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Phytochemical Evaluation of Total Phenolic and Flavonoiuds of *Eulophia nuda* Lindl.tubers.

Wayal Y.P. and Patil S.S

Shri D. M. Burungale Science & Arts College, Shegaon (M.S.)

Head / Director, Dept. of Lifelong Learning & Extension (Adult and Continuing Education and Extension Service),Sant Gadge Baba Amravati University, Amravati

Abstract:

Phytochemicals have been classified in diverse forms but classically they are divided based on the primary and secondary metabolites based on their roles in plant metabolis. The primary metabolites consist of proteins, sugars, amino acids, purines, and pyrimidines while, the secondary metabolites include steroids, terpenoids, flavonoids, phenolics, alkaloids, saponins and lignin. Based on the occurrence of phytochemicals.The present investigation was carried out on an ethanomedicinal plant *Eulophianuda* belongs to the family Orchidaceae. This plant used ethnomedicinally for various purpose. Phytochemical evaluation of tubers extract was carried out in various solvents such aschloroform and ethanol. phytochemical screening revealed the presence of Alkaloids, Carbohydrates, Glycosides, Saponins, Tannins and flavonoids.

Key words: Phytochemical, alkaloids, Orchidaceae, *Eulophianuda*. Flavonoids, phenols

Introduction:

Orchids are a unique group of plants belonging to the family Orchidaceae. Orchids are largest and most diverse group among angiosperms (Sigh and Duggal, 2009). Orchid plants belong to family Orchidaceae which exihibits the large variety in colour, shape and size(Kumar and Singh, 2023).These orchids have adapted themselves to a variety of ecological niches due to their habitat specificity. They grow as lithophytes, epiphytes, and terrestrial (Chauhan, 2022). This monocotyledons family contains 600-800 genera with valuable medicinal properties (De and Medhi, 2015).Although orchids are found in natural habitats in several parts of the world, their presence is decreasing due to great demand by the population (Fonge, *et al*, 2019).

They are habitat specific and often serve as indicator plants of a healthy ecosystem (Misra, 2004). Orchids are grown primarily as ornamentals and are valued as cut flowers because of their exotic beauty and their long lasting blooming period (Hew *et al*., 1997).The literal meaning of the term orchid (orchis), in Greek is testicles and it was Theophrastus who first coined the term as the anatomy of plant resembles testicles [Stewart and Griffith, 1995). India is very rich in orchid genetic resource.

Phenolic compounds are one of the largest group of phytochemicals observed in plants. They are bioactive secondary metabolites of high importance with a wide array of biological activities (Al Mamari, 2022). These are hydroxyl containing compounds where the -OH group is directly bonded to an aromatic hydrocarbon. They are synthesized in plant cells through shikimic acid pathway or malonate/acetate pathway leading to the synthesis of phenolic acids (Gonzalez Mera *et al.*, 2019).

Material and Methods:

Collection & Authentication of plant materials

The phytochemical study of the selected tuber extracts was performed based on the standard protocols. Phytochemical tests for the screening of the extracts obtained using Soxhlet extraction were evaluated for the identification of the biochemical constituents (Harborne, 1973; Trease and Evans, 1989; Sofowara, 1993).

Preliminary phytochemicals screening

The chloroform and ethanol crude extracts (1 g) was completely dissolved in 100 mL of its own mother solvents. It was prepared the stock solution. The obtained stock solution was used for phytochemical screening following the methodology of Harborne and Kokate[14],[15].

A. Test for alkaloids

One gram powder samples of plant was taken in a conical flask and added ammonia solution (3 mL). It was allowed to stand for few minutes to evaluated free alkaloids. Chloroform (10 mL) was added to the conical flask shaken by hand and then filtered. The chloroform was evaporated from the crude extract by water bath and added Mayer's reagent (3 mL). A cream colour precipitation was obtained immediately that showed the presence of alkaloids.

B. Test for flavonoids

The stock solution (1 mL) was taken in a test tube and added few drop of dilute NaOH solution. An intense yellow colour was appeared in the test tube. It became colourless when on addition of a few drop of dilute acid that indicated the presence of flavonoids.

C. Test for saponins

The stock solution (1 mL) was taken in a test tube and diluted with 20 mL of distilled water. It was shaken by hand for 15 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins.

D. Test for steroids

The crude plant extracts (1 mg) was taken in a test tube and dissolved with chloroform (10 mL), then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

E. Test for tannins

The stock solution (3 mL) was taken in a test tube and dilutedwith chloroform and added acetic anhydride (1 mL). Finally, sulphuric acid (1 mL) was added carefully by the side of test tube to the solution. A green colour was formed which showed the presence of tannins.

F. Test for triterpenoids

The dry crude plant extract (5 mg) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids.

Determination of total phenol by Folin-reagent method

Total phenol content was determined by Folin-Ciocalteu reagent method with modification. From each crude extracts (1 mg) was dissolved in methanol (1 mL). A total of 10% Folin-Ciocalteu reagent was prepared by adding Folin-Ciocalteu reagent (10 mL) in water (90 mL). Then, 5% Na2CO3 (3 g) was prepared by dissolving Na2CO3 (3 g) in water (50 mL). Each crude sample (200 µL) was taken in a test tube and added 10% Folin-Ciocalteu reagent (1.5 mL). Then all the test tube was kept in a dark place for 5 min. Finally, 5% Na2CO3 (1.5 mL) was added to the solution and mixed well by hand. Again all the test tube was kept in the dark for 2 h. The absorbance was measured for all solution by using UV-spectrophotometer at constant wavelength 750 nm.Gallic acid calibration crude was prepared by Folin-Ciocalteu reagent method with different concentrations.

Determination of total flavonoids by colorimetric method

The chloroform and ethanol crude extracts were used to determine the total flavonoids contents. The total flavonoids contents of different crude extracts were estimated by aluminium chloride colorimetric method as described by Hossain et al. Sodium nitrate (2.5 g) was taken in a volumetric flash (50 mL) and added water upto the mark that was 5% sodium nitrate. Sodium hydroxide (2.5 g) was taken in another volumetric flash (50 mL) and added water upto the mark that was 4% sodium hydroxide. Then 10% aluminium chloride solution was prepare the same procedure. The different crude extracts (0.25 mg) were taken in a test tube and added water (1.25 mL) and sodium nitrate (0.75 µL) then mixed together. All the test tubes were kept in the dark place for 6 min. Then 10% aluminium chloride (0.150 µL) was added into the test tube and wait for 5 min in the dark for complete reaction. Finally, 5% sodium hydroxide (0.5 mL) and water (0.275 mL) were added to the test tube. The absorbance was measured of all samples at a fixed wavelength 510 nm on spectrophotometer. Rutin standard was used for the calibration curve. The estimation of total flavonoids contents in the crude extracts was carried out in triplicate and the results were averaged.

Results and Discussion

The amorphous solid masses were obtained by evaporation of chloroform and ethanol. The result for phytochemical screening of The chloroform and ethanol extracts were from the tubers showed the presence of flavonoids, saponins and steroids, but alkaloids, tannins, and triterpenoids not present in the crude extract. The detailed results are shown below;

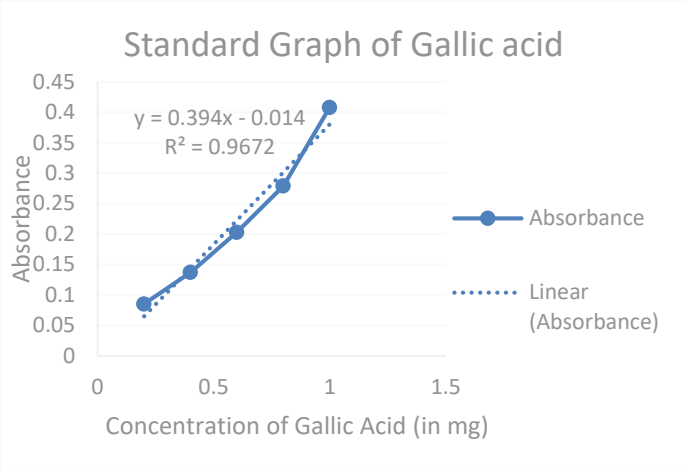
Table : Phytochemical test of *E. nuda*

Determination of Total Phenolic and Flavonoid content

The study for the determination of total phenolic and total flavonoid content was also performed. The total flavonoid content varied from 0.53±0.18 to 1.25±0.20 for chloroform extract and the ethanol extract. While, the total phenol content varied from 1.58 ±0.34 to 10.52±1.32 for ethanolic and chloroform extract of *E. nuda*. Standard graphs of both gallic acid (total phenol) and rutin (total flavanoids) is shown below;

Solvent	**Total Flavonoid in E. nuda (RE/g)**	**Total Phenol in E. nuda GAE/g**
Ethanol	1.25±0.20	**1.58** ±0.34
Chloroform	0.53±0.18	**10.52**±1.32

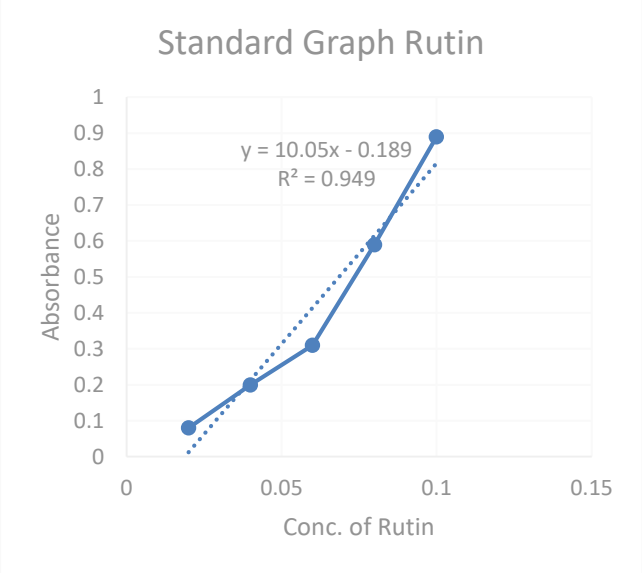
Where, RE: Rutin equivalent and GAE: Gallic acid equivalent



Graph: Standard Graph of Gallic acid

Table : Gallic acid and Rutin Standard graph table

Concentration of Gallic Acid (in mg)	**Absorbance**		**Concentration of Rutin (in mg)**	**Absorbance**
0.2	0.085		0.2	0.085
0.4	0.137		0.4	0.137
0.6	0.203		0.6	0.203
0.8	0.279		0.8	0.279
1.0	0.408		1.0	0.408



Graph: Standard Graph of Rutin

Secondary metabolites are bioactive compounds that are derived from plant, animals and living organisms. The study of plant secondary metabolites involves extraction and isolation of compounds from plant samples, characterization through spectroscopic studies and determination of the structure of the bioactive compound. The secondary metabolites present in the plants serve as a defence mechanism for them by protecting plants from predators and microbial infections. Some of the primary metabolites observed in the medicinal plants include presence of alkaloids, flavonoids, terpenoids, steroids, phenols and tannins that serve as the defense system of the plants. The medicinal properties of the plants are majorly due to the presence of these bioactive compounds. The isolation and identification of these compounds help in the discovery of novel pharmaceutical drugs that can further be used for curing several human ailments.

Tiwari *et al*., (2012) studied the preliminary phytochemical analysis of the quantification of the bioactive compounds. Aqueous, methanolic and ethanolic extracts of the tubers were prepared using Soxhlet extraction. The phytochemical tests for alkaloids, flavonoids, phenols and tannins, carbohydrates, steroids and glycosides were performed. The results of the study depicted presence of alkaloids, flavonoids, steroids, saponins and glycosides in the respective three extracts.

Nagulwar*et al*. (2017) reported the phytochemical screening of three extracts of *E. nuda* for the investigation of the presence of bioactive compounds. The preliminary tests were performed by Soxhlet extraction of ethanol, acetone and chloroform extracts of tuber of *E. nuda*. The phytochemical screening revealed presence of alkaloids, flavonoids, phenols and tannins, glycosides, steroids, carbohydrates and saponins. The ethanolic extract of the tuber showed presence of flavonoid, alkaloid, glycosides, saponins and tannins while, the acetone extract depicted presence of cardiac glycosides, alkaloids and saponin.

Nanekar *et al*., (2019) performed the preliminary phytochemical screening of aqueous, methanol, petroleum ether, and ethyl acetate extracts of *E. nuda* tubers for the estimation of the presence of the bioactive compounds.

The results of the study depicted presence of alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, coumarins, and glycosides. Dawande and Gurav (2021) investigated the phytochemical analysis of the methanolic extract of the tubers of *E. nuda*. The results of the phytochemical investigation depicted the presence of alkaloids, steroids, flavonoids, glycosides, carbohydrates, saponins, tannins and phenols.

References:

Bulpitt.. C. J. (2005). The uses and misuses of orchids in medicine. *QJM: An International Journal of Medicine*; 98:625-631.

Chauhan, P.P. (2022). Ethnobotany and Occurrence of Two Orchid Species in Pabbar Valley, Distt Shimla, Himachal Pradesh, India. Annals of Plant Sciences; 11(02): 4777-4782.

De, L.C. and R.P. Medhi (2015). Orchid- A diversified component of farming systems for profitability and Livelihood security of small and marginal farmers. *Journal of Bioscience*; 4(2): 1393-1406.

Evans, W. C. (2005). Trease and Evan's Pharmacognosy. 15th ed., London, W.B. Sounders Company Ltd; pp. 545-547.

Fonge, B. A., S. E. Essomo, T. E. Bechem, P.T. Tabot. B.D. Arrey, Y. Afanga and E. W. Assoua (2019) Market trends and ethnobotany of orchids of Mount Cameroon. *Journal of Ethnobiology and Ethnomedicine*; 15(1): 1–11.

Hew CS, Arditti J, Lin WS (1997). Orchid cut-flower production in ASEAN countries. In: Arditti, J(Ed.), Orchid Biol. Rev. Perspect. 6:363-401.

Hossain MA, Muhammad MD, Charles G, Muhammad I. In vitro total phenolics, flavonoids contents and antioxidant activity of essential oil, various organic extracts from the leaves of tropical medicinal plant Tetrastigma from Sabah. Asian Pac J Trop Med. 2011;4(9):717–721.

Khandelwal, K. R. (2006). Practical pharmacognosy techniques and experiments. Nirali Prakashan, Pune, 15th ed. pp. 15–163.

Kumar A., and L. Singh (2023). Ethnomedicinal uses of orchids and its species under threat: A review. Research journal of Pharmacy and Technology; 16 (09), 4469-4478.

Misra, S. Orchids of orissa. Bishen Singh Mahendra Pal Singh, Dehradun, India 2004).

Narkhede, A. N., D. M. Kasote, A. A.Kuvalekar,A. M.Harsulkar, and S. D. Jagtap (2016). Amarkand: A comprehensive review on its ethnopharmacology, nutritional aspects, and taxonomy. Journal of Intercultural Ethnopharmacology; 5(2): 198–204.

Ram Pal, N. K. Meena, M. Dayamma &D. R. Singh (2022). Ethnobotany and Recent Advances in Indian Medicinal Orchids, Orchids Phytochemistry, Biology and Horticulture; Reference Series in Phytochemistry. Springer, Cham. pp 361–387.

SinghA. and S. Duggal (2009). Medicinal Orchids - An Overview. *Ethnobotanical Leaflets;*13: 399-41216(9):

Stewart, J &M.,Griffith (1995). Manual of Orchids.Timber Press Portland, Oregon.