PATNA WOMEN'S COLLEGE 1940

Botany

Explore–Journal of Research for UG and PG Students
ISSN 2278 – 0297 **(Print)**
ISSN 2278 – 6414 **(Online)**

http://www.patnawomenscollege.in/journal

Analysis of phytocompounds and study of antioxidant and antifungal properties of *Vitis vinifera* leaves

• **Mallick Tahreem Eqbal** • **Nidhi** • **Preeti**
• **Urvashi Sinha**

Received : November 2014
Accepted : March 2015
Corresponding Author : Urvashi Sinha

Abstract : *The present study deals with the qualitative analysis of phytochemical, antioxidant and antifungal activities of aqueous leaf extract of Vitis vinifera. All the active phytocompounds flavonoids, phenols, alkaloids, terpenoids, tannins, proteins amd soluble sugar tested were present in the aqueous extract. The antioxidant property was evaluated using hydrogen peroxide scavenging assay and the percentage inhibition was calculated as 16.439. The antifungal property of the extract was found to be effective only against the pathogenic fungal strain, Aspergillus niger and was ineffective against the non pathogenic fungal strain, Mucor. 14 mm zone of inhibition was obserbed against Aspergillus niger.*

Keywords: *Vitis vinifera, phytochemical screening, antioxidant activity, antifungal assay.*

Mallick Tahreem Eqbal
B.Sc. III year, Botany (Hons.), Session: 2012-2015,
Patna Women's College, Patna University, Patna,
Bihar, India

Nidhi
B.Sc. III year, Botany (Hons.), Session: 2012-2015,
Patna Women's College, Patna University, Patna,
Bihar, India

Preeti
B.Sc. III year, Botany (Hons.), Session: 2012-2015,
Patna Women's College, Patna University, Patna,
Bihar, India

Urvashi Sinha
Assistant Professor, Deptt. of Botany,
Patna Women's College, Bailey Road,
Patna–800 001, Bihar, India
E-mail : urvashi_vrm@yahoo.co.in

Introduction :

Grape leaves are of the plant, *Vitis*, a genus of over sixty species of vining plants in the flowering plant family Vitaceae. *Vitis vinifera* (common grape vine) is a species of *Vitis*, native to the Mediterranean region, Central Europe and South-Western Asia, from Morocco and Portugal in the north to southern Germany and in the east to northern Iran (Davis 1997). Grape leaves boost intake of vitamins and provide a particularly rich source of fat-soluble vitamins A and K. Grape leaves also provide us with calcium and iron, two essential minerals. Other uses for grape leaves include the treatment of diarrhoea, heavy menstrual bleeding, uterine haemorrhage, canker

sores and excess vaginal discharge, sore breasts, rheumatism, headache and fever. Grape contains phenolic compounds, including resveratrol, flavon-3-ols, caffeic acid, ellagic acid and quercetin. Phenolic compounds have anticancer properties and have been correlated with the inhibition of various cancers. Resveratrol was found to act as an antioxidant and antimutagen and to induce phase II drug-metabolizing enzymes; it mediated anti-inflammatory effects and inhibited cyclooxygenase and hydroperoxidase functions. Grape seeds are rich in anticancer phytochemicals (Lardos et al 2000). The present study was carried out to screen the phytocompounds present in the leaves of *Vitis vinifera* and check its antifungal activity against the pathogenic fungi, *Aspergillus niger* and non pathogenic fungi, *Mucor.*

Materials and Methods:

Fresh leaves of *Vitis vinifera* were collected from a local area in Patna. The fresh leaves were cleaned and kept in Hot air oven at 45ºC for drying. Dried leaves were then powdered. It was then dissolved in distilled water in 10:1 (v/w) (Saklani et al 2011). The mixture was centrifuged at 2500 rpm for 10 minutes. The filterate obtained was used as final extract and was subjected to different phytochemical tests for detection, analysis and assessment of primary and secondary metabolites, antioxidant and antifungal properties.

Phytochemical screening of the extract: The extract was subjected to the Phytochemical screening using the method adopted by Manjunath et al (2006) to test presence of flavonoids, alkaloids, phenols, terpenoids, tannins, and proteins in aqueous extract. To test the presence of soluble sugar method of Devmurari and Jivani (2010) was followed.

Test for Flavonoids (Concentrated sulphuric acid test): 1 ml of extract was taken and 2 drops of concentrated sulphuric acid was added in test tube. Formation of crimson colour indicated the presence of flavonoids.

Test for Alkaloids (Mayer's test): 1 ml of extract was taken and treated with Mayer's reagent. Formation of cream precipitate indicated the presence of alkaloids.

Test for Phenols (Ferric chloride test): 1 ml of extract was taken in test tube and few ml of ferric chloride solution was added. Formation of black precipitate was an indication of presence of phenols.

Test for Terpenoids (Salkowski's test): 1 ml of extract was taken and 2 ml of chloroform along with 3 ml of concentrated. Sulphuric acid was added. Appearance of reddish brown coloration was an indication of presence of terpenoids.

Test for Tannins (Ferric chloride test): 1 ml of extract was taken and few drops of ferric chloride solution were added. Formation of black precipitate was an indication of presence of tannins.

Test for soluble sugar: 1 ml extract was taken and 2-3 drops of 1% alcoholic α-napthol solution along with 2 ml of concentrated. Sulphuric acid was added. Appearance of violet coloured ring was an indication of presence of soluble sugar.

Test for Proteins: 1 ml of extract was taken and 1 ml of concentrated. Nitric acid was added. A white precipitate was obtained. The solution was heated for 1 minute and cooled under tap water. It was made alkaline using excess of 40% sodium hydroxide. Formation of orange precipitate was an indication of presence of proteins.

Spectrophotometric estimation of protein and soluble sugar: Protein estimation was done following the method of Lowry et al (1951). For sugar and phenol methods of Dubois et al (1956) and Park et al (2001) respectively were followed using UV-VIS spectrophotometer.

Antioxidant properties of leaf extract: Hydrogen peroxide scavenging activity

Ability of the extracts to scavenge hydrogen peroxide was determined as described by Govindarajan et al (2003). 1 ml of extract was rapidly mixed with 2 ml of 10 mM phosphate buffered (0.1M, pH 7.4) hydrogen peroxide solution. The absorbance was measured at 230 nm in the UV Spectrophotometer after 10 minutes of incubation at 37°C against a blank (without hydrogen peroxide). The percentage of inhibition of hydrogen peroxide was calculated using the following formula:

% inhibition H_2O_2 = [AO] – [A1] / [AO] × 100
Where, (AO – Absorbance of control;
A1 – Absorbance of sample)

Antifungal assay of the leaf extract:

The leaf extract was subjected to antifungal test by disc diffusion method (Esteban et al 2005). For this study pure culture of a pathogenic strain *Aspergillus niger* and a non-pathogenic strain of *Mucor* were taken. Potato dextrose agar (PDA) was poured on two sterilized petriplates. In an opaque vial 5 ml of saline water was taken and sterilized by autoclaving at 121ºC and 15lbs/inch^2 pressure for 15 minutes. Then, a loop full of each isolate of fungi were transferred in to the opaque vials and mixed well using vertex to develop turbidity. With the help of sterilised pipette, 1 ml of each isolate was pipette out and dropped on the plate. It was then evenly spread over the surface of the media with the help of a sterile glass spreader. The Plates were kept at room temperature for 15 minutes to allow surface moisture to be absorbed before loading the impregnated discs. Then, two discs were loaded per plate by means of sterile forceps, strictly under aseptic condition. The discs were placed on the plate in such a manner that centres were at least 24mm apart. Then within 15 minutes after the discs were loaded, the plates were placed in incubator at 27°C for 48 hrs (Tiwari et al 2011).

Results and Discussion:

The phytochemical screening of the aquous extract of the leaves revealed that all the major constituents of the leaf are found to be present.The results are depicted in table.1

Table-1: Phytochemical screening of leaf extract of *Vitis vinifera*

Phytochemical constituents	Aqueous extract
Flavonoid	+
Alkaloids	+
Phenol	+
Terpenoids	+
Tannins	+
Proteins	+
Soluble sugar	+

Present (+), Absent (–)

Phytochemical analysis of the leaf extract of *Vitis vinifera* showed the presence of primary as well as secondary metabolites. Table 1 shows the presence of Protein, soluble sugar, tannin, phenol, flavonoid, alkaloids and terpenoids in aqueous extract. The result was found to be similar to that of the phytochemical screening reported by Devmurari and Jivani (2010).

Table-2: Estimation of protein, soluble sugar and phenol by spectrophotometer

Phytocompounds	Concentration (µg/ml)
Proteins	219
Soluble sugar	73.32
Phenol	8

The result of Table 2 showed the concentration of proteins, soluble sugar and phenol in aqueous leaf extract of *Vitis vinifera*. Concentration of proteins was found to be highest where as phenol concentration was least.

Table-3: Antioxidant activity of the leaf extract

Sample	Absorbance of control	Absorbance of sample	% of inhibition
Aqueous extract	4.988	4.168	16.439

The leaf extract of *Vitis vinifera* showed antioxidant activity. Table 3 indicated that the absorbance of sample in aqueous extract was **4.168** and the absorbance of control was **4.988.** The inhibitive effect of the plant sample was subjected to H_2O_2 scavenging assay and the percentage inhibition was calculated as **16.439**.

Table-4: Antifungal activity of the sample

Sample	*Aspergillus niger*	*Mucor*
Aqueous extract	Effective	Ineffective

The result of Table 4 showed that the aqueous leaf extract of *Vitis vinifera* was effective against the pathogenic fungi *Aspergillus niger* and ineffective against the non- pathogenic fungi *Mucor*. The zone of inhibition was observed 14 mm against the pathogenic fungi. The result was similar to the work mentioned by Tiwari et al (2011) who has suggested presence of antioxidant and antifungal properties in aquous leaf extract of Menthe plant based on the presence and absence of various phytoconstituents.

Conclusion:

The results of the study showed that the leaves of *Vitis vinifera* is a potential source of vital chemical constituents like alkaloids, flavonoids, phenols, terpenoids, tannins, soluble sugar and proteins. Soluble sugar and proteins present in the leaf extract of *Vitis vinifera* add to the nutritional value and thus on consumption ensures a healthy and active life. Presence of secondary metabolites like alkaloids, flavonoids and tannins indicate that the leaf extract is very effective against the parasitic fungus *Aspergillus niger*. The results of the study also indicated that leaf extract of *Vitis vinifera* possesser antioxidant properties and thus contribute to dietary supplements. Free radical scavenging activity of secondary metabolites help in preventing human neurodegenerative disorders, cardiovascular diseases and cancer. Hence, more research work needs to be done in this field to bring forth the latent potential of the leaves of *Vitis vinifera* which may prove to be as useful as its fruit.

Acknowledgement:

We express our great sense of respect and gratitude to our Principal, Dr. Sister Doris D'Souza A.C, for giving us the opportunity to do this research work. We are also thankful to Ms. Sheila Bedi, H.O.D. of Botany, for her guidance.

References:

Davis PH. (1997). *Flora of Turkey and East Aegean Islands.* Edinburgh : Edinburgh University Press. **2**: 521-522.

Devmurari VP. and Jivani N P. (2010). 'Phytochemical screening and antibacterial activit of ethanolic extract of Artemisia Nilagiric'. *Annals of Biological Research*, **1 (1)**: 10-14.

Dubois M, Gilles K, Hamilton J, Rebers P and Smith F. (1956). 'Colorimetric method for determination of sugars and related substances'. *Anal. Chem*, **28(3)**: 350–356.

Esteban A, Abarca ML, Cabanes FJ. (2005). 'Comparison of disk diffusion method and broth microdillution method for antifungal susceptibility testing of dermatophytes'. *Med Mycol*. **43**: 61-6.

Govindarajan R, Rastogi S, Awat AKS, Vijayakumar M, Shirwaikar A, Mehrotra S. and Palpu P. (2003). 'Studies on the Antioxidant Activities of Desmodium gangeticum'. *Biolog. Pharmaceut. Bulletin.* **26**: 1424-1427.

Lardos A, Kreuter MH. (2000). 'Red vine leaf.' In: Kreuter, M.H. (Ed.). *Phytopharm. and Phytochem. Products.* Zurich : Flachsmann AG. pp.1-7.

Lowry, O.H.; Rosenbrough, N.J.; A.L.and Randall, R.J. (1951). 'Protein measurement with the Folin Reagent'. *J Biol Chem* **193**: 265-275.

Manjunath KP, Kulkarni GT, Patil KS. (2006). 'Preliminary Phytochemical investigation and wounds healing activity of the root of Swertia chirata Buch. Ham. (gentianaceae)'. *Indian Drugs*.**43**: 535-537.

Park YK, Alencar. de SM., Aguiar CL, Scamparini ARP González M. and Molina MAA. (2001) *www.apacame.org.br Mensagem Doce. 61 on line*.

Tiwari P. Rai H. Upret DK. Trivedi IS and Shukla P. (2011). 'Antifungal Activity of a Common Himalayan Foliose Lichen Parmotrema tinctorum (Despr. ex Nyl.) Hale'. *Nature and Science*,**9(9):** 167-171.

Saklani Sarla, Chandra Subhash and Mishra A P. (2011). 'Evaluation of Nutritional profile, medicinal value and quantitative estimation in different parts of Pyrus pashia Ficus palmata and Pyracantha crenulata'. *JGTPS*, **2(3):** 350-354.