P	P	P	P
		Wagner's Test	P	P	P	P
2	Flavonoids	Alkaline Reagent Test	P	P	P	P
		Zinc Hydrochloride Reduction Test	P	P	P	P
		Ferric chloride test	P	P	P	P
3	Phenols	Ferric Chloride Test	P	P	P	P
		Lead Acetate Test	P	P	P	P
		Potassium Dichromate Test	P	P	P	P
4	Tannins	Ferric chloride Test	P	P	P	P
		Lead acetate Test	P	P	P	P
		Potassium Dichromate Test	P	P	P	P
5	Saponins	Frothing Test	A	A	P	A
6	Steroids	Libermann - Buchard Test	P	P	P	P
		Acetic anhydride Test	P	P	P	P
		Libermann - sterol Test	P	P	P	P
7	Glycosides	Keller - Killiani Test	P	P	P	P

DPPH ASSAY

The results of DPPH Assay done for the *Vinca* varieties leaf extract (*V. alba* and *V. rosea*) the DPPH Assay showed that the *V. rosea* variety showed more antioxidant potentials than that of *V. alba*. The *V. alba* variety showed the following activity at 0.2, 0.4, 0.6, 0.8 and 1mg/ml with % Inhibition 53.2± 0.1, 55.43±1.04, 56.97±0.27, 59.97±0.68 and 69.77±0.2 respectively. The *V. rosea*

variety showed the following activity at 0.2, 0.4, 0.6, 0.8 and 1mg/ml with % Inhibition 52.9±0.15, 53.37±0.03, 54.03±0.13, 54.97±0.34 and 67.1±0.44 respectively **(Fig. 3)**.

The free radical scavenging activity using DPPH Assay for *V. rosea* variety show much better antioxidant potentials than the *V. alba* variety. The concentration series for the experiment showed the following results for leaves extracts of *V. alba* and *V. rosea* variety i.e., for 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml had % Inhibition were found as 53.2±0.1, 55.43±1.04, 56.97±0.27, 59.97±0.68 and 69.77±0.2% for *V. rosea*; 52.9±0.15, 53.37±0.03, 54.03±0.13, 54.97±0.34 and 67.1±0.168 % for *V. alba* **(Fig. 3)**. Henceforth it can be concluded that the *V. rosea* variety has better free radical scavenging potentials than *V. alba* variety leaves methanol extracts. Such results were obtained by Sharma V and Sain M, 2013, Dixit P *et al.*, 2013, Nejat N *et al.*, 2015, Punia S *et al.*, 2014 and Gajalakshmi S *et al.*, 2013.

According to Dixit P *et al.*, 2013 the antioxidant activity of *V. rosea* showed highest activity i.e., 97. 44% at conc. of 800µg/ml which was more than that of the standard considered (L- Ascorbic acid) i.e., 94%. But both the varieties *V. rosea* and *V. alba* have found to be showing the % inhibition as 75% and 60% respectively at a concentration of 200µg/ml according to Bhutkar MA *et al.*, 2011. The results do not match with that of the experimental results as at concentration 1mg/ml the % inhibition is 69.77±0.2% and 67.1% respectively. While the work done by Abdul-Lateef Molar and Mahdy Abdulkhaliq Saleh *et al.*, 2014 the DPPH activity of *Vinca* extracts showed 44.6% inhibitory activity at the maximum concentration considered which almost near to the experimental result. According to the research work done by Yadao N *et al.*, 2015 the DPPH assay for leaf extracts of *Vinca rosea* showed the IC_{50} value as 77.41µg/ml, while the experimental results do not match with the same variety showed the inhibitory concentration to be 0.119mg/ml. The DPPH assay for Methanol leaf extracts of *V. rosea* leaves showed an IC_{50} value of 358.411µg/ml as per work done by Wahyu Widowati *et al.*, 2010 while for the present experiment the results obtained were 0.119mg/ml for *V. rosea* leaf and for *V. alba* leaf the IC_{50} value came to be 0.168mg/ml which means for the scavengimg of 50% of free radicals of DPPH 0.119mg/ml of *V. rosea* leaf extract and 0.168mg/ml of *V. alba*. While according to Singh Sukhdev *et al.*, 2014 the leaf extracts show higher antioxidant potentials showing an IC_{50} value of 170µg/ml, while the activity was not specified.

The free radical scavenging activity using DPPH Assay was experimentally studied for the three varieties leaves of *Thevetia nerifolia* (orange variety) show much better free radical scavenging activity than *Thevetia nerifolia* (white variety) and *Thevetia nerifolia* (yellow variety). The concentration series for the experiment showed the following results for leaves extracts *Thevetia nerifolia* varieties i.e., for 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml had % Inhibition were found as 30.06±0.12, 31.44±0.06, 34.78±0.75, 34.49±0.2, 35.11±0.03% (orange variety);

28.87±0.1, 29.83±0.22, 30.6±0.22, 31.18±0.11, 31.76±0.22% (white variety) and 30.87±0.07, 31.83±0.22, 32.27±0.09, 32.7±0.12, 33.5% (yellow variety) **(Fig. 4)**.

The inhibitory activity of *Thevetia nerifolia* (orange variety) begins with 30.06±0.12% at 0.2mg/ml with the highest activity shown at the concentration 1mg/ml got to be 35.11±0.03%; white variety the inhibitory activity begins with 28.81±0.1% at 0.2mg/ml with the highest activity shown at the concentration 1mg/ml as 31.76±0.22%. With the changing concentration from 0.2mg/ml to 1mg/ml there was an increase of almost 2% free radical scavenging potentiality in its leaf extracts and for yellow variety the inhibitory activity begins with 30.87±0.07% at 0.2mg/ml with the highest activity shown at the concentration 1mg/ml got to be 33.5% **(Fig. 4)**.

The IC_{50} values of the three varieties where they showed or could show possible free radical scavenging activity came to be 6.086mg/ml for White variety, 6.39mg/ml for yellow variety and 3.158mg/ml for orange variety. Hence it can be observed that the 50% inhibitory concentration for *Thevetia* leaf extracts of orange variety was much better than the rest of the two varieties; whereas the extract of *Thevetia* white variety require almost 6mg/ml of extract in order to show 50% inhibition **(Fig. 4)**.

In reference to the work done by Dixit Anupam *et al.*, 2015 roots of *Thevetia peruviana* (Pers.) K. Schm. showed good activity as compared to its leaves with an IC_{50} value of 170.18±0.77 and 151.02±0.24µg/ml. According to Ayoola GA *et al.*, 2008 the leaves of *Thevetia nerifolia* whose extracts were prepared using solvent methanol still the extracts do not show free radical scavenging activity on screening with DPPH. In the work done by G. Savitha and S. Balamurugan 2015 the methanol extracts of leaves of *Thevetia nerifolia* for series 125, 250, 500 and 1000µg/ml with percentage inhibition of 32.34±0.042, 42.51±0.023, 52.41±0.046 and 62.17±0.043 respectively and the IC_{50} value came to be 620 µg/ml. These results support the experimental results while the specification not given as to which cultivar was used for their study. The DPPH assay done by Nesy EA and Lizzy Mathew, 2014 the crude methanol extracts of leaves showed the an IC50 value 0.46mg/ml, wherein a vast variation was observed with the IC_{50} values beginning from 3.158mg/ml, 6.086mg/ml and 6.39mg/ml **(Fig. 4)**.

The free radical scavenging activity using DPPH Assay was performed for the four varieties of *Nerium* (Armin, Turner's Katherine Childer's, Magaly and Mrs. John Adriance) variety Armin showed much better free radical scavenging activity than the leaf extracts of the remaining three varieties. The concentration series for the experiment showed the following results for leaves extracts of all the varieties i.e., for 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1mg/ml had % Inhibition were found as 49.22±0.15, 53.88±0.17, 54.84±0.23, 57.89±0.35, 58.18±0.31% (variety-Armin); 47±0.05, 47.51±0.11, 47.99±0.14, 48.38±0.26, 48.64±0.05% (variety-Turner's Katherine Childer's)

and 48.06±0.01, 48.48±0.17, 49.15±0.2, 49.57±0.14, 50.21±0.14% (variety-Magaly) and 46.45±0.26, 47.16±0.21, 47.29±0.05, 48.32±0.14, 49.25±0.28% (variety-Mrs. John Adriance) **(Fig. 5)**.

The present study results reveal that the antioxidant potentials of the variety-Armin of *Nerium* showed highest scavenging activity as compared to the other varieties selected for the study. The observations of these results show that *Nerium* leaves show antioxidant activity as the reviewed research work done by Moteriya Pooja *et al*., 2015 and Patel *et al.*, 2011. The results of Sudha and Vinayagam 2011, showed that the *Nerium indicum* leaves methanol extracts had a % Inhibition of 72.8% which does not match with the above results i.e., 58.18±0.31, 48.64±0.05, 50.21±0.14 and 49.25±0.28% at highest concentration considered for the experiment wherein none of the mentioned varieties had similar scavenging potentials.

The IC_{50} values for the selected varieties were 0.162, 1.614, 0.939 and 1.284mg/ml **(Fig. 5)**. While according to Germi *et al*., 2013 the IC_{50} value for *Nerium oleander* leaf methanol extract came to be 0.27mg/ml even though the stock solution was prepared with the same dilution (1mg/ml) still it does not match with the experimental evaluation. A vast difference was observed when the results were compared to that of work done by Dey Priyankar *et al.*, 2012 where the % Inhibition and IC_{50} values were 33.14% and 217.15±18.39µg/ml respectively. According to Zibbu Garima and Batra Amla, 2011 the DPPH assay for *Nerium oleander* came to be 48.94±0.14µ/ml.

From the results of the DPPH assay it can be observed that the white varieties showed comparatively lower activity as compared to the colored varieties leaves extracts. Hence the presence of saponins in white varieties lowered the DPPH free radical scavenging activity. Phenols being present in the plants of the entire three genus varieties indicated the presence of the antioxidant potential. Phenolic compounds are known to have the ability to scavenge the free radical molecules present around these compounds.

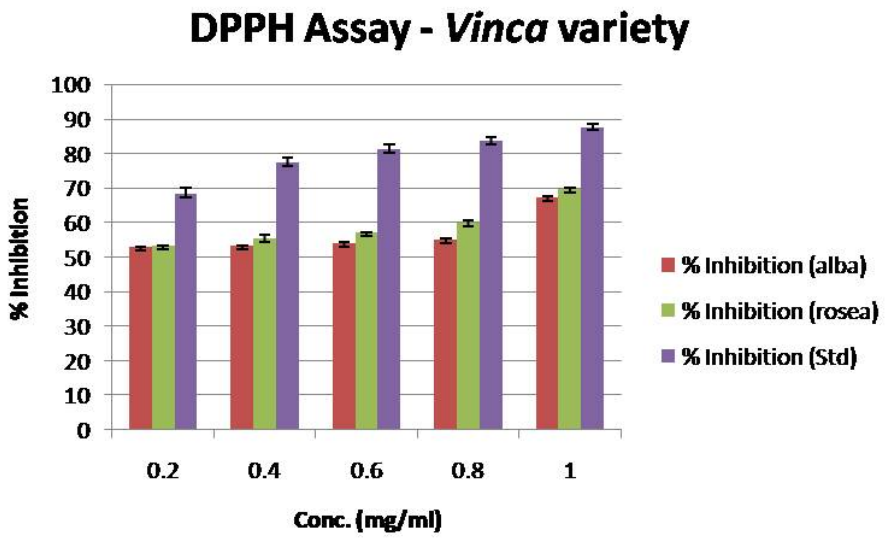

Fig. 3: DPPH Assay of Genus: *Vinca* varieties leaves compared with Standard (Ascorbic acid) (n=3).

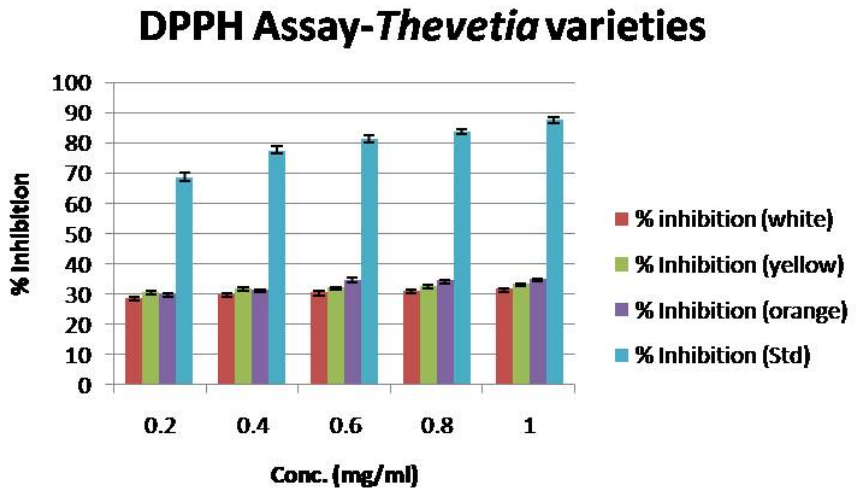

Fig. 4: DPPH Assay of Genus: *Thevetia* three varieties leaves compared with Standard (Ascorbic acid) (n=3).

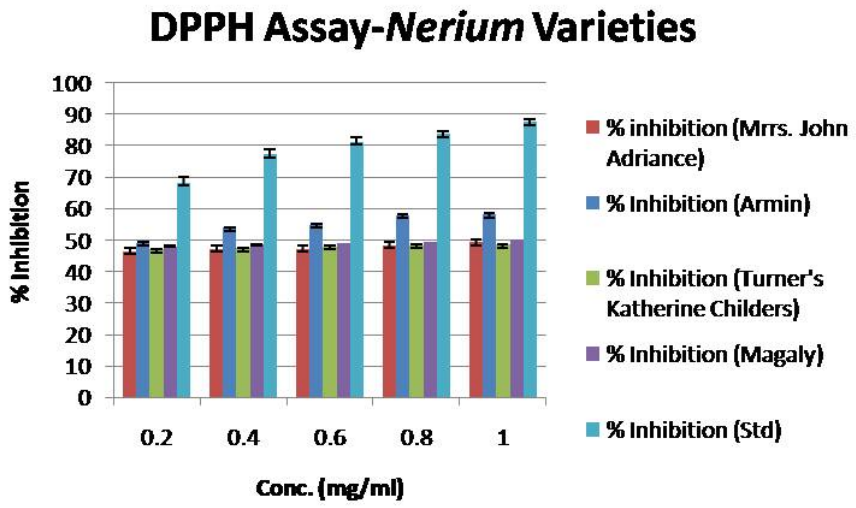

Fig. 5: DPPH Assay of leaves of four Varieties of Genus: *Nerium* compared with Standard (Ascorbic Acid) (n=3).

CONCLUSION

The screening and the DPPH assay shows that there can be variations in the phytochemicals (secondary metabolites) presence as well as the antioxidant potentials in the plants within the same family same genus but different variety. This provides us with some links for future studies as to which variety could provide us with which metabolites in addition to it the antioxidant activity to scavenge free radicals with changing plant variety belonging to a specific genus is an eye opener to the upcoming Scientists to work upon.

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