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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical and Antioxidant activity of Indian Medicinal Plant *Vicoa indica* LINN.

S. Gopi Krishnan*, M. Thanga Kokila[1], Parthiban S[2], Boopathi T[2], Inigo.P[3]
*[1]Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, Tamilnadu
[2]Department of Pharmacology, Karpagam College of Pharmacy, Coimbatore
[3]Department of Pharmaceutics, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi
*Corresponding author email: medgopi111@gmail.com

ABSTRACT
The current study is focused on investigating the antioxidant effect of Vicoa indica. The whole plant was shade dried powdered and extracted serially using solvents of increasing polarity such as chloroform and ethyl acetate. The antioxidant potential of obtained extracts was studied using DPPH˙ Radical Scavenging Activity, Ferric Reducing Antioxidant Power Assay and Phosphomolybdenum Assay. The extracts are then subjected to phytochemical screening which showed the presence of Carbohydrate, Protein, Amino acid, Alkaloids, Sterols, Saponins, Tannins and Phenolic compounds in ethyl acetate extract of the plant. Protein, Amino acid, Alkaloids, Sterols in chloroform extract of plant. Since the results are promising this work forms a firm base for the further researches to explore the antioxidant characteristic of the selected plant to discover a potent herbal drug for cancer.
Keywords: *Vicoa indica, DPPH Radical Scavenging Activity, Ferric Reducing Antioxidant Power Assay and Phosphomolybdenum Assay.*

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INTRODUCTION

An antioxidant is the substrate that prevents the oxidation of molecules inside a cell. It is a well-known chemical process that allows the removal of electrons or hydrogen from a substance. Free radicals are produced during the biological oxidation reaction. Because the radicals are reactive, they start the chain reaction simultaneously. This can lead to the damage or even the death of a cell. In biological system, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals, can damage the DNA and lead to the oxidation of lipid and proteins in cells (1,2,3). When there is an imbalance between the ROS and endogenous antioxidants, exogenous antioxidants taken with food can be an effective factor in the fight against free radicals. Excellent alternative sources of natural antioxidants are plant products, such as fruits, vegetables and herbs (4,5,6,7). Medicinal herbs and spices have been valued since ancient times for their medicinal effects, as well as their flavor and aroma. The benefits arising from, among other things, the presence of antioxidants also make them widely used today, not only in medicine but also in cooking (8). The biological activity of medicinal herbs and spices is influenced by the phenolic compounds, vitamins and trace elements present in them, as well as the volatile constituents of essential oils (9). Moreover, to further assess the antioxidant capacities of extracts from natural products, especially those frequently consumed by people, different evaluation assays have been developed, e.g., Trolox equivalence antioxidant capacity (TEAC) assay, ferric ion reducing antioxidant power (FRAP) assay, oxygen radical absorbance capacity (ORAC) assay, inhibiting the oxidation of low-density lipoprotein (LDL) assay, cellular antioxidant activity assay, etc. These assays have been used for ranking the antioxidant plants and recommending best antioxidant foods for consumption.

MATERIAL AND METHODS

Materials

The whole plant materials of *Vicoa indica* was collected from young matured plant from the village belt around Thenmalai ,Rajapalayam and identified and authenticated by Dr.M.Syed Ali Fathima, Assistant Professor & Head, Department of Botany, Assistant Professor & Head ,Department of Botany, Sadakathullah Appa College (Autonomous),Tirunelveli–627011. The whole plant of *Vicoa indica* was collected in bulk,

wash to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The powder was passed through sieve no: 40 and used for further studies. The freshly collected whole plant were chopped into pieces and shade under dried at room temperature (32-35°C) to constant weight for 5 days. The plant material was extracted with Pet ether, Chloroform and Ethyl Acetate by cold Maceration method. Leaves, stems and roots of *V. Indica* were shade dried and powdered. These coarse powders were then subjected to successive extractions by various solvents of gradual increasing polarities. The collected extracts were then taken up for further investigations.

Extraction

Extraction is the first step to separating the desired natural products from the raw materials. Extraction methods include solvent extraction, distillation method, pressing, and sublimation according to the extraction principle.

Methods

In maceration (for fluid extract), whole or coarsely powdered plant- drug is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved. This method is best suitable for use in case of the thermo labile. Air dried aerial parts of *Vicoa indica D C,* the whole plant was extracted with chloroform by cold percolation method. The solvents were removed under vacuum and the green semisolid extract was obtained.

PHYTOCHEMICAL STUDIES

Qualitative Phyto-chemical analysis [10, 11, 12]

The extract was tested for the presence of bioactive compounds by using following standard methods

TEST FOR PROTEINS

Millon's test

Crude extract when mixed with 2ml of Millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

TEST FOR CARBOHYDRATES

Fehling's test

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's test

Crude extract when mixed with 2ml of Benedict's reagent and boiled, are d dish brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test

Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Iodine test

Crude extract was mixed with 2ml of iodine solution. A dark blue or purple coloration indicated the presence of the carbohydrate.

TEST FOR PHENOLS AND TANNINS

Crude extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

TEST FOR FLAVONOIDS:

Shinoda test

Crude extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet colour appeared after few minutes which of flavonoids.

Alkaline reagent test

Crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

TEST FOR SAPONINS

Crude extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

TEST FOR GLYCOSIDES

Liebermann's test

Crude extract was mixed with each of 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added. A colour change from violet to blue to green indicated the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Salkowski's test

Crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H2SO4 was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring, i.e. ,glycone portion of the glycoside.

Keller-kilani test

Crude extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of 2% solution of FeCl3. The mixture was then poured into another test tube containing 2ml of concentrated H2SO4. A brown ring at the interphase indicated the presence of cardiac glycosides.

TEST FOR STEROID

Crude extract was mixed with 2ml of chloroform and concentrated H2SO4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2ml of chloroform. Then 2ml of each of concentrated H2SO4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

TEST FOR TERPENOIDS

Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H2SO4 was added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

TEST FOR ALKALOIDS

Crude extract was mixed with 2ml of 1% Hcl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

ANTIOXIDANT ASSAY

DPPH RADICAL SCAVENGING ACTIVITY [13, 14]**:**

The radical scavenging capacity of the sample was measured based on DPPH (1, 1- diphenyl 2-picrylhydrazyl) radical scavenging activity. One mL of 0.1mM DPPH solution in methanol was mixed with 1 mL of various concentrations (50-300 µg/mL) of samples. The mixture was then allowed to stand for 30 min incubation in dark. One mL methanol mixed with 1 mL DPPH solution was used as the control. The decrease in absorbance was measured at 517 nm.

FERRIC REDUCING ANTIOXIDANT POWER ASSAY[15]

Ferric reducing antioxidant power assay method that uses antioxidants as reductants in a redox-linked colorimetric reaction. Different concentrations of the extracts (50-300µg/mL) were mixed with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1mL of 1% potassium ferricyanide. The mixture was incubated at 50° C for 20 minutes. 1 mL of 10% trichloroacetic acid was added to the mixture. Then 1 mL of 0.1% of freshly prepared ferric chloride was added and the absorbance of the resultant solution was measured at 700 nm.

% = [(Abs (sample) – Abs (control)/Abs (sample)] x 100

PHOSPHOMOLYBDENUM ASSAY[16]

The antioxidant activity was evaluated by reduction assay method which is based on the formation of green phosphomolybdenum complex. 50-300µg/mL various concentrations of extracts were combined with 1 mL of phosphomolybdenum reagent solution. The tubes were capped and incubated in a water bath at 95°C for 30 minutes. The samples were cooled to room temperature and the absorbance of the mixture was measured at 695 nm against blank.

% = [(Abs (sample) – Abs (control)/Abs (sample)] x 100

RESULTS AND DISCUSSION

Phytochemical screening of various extracts shows the presence of Secondary metabolities like Alkaloids, Steroids, Carbohydrates, Proteins, Amino acids, Flavanoids, Tannins, Phenolic compounds, Saponins and others are shown in the Table 1. The various extract of *Vicoa indica* were used to evaluate the *In-Vitro* Anti oxidant activity showed the effect of in different concentration 50 µg/ml 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml,300 µg/ml by Various methods like DPPH˙ Radical Scavenging Activity, Ferric Reducing Antioxidant Power Assay, Phosphomolybdenum Assay. When compared with the three methods Phosphomolybdenum Assay shows maximum percentage of reduction up to 85 and 86% based on the concentration of the plant extract both in chloroform and ethyl acetate.

Table 1. Preliminary Phytochemical screening of various extracts (Chloroform, Ethyl acetate) of powdered plant of *Vicoa indica*

Sl. No	Chemical Constituents	Ethyl Acetate	Chloroform
1.	Carbohydrate	+	-
2.	Protein	+	+
3.	Aminoacid	+	+
4.	Alkaloids	+	+
5.	Sterols	+	+
6.	Glycosides	-	-
7.	Saponins	+	-
8.	Tannins & phenolic compound	+	-
9.	Flavonoids	-	-

Table 2. DPPH Radical Scavenging Activity - Ethyl acetate extract

S. No	Conc. (µg/m L)	Absorbance @ 517nm	% of Inhibition
1.	**Control**	0.242	**-**
2.	**50**	0.225	**7.02**
3.	**100**	0.208	**14.04**
4.	**150**	0.196	**19**
.5	**200**	0.175	**27.68**
6.	**250**	0.172	**28.92**
7.	**300**	0.139	**42.56**

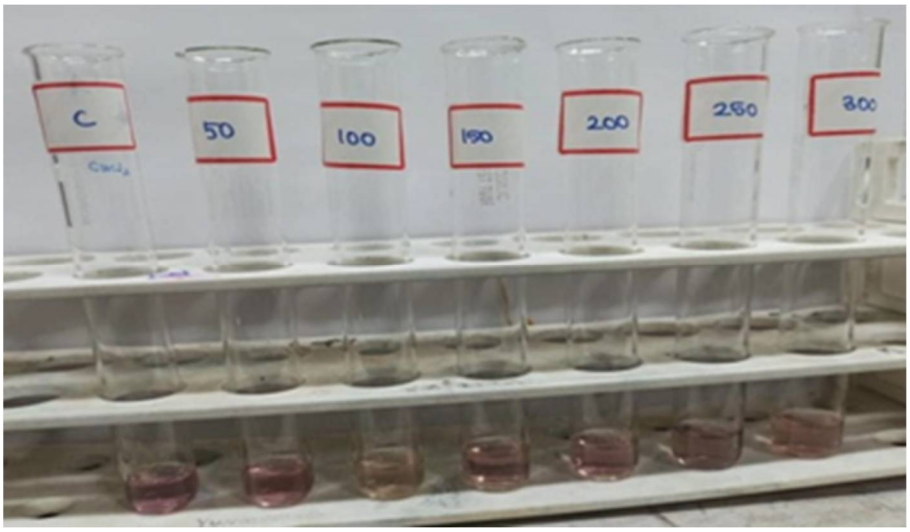

Figure 1. DPPH Radical Scavenging Activity of Ethyl acetate extract of *Vicoa indica*

Table 3. DPPH Radical Scavenging Activity – Chloroform Extract

S. No	Conc. (µg/m L)	Absorbance @ 517nm	%of Inhibition
1.	**Control**	0.254	**-**
2.	**50**	0.216	**14.96**
3.	**100**	0.196	**29.59**
4.	**150**	0.183	**27.95**
.5	**200**	0.152	**40.15**
6.	**250**	0.141	**44.48**
7.	**300**	0.123	**51.57**

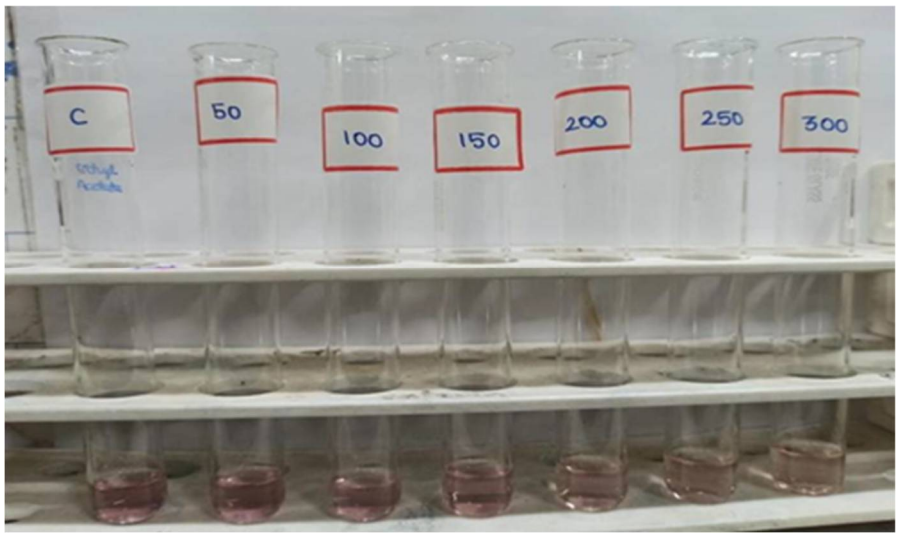

Figure 2. DPPH Radical Scavenging Activity of Chloroform extract of *Vicoa indica*

Table 4. Ferric Reducing Antioxidant Power Assay - Ethyl acetate extract

S. No	Conc. (µg/m L)	Absorbance @ 700nm	% of Reduction
1.	**Control**	0.455	**-**
2.	**50**	0.489	**6.95**
3.	**100**	0.501	**9.18**
4.	**150**	0.520	**12.5**
.5	**200**	0.563	**19.18**
6.	**250**	0.600	**24.66**
7.	**300**	0.621	**26.73**

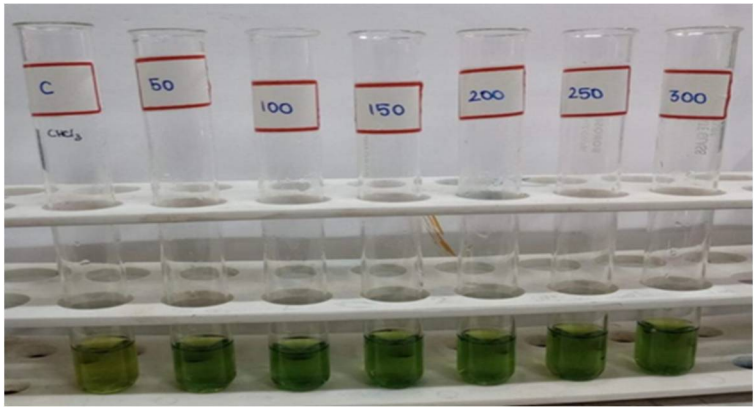

Figure 3. Ferric Reducing Antioxidant power assay Activity of Ethyl acetate extract of *Vicoa indica*

Table 5. Ferric Reducing Antioxidant Power Assay - Chloroform extract

S. No	Conc. (µg/m L)	Absorbance @ 700nm	% of Reduction
1.	**Control**	0.470	**-**
2.	**50**	0.486	**3.29**
3.	**100**	0.493	**4.66**
4.	**150**	0.508	**7.48**
.5	**200**	0.537	**12.47**
6.	**250**	0.589	**20.20**
7.	**300**	0.614	**23.45**

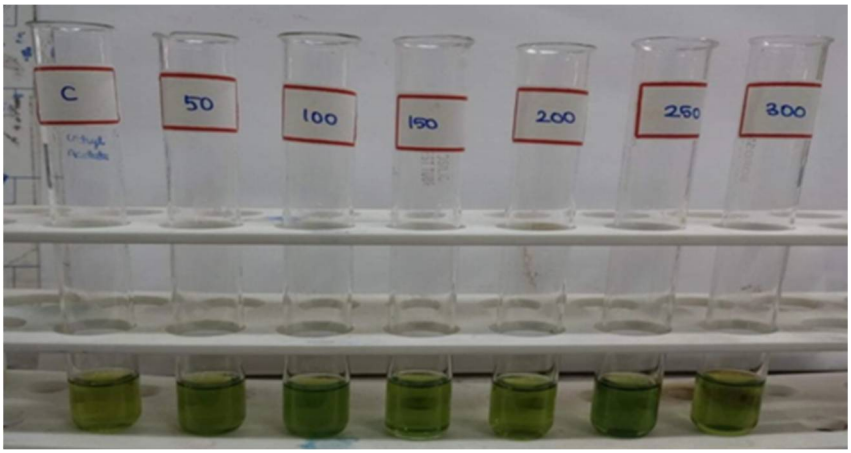

Figure 4. Ferric Reducing Antioxidant power assay Activity of Chloroform extract of *Vicoa indica*

Table 6. Phosphomolybdenum Assay - Ethyl acetate extract

S. No	Conc. (µg/m L)	Absorbance @695nm	% of Reduction
1.	**Control**	0.039	**-**
2.	**50**	0.058	**32.75**
3.	**100**	0.091	**57.14**
4.	**150**	0.181	**78.45**
.5	**200**	0.200	**80.5**
6.	**250**	0.241	**83.81**
7.	**300**	0.260	**85**

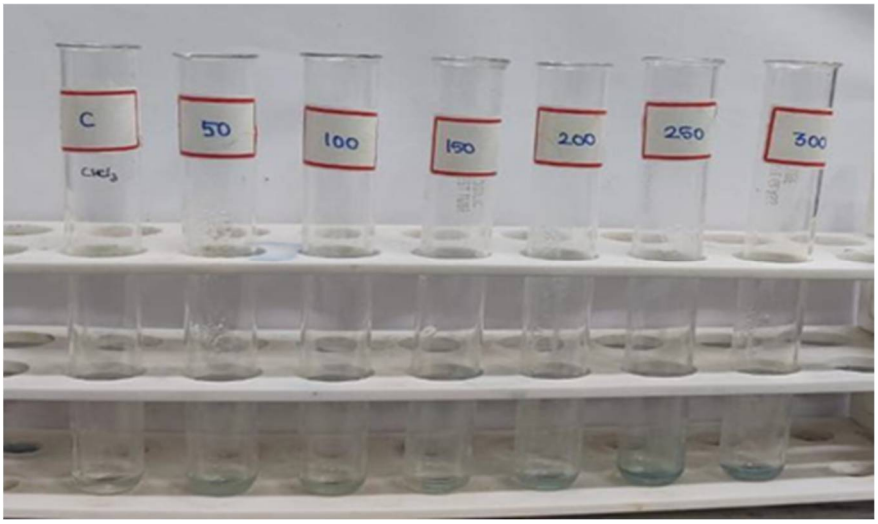

Figure 5. Phosphomolybdenum assay Activity of Ethyl acetate extract of *Vicoa indica*

Table 7. Phosphomolybdenum Assay – Chloroform extract

S. No	Conc. (µg/m L)	Absorbance @695nm	% of Reduction
1.	**Control**	0.039	**-**
2.	**50**	0.058	**32.75**
3.	**100**	0.091	**57.14**
4.	**150**	0.181	**78.45**
.5	**200**	0.200	**80.5**
6.	**250**	0.241	**83.81**
7.	**300**	0.260	**85**

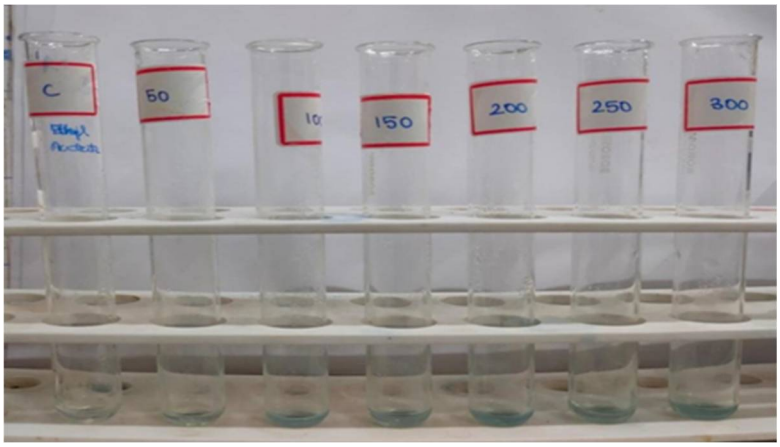

Figure 6. Phosphomolybdenum Assay Activity of Chloroform extract of *Vicoa indica*

CONCLUSION

Antioxidant studies of *Vicoa indica* indicates its remarkable role in DPPH Radical Scavenging Activity, Ferric Reducing Antioxidant Power Assay, Phosphomolybdenum Assay in Both the chloroform and ethyl acetate extract due to the presence of secondary metabolites. The present study has provided a platform for further researches to probe on plant *Vicoa indica* to bring out its further investigations.

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