J. Phytol. Res. **37**(2): 163-171, 2024 **ISSN 0970-576**

QUANTITATIVE ESTIMATION OF VARIOUS PRIMARY METABOLITES AND ENZYMES INVOLVED IN THE BIOCHEMICAL DEFENSE MECHANISM OF *CATHARANTHUS ROSEUS* L.

PRIYANKA TOLAMBIYA[1]*, BHAGAWATILAL JAGETIYA[2] and SUJATA MATHUR[3]
[1]Department of Botany, Govt PG College, Kekri-305404 Rajasthan, India
[2]Phytotechnology Research Laboratory, Department of Botany, M.L.V. Government College, Bhilwara-311001, Rajasthan, India
[3]Department of Botany, B.B.D. Government PG College, Chimanpura (Shahpura)-303103, Jaipur, Rajasthan, India
***Corresponding Author's Email:** ptolambiya@gmail.com

The intermediate products of metabolism are called metabolites. Type of metabolites that are specifically engaged in appropriate growth, development, and reproduction are called primary metabolites. These are typically carried out by organism's physiological tasks. Primary metabolites include a wide variety of chemical molecules, including proteins, lipids, and carbohydrates; all of which are extremely important to the growth and development of plants. To assess different primary metabolites and enzymes, various plant organs such as leaves, stems, roots, and flowers of *Catharanthus roseus* L. have been dried and ground into powder. Carbohydrates and lipids with certain enzymes such as indole acetic acid oxidase, polyphenol oxidase, and peroxidase assay (POX) were performed. Highest concentration of lipids (0.020 ± 0.004 mg mg g-1 dw) and carbohydrates (1.546 ± 0.1666 mg mg g-1 dw) were found in the blossom of *C. roseus*. Leaves showed highest polyphenol oxidase and peroxidase activities with the mean values 2.729 ± 0.03 OD $min^{-1}mg^{-1}$fresh weight and 2.4640± 0.010 $mM^{-1}cm^{-1}$, respectively. Highest IAA oxidase activity was recorded in the stem, with a maximum of 0.481 ± 0.02 OD $min^{-1}mg^{-1}$fresh weight.

Keywords: Carbohydrates, Lipids, Polyphenol oxidase, Peroxidase Assay (POX), Indole Acetic acid oxidase.

Introduction

Metabolites are the intermediates products of metabolism, and the term metabolite is usually restricted to small molecules. A primary metabolite is a kind of metabolite that is directly involved in normal growth, development and reproduction. It usually performs a physio-logical function in the organism. It is also referred to as a central metabolite. Primary metabolites comprise many different types of organic compounds, mainly nutritional components of plants such as common sugars, amino acids, proteins, nucleic acids, and chlorophyll[26]. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle. Examples of primary metabolites include energy rich fuel molecules, such as sucrose and starch, structural components such as cellulose, informational molecules such as DNA (deoxyribonucleic acid) and RNA (ribonucleic acid), and pigments such as chlorophyll. Primary metabolites are

precursors (starting materials) for the synthesis of secondary metabolites[33-37]. Plants based metabolites are widely used in agricultural, food items and pharmaceutical products[8]. Saccharides, often known as carbohydrates, are the most prevalent metabolites. There are several functions that carbohydrates fulfil in living things. Polysaccharides are used as structural elements (such as chitin in arthropods and cellulose in plants), and for the storage of energy (e.g. starch, and glycogen). A crucial part of coenzymes like ATP, FAD, and NAD as well as the structural backbone of the genetic material known as RNA is the 5-carbon monosaccharide ribose. The corresponding deoxyribose is found in DNA. The human body uses carbohydrates as an energy source, aids in the regulation of blood sugar and insulin metabolism, takes part in the metabolism of triglycerides and cholesterol, and facilitates fermentation[11]. Numerous other significant macromolecules that are essential for the immune system, fertilization, blocking disease, blood coagulation, and other processes are either saccharides or their derivatives.

A class of naturally occurring compounds known as lipids is made up of fats, waxes, cholesterol, fat-soluble vitamins (including A, D, E, and K), phospholipids, and other molecules. According to[9,15] lipids' primary biological roles include energy storage, signaling, and serving as structural elements of cell membranes. Lipids are classified as tiny molecules that are either hydrophobic or amphiphilic. Some lipids are amphiphilic, which enables them to form structures like membranes in an aqueous environment, vesicles, and multi- or unilamellar liposomes. The ketoacyl, and isoprene groups are two different kinds of biochemical subunits or 'building-blocks' that can contribute wholly or partially to the formation of biological lipids[9]. Fatty acids, glycerolipids, glycerol- phospholipids, sphingolipids, saccharolipids, polyketides (derived from condensation of ketoacyl subunits), sterol lipids, and phenol lipids (derived from condensation of isoprene subunits) are the eight categories into which lipids can be classified[9]. Triglycerides are a subclass of lipids known as fats, despite the fact that the term 'lipid' is occasionally used interchangeably with fats. Lipids[21] also includes molecules like fatty acids and their derivatives, such as tri- and di-mono-glycerides and phospholipids as well as various metabolites that contain cholesterol. Lipids are used in nanotechnology, the food and cosmetics industries, and other fields[18]. Plants have antioxidant defenses that can detoxify reactive oxygen species. Chemical reactions can be accelerated by enzymes, which are macromolecular biological catalysts. Enzymes can operate on molecules known as substrates at the start of a process, and they change these molecules into other molecules known as products. Enzymes are required for nearly all metabolic activities in cells to occur at rates rapid enough to support life. Which metabolic pathways are active in a given cell depends on the set of enzymes that cell produces.

One of the many enzymes found in nature that belong to the class of oxidoreductases is peroxidase. By interacting with hydrogen peroxide and related compounds, it can accelerate the oxidation of a variety of organic and inorganic substrates. Peroxidases (E.C. number 1.11.1.7) are haem-containing enzymes that catalyze a variety of oxidative processes by using hydrogen peroxide as the electron acceptor. It is known that peroxidase contributes to a plant's increased defenses against infections[14]. Participate in the synthesis of phytoalexins, cross-linking of cell wall components, and the production of lignin, and suberin, among other physiological processes; or engage in the metabolism of ROS and RNS, both of which

activate the hypersensitive response (HR), a type of programmed host cell death at the infection site linked to restricted pathogen development[1]. Because of their broad range of catalytic activity, peroxidases can be used in chemical synthesis, the elimination of phenolic compounds and peroxides, and according to recent research, the destruction of mycotoxin[22].

Plant polyphenol oxidases (PPOs) are extensively distributed, well-researched oxidative enzymes, and for a long time, it has been known how they affect the coloring of diseased and injured plant tissues. Catechol oxidase (E.C. 1.10.3.2), a type of PPO has been described in detail by many authors[5,20]. PPOs are widely distributed enzymes that contain copper and oxidize common ortho-diphenolic chemicals like catechol and caffeic acid to their corresponding quinines using molecular oxygen. Tyrosinases is another name for PPOs[20]. PPO plays a significant part in physiological metabolism and plant resilience to stress[41].

One kind of peroxidase that is involved in ripening is called indole acetic acid oxidase **(**IAA oxidase). The enzyme recognized best for its natural auxin, IAA oxidase, is involved in several stages of growth and differentiation[2,10]. The most well-known naturally occurring plant hormone is indole-3-acetic acid, or IAA. It takes part in the genetic, physiological, and metabolic processes that allow plants to grow and differentiate. It is thought that the main precursor of IAA in plants is tryptophan an amino acid[19,23.24,32]. Studies on primary metabolites involved in development and growth, and in the regulation of plant defense responses are very scarce[27]. Similarly, a very diminutive information is available on quantitative estimation of some of the significant primary metabolites (carbohydrates and lipids) and defense enzymes (peroxidase, polyphenol oxidase and indole acetic acid oxidase) in vital organs of test plants[40]. The outcomes of the present findings will unveil the relative presence of primary metabolites in *C. Roseus*.

To gain a better understanding of an organism's growth, development, and reproduction, primary metabolites and various enzymes are extracted and quantified. Primary metabolites, intermediate byproducts of anabolic metabolism, are used by cells to make essential macromolecules. Among the primary metabolites are proteins, fats, carbohydrates, vitamins, and nucleic acid components. Understanding the function of peroxidase and polyphenol oxidase (PPO) in food quality, bioremediation, soil detoxification, waste-water treatment, the paper industry, and biomedical and pharmaceutical applications is the aim of their extraction from plants.IAA increases root growth and extension, which enables the plant to use water and nutrients more efficiently.

Material and Methods

Quantification of Carbohydrates

The dried experimental plant material (0.1 g each) was homogenized in a pestle and mortar with 20 ml of 80% ethanol on its own, and it was left overnight. Each sample was centrifuged for 15 minutes at 1200 rpm. The Loomis and Shull (1937) procedure was then used to separate and concentrate the supernatants on a water bath. Distilled water was added to get the volume up to 50 ml, and the combination was then further processed for quantitative analysis.

The phenol sulphuric acid method of Dubois et al.[6] (1951) was used to estimate the amount of carbohydrates in 1 ml of aliquot of each sample. A distilled water stock solution containing 100 µg of glucose per ml was created, and 0.1 to 0.8 ml were pipetted into

distinct test tubes and volume were added with distilled water to make 1 ml, and the tubes were placed on ice. To each tube 1 ml of 5% phenol was added and carefully shaken. Now, 5 ml of concentrated sulfuric acid was added quickly causing the liquid to steam while the tubes were gently shaken continually. Lastly, the mixture was let to stand for 20 minutes at 26–30 ^{0}C on a water bath. The color turned to a mix of yellow orange. After adjusting the spectrophoto- meter (Hitachi UH 5300) for 100% transmission against a blank, the optical density was measured at 490 nm. Using Beer's Lambert Law as a guide, a standard regression curve was created between the known glucose concentration and each optical density. Each plant sample was examined in a similar way.

Quantification of lipids

The test samples were dehydrated, ground into powder, and then 100 mg was macerated in 10 ml of distilled water. Samples were then put into a conical flask with 20 ml of methanol and chloroform[13]. For full extraction, the blends were well combined and left in the dark at room temperature overnight. Afterwards, 2 ml of water and 20 ml of chloroform were added, and the mixture was centrifuged. Two layers were separated, the colorful aqueous layer of methanol, which contained all the water-soluble compounds, were discarded, and the lower layer of chloroform, which contained all the lipids, was carefully collected in the pre-weighed glass vials. After being vacuum-dried, the lipids were weighed. The mean values of each treatment were computed after it was repeated three times.

Enzymes extraction and quantitative estimation

Peroxidase Assay (POX):

The method of assay measures the oxidation of pyrogallol to purpurogallin by peroxidase when catalyzed by peroxidase at 420 nm and at 20 ^{0}C.

$$\text{Pyrogallol} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Purpurogallin}$$

200 mg plant sample was homogenized with 10 ml of phosphate buffer and centrifuged for 20 minutes at 10,000 rpm. The enzyme extract was extracted from the clear supernatant. With the following adjustments, the activity was tested using Chance and Maehly[4] methodology. 0.3 ml of pyrogallol, 0.2 ml of H_2O_2, and 2.4 ml of phosphate buffer were added. After adding 0.1 ml of enzyme extract, the absorbance at 420 nm was measured to estimate the amount of purpurogallin that had produced. The enzyme activity was calculated using the 2.8 mM $^{-1}$cm^{-1} extinction that was expressed in terms of mmol per minute per gram dry weight.

Estimation of Polyphenol oxidase

Polyphenol oxidase was estimated using the method described by Shinshi and Noguchi[30]. 1g of plant material was crushed (leaves, stems, roots, and flowers) with mortar and pestle by adding 10 ml of 1M phosphate buffer (pH 6.0) then centrifuged for ten to fifteen minutes at 3000 rpm. Enzyme extracts from the supernatant were collected. Using 1 ml of enzyme extract and 3 ml of tannic acid solution, optical density was measured at 495 nm for every 15 seconds. For enzyme activity, the average value of three replicates was expressed.

Estimation of IAA oxidase

Salkowski reagent is a chemical mixture that detects the presence of indole-3-acetic acid, a type of auxin. The IAA oxidase activity was estimated using the Saenger (1984) method. Residual IAA along with dark reaction with the Salkowski reagent was used to measure the activity of mono-phenolic compounds. Ten ml of 1M acetate buffer pH (4–7) were used to macerate 0.5 g of plant material. Centrifuging at 3000 rpm

for 10 to 15 minutes and supernatant was used as a source of enzymes. 2.0 ml of reagent and 1.0 ml of reaction mixture were mixed. At 530 nm, optical density was measured using all the reagents except IAA in a blank. IAA oxidase activity data were expressed as OD min^{-1} mg^{-1} tissue as average of three replicates**.**

Results and Discussion

Carbohydrates:

Results of present investigation (Table-1) showed that flower parts of *C. roseus,* have the highest level of carbohydrates 1.546 mg g^{-1} dw ± 0.1666. However, some researchers claim that maximum soluble sugars, starch, are found in leaf parts[3,12]. Due to the availability of carbohydrates in its leaf and flower parts are traditionally used as direct treatment of diabetes. Plant sugar can be used as artificial sweetener, and they can even benefit in diabetes by supporting the body in its rebuilding.

Lipids:

Highest lipid 0.020 mg g^{-1} dw ± 0.004 contents were observed in leaves of test plant (Table-1). Similarly, Jain et al[12] found the highest lipid contents in leaves. Lipid is a diverse group of primary metabolites, including reserve plant material such as fats, essential oils, waxes terpenoids and oleoresin. Plant lipids have been used for culinary, medicinal or cosmetic purposes[39]. They can also be used as fungicide, pesticides, an antiseptic, disinfectant and in the manufacture of resins, explosives, plastics, detergents and pharmaceutical substances[28]. They are also used as raw material and food additives[29].

Table-1: Carbohydrate and lipid quantities in plant organs of *Catharanthus roseus* L.

Plant Organ	Carbohydrates (mg g^{-1} dw)	Lipids (mg g^{-1} dw)
Leaf	1.082 ± 0.0025	0.020 ±0.004
Stem	0.705 ± 0.005	0.010 ±0.007
Root	0.891 ± 0.0033	0.010 ±0.003
Flower	1.546 ± 0.1666	0.010 ±0.007

Table-2: Peroxidase, Polyphenol oxidase and IAA oxidase activity values in plant organs of *Catharanthus roseus* L.

Plant Organ	Peroxidase activity ($mM^{-1}cm^{-1}$)	Polyphenol oxidase activity (OD $min^{-1}mg^{-1}$ tissue)	IAA oxidase activity (OD $min^{-1}mg^{-1}$ tissue)
Leaf	2.464 ± 0.010	2.729 ± 0.03	0.375± 0.05
Stem	2.254 ± 0.036	1.906 ± 0.01	0.481± .02
Root	1.456 ± 0.0045	2.508 ± 0.21	0.0623± 0.06
Flower	1.064 ±0.0005	1.905 ± 0.03	0.27± 0.017

Peroxidase Enzyme

Highest peroxidase value observed 2.4640 ± 0.010 $mM^{-1}cm^{-1}$ in leaves followed by stem, root and flower for peroxidase enzyme (Table-2). It is indicative that leaves may have a higher capacity to inhibit the free radicals. Peroxidase can be used for the treatment of industrial wastewater. For example, phenols, which are important pollutants, can be removed by enzyme-catalyzed polymerization using horseradish peroxidase. Phenols are oxidized to phenoxy radicals, which participate in reactions where polymers and oligomers are produced that are less toxic than phenols. It also can be used to convert toxic materials into less harmful substances. There are many investigations into the use of peroxidase in many manufacturing processes like adhesives, computer chips, car parts, and linings of drums and cans. Other studies have shown that peroxidase may be used successfully to polymerize anilines and phenols in organic solvent matrices[38].

Polyphenol oxidase Enzyme:

Results of present study indicate that leaves of *C. roseus* have maximum polyphenol oxidase value which was 2.729 ± 0.03 OD^{-1} min $^{-1}$mg tissue (Table-2)**.** Polyphenol oxidase are copper containing proteins which catalyses the aerobic oxidation of certain phenolic compounds to quinones. They are auto oxidized to dark brown pigments and play an important role in plant defense mechanisms. The enzyme is most found in fresh fruit and vegetables etc. They have been reported from a variety of plant organs[25].

IAA oxidase Enzyme:

IAA oxidase activity was observed maximum in stem is 0.481± 0.02 OD^{-1} min $^{-1}$mg tissue during this investigation (Table-2). IAA oxidase catalyses various biochemical and physiological activities. In buds, flower formation is accompanied by enhanced activities of peroxidase, polyphenol oxidase and higher contents of phenolic compounds as well as lower levels of IAA[7].

Conclusion

Primary metabolites comprise many different types of organic compounds such as carbohydrates, lipids and protein having monumental role in growth and development of plant. Carbohydrates and lipids increase the food quality and are also used as a raw material and food additives. Highest carbohydrate level was observed 1.546 mg g^{-1} dw ± 0.1666 in flower whereas lipids were 0.020 mg g^{-1} dw ±0.004 detected highest in leaves. Peroxidase and polyphenol oxidase enzymes activity have been found highest in Leaf samples, while indole acetic acid oxidase activity was observed in stem tissues. Generally, the IAA level is found higher in shoot tips and present results corroborate the fact.

Future Scope

This study can be used as a guide for future workers as it will serve baseline data of carbohydrates, lipids and enzymes (peroxidase, polyphenol oxidase, indole acetic acid oxidase activity) in various organs of *C. roseus*. Further, it may be helpful to understand the plant defense mechanism in detail.

Acknowledgement

The Authors are thankful to University of Rajasthan, Jaipur India for providing sophisticated laboratory facilities and to explore relevant literature to conduct the investigation.

References

1. Almagro L, Gómez Ros LV, Belchi-Navarro S, Bru R, Ros Barceló A and Pedreño MA 2009, Class III peroxidases in plant defence reactions.

Journal of Experimental Botany. **60** (2) 377-390.
2. Bryant DS and Lane EF 1979, Indole-3-acetic Acid Oxidase from Peas I. Occurrence and distribution of peroxidative and nonperoxidative forms. *Plant Physiology*. **63** 696-69.
3. Bhumi G and Savithramma N 2014, Screening of Pivotal Medicinal Plants for Qualitative and Quantitative Phytochemical Constituents. *International Journal of Pharmacy and Pharmaceutical Sciences.* **6**(3) 63-65.
4. Chance B and Maehly AC 1955, Assay of catalase and peroxidases. *Methods in Enzymology*. **2** 764-775.
5. Constabel CP, Bergey DR and Ryan CA 1996, Polyphenol oxidase as a component of the inducible defense response in tomato against herbivores. In: *Phytochemical Diversity and Redundancy in Ecological Interactions.* (Eds.) Romeo JT, Saunders JA and Barbosa P. Plenum Press, New York, Pp 231–252.
6. Dubois M, Gilles K, Hamilton JK, Rebers PA and Smith F 1951, A colorimetric method for the determination of sugar. *Nature*. **168** (4265) 167.
7. Ebrahimzadeh H and Abrishamch P 2001, Changes in IAA, Phenolic Compounds, Peroxidase, IAA Oxidase, and Polyphenol Oxidase in Relation to Flower Formation in Crocus sativus. *Russian Journal of Plant Physiology*. **48**(2) 190-195.
8. Elshafie HS, Camele I and Mohamed AA 2023, A Comprehensive Review on the Biological, Agricultural and Pharmaceutical Properties of Secondary Metabolites Based-Plant Origin. *Int J Mol Sci.* **24**(4) 3266.
9. Fahy E, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, Van Meer G, Wakelam MJ and Dennis EA 2009, Update of the LIPID MAPS comprehensive classification system for lipids. *Journal of Lipid Research.* **50** (1) 9-14.
10. Feng Fu S, Wei JY, Chen HW, Liu YY, Lu HY and Chou JY 2015, Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. *Plant Signal Behav*. **10**(8) e1048052.
11. Holesh JE, Aslam S and Martin A 2023, *Physiology, Carbohydrates.* Treasure Island (FL): Stat Pearls Publishing.
12. Jain AK, Meena and Kumar D 2014, *In-vivo* and *in-vitro* comparative study of primary metabolites and antioxidant activity of *Catharanthus roseus. Asian Journal of Plant Science and Research*, **4**(2) 42-46.
13. Jayaraman J 1958, *Laboratory Manual in Biochemistry.* Wiley Eastern Limited, New Delhi: Pp. 96-97.
14. Karthikeyan J, Kavitha V, Abirami T and Bavani G 2017, Medicinal plants and diabetes mellitus: *A review. Journal of Pharmacognosy and Phytochemistry*. **6** (4) 1270-1279.
15. Kim HU 2020, Lipid Metabolism in Plants. *Plants (Basel).* **9**(7) 871.
16. Loomis WE and Shull CA 1937, *Methods in Plant Physiology: A Laboratory Manual and Research.* Mc Graw - Hill publication, New York. Pp 290.
17. Lowry OH, Rosebrough NJ, Farr AL and Randll RJ 1951, Protein measurement with the Folin-phenol reagent. *Journal of Biology Chemistry.* **193**(1) 265-275.

18. Mashaghi S, Jadidi T, Koenderink G and Mashaghi A 2013, Lipid nano-technology. *International Journal of Molecular Sciences.* **14** (2) 4242–82.
19. Mathur S 2002, *Biotechnological Investigations of the tree with Immense medicinal potentials.* Ph.D. Thesis, University of Rajasthan, Jaipur.
20. Mayer AM 2006, Polyphenol oxidases in plants and fungi: going places? A review. *Phytochemistry*. **67**(21) 2318–2331.
21. Maton A, Hopkins J, McLaughlin CW, Johnson S, Warner MQ, LaHart D and Wright JD 1993, *Human Biology and Health*. Englewood Cliffs, New Jersey, USA: Prentice Hall. Pp. 232-249.
22. Oliveira FK, Santos LO and Buffon JG 2021, Mechanism of action, sources, and application of peroxidases. *Food Res Int*. **143** 110266.
23. Parry G, Marchant A, May S, Sarup R, Swarup K, James N, Graham N, Allen T, Martucci T, Yemn A, Napier R, Manning K, King G and Bennett M 2001, Quick on the uptake: Characterization of a family of plant auxin influx carriers. *Journal of Plant growth Regulation.* **20** 217-225.
24. Quesada MA, Roldán CS, Heredia A, Valpuesta V and Bukovac Martin J 1992, Peroxidase and IAA oxidase activities and peroxidase isoenzymes in the pericarp of seeded and seedless "Redhaven" peach fruit. *Journal of Plant Growth regulation.* **11** 1-6.
25. Rao TVP and Venkateswarlu V 1965, Chemical examination of *Embelia ribes*-VII: Synthesis of some new N-Bis(anhydrobenzoquinones). *Tetrahedron.* **21**(3) 389-390.
26. Rungsung W, Ratha KK, Dutta S, Dixit AK and Hazra J 2015, Secondary metabolites of plants in drugs discovery. *World J Pharm Res*. **4**(7) 604–13.
27. Salam U, Ullah S, Tang ZH, Elateeq AA, Khan Y, Khan J, Khan A and Ali S 2023, Plant metabolomics: An overview of the role of primary and secondary metabolites against different environmental stress factors. *Life(Basel)* **13**(3) 706.
28. Sangwan S, Rao DV and Sharma RA 2010, Biochemical estimation of primary metabolites from *Pongamia pinnata* L. An important biodiesel plant. *International Journal of Pharmaceutical Sciences Review and Research*. **5**(1) 146-149.
29. Sarin R and Samria S 2014, Biochemical estimation of selective primary metabolites from two medicinally important plants of Cucurbitaceae. *World journal of pharmacy and pharmaceutical sciences*. **3**(6) 1161-1169.
30. Shinshi H and Noguchi M 1975, Relationship between peroxidases, IAA oxidases and polyphenol-oxidases. *Phytochem.* **14**(5/6) 1255–1258.
31. Sofowora A 1980, The present status of knowledge of the plants used in traditional medicine in western Africa: A medical approach and a chemical evaluation. *J Ethnopharmacol*. **2**(2) 109–18.
32. Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K and Bennett M 2001, Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the Arabidopsis root apex. *Genes and Development.* **15**(20) 2648–2653.

33. Tolambiya P, Jagetiya B and Mathur S 2023. Estimation of total phenolic and flavonoid content and antioxidant activity in *Catharanthus roseus* L. (Alba) plant extract. *Biological Forum – An International Journal*.**15**(2) 1101-1106.
34. Tolambiya P, Jagetiya B and Mathur S 2023, Extraction and identification of various phytopharmaceutical constituent of *Catharanthus roseus* L. (Alba) leaves. *Plant Archives.* **23**(2) 58-165.
35. Tolambiya P and Mathur S 2016, A study on potential phytopharma-ceuticals assets in *Catharanthus roseus* L. (Alba). *International Journal of Life Sciences Biotechnology and Pharma Research*. **5**(1) 1-6.
36. Tolambiya P and Mathur S 2016, Antimicrobial attributes *in-vivo* grown *Catharanthus roseus* L. *EJBPS.* **3**(11) 217-223.
37. Tolambiya P and Mathur S 2017, Identification of cost-effective drug through *in-silico* approach of *Catharanthus roseus* plant ligand-receptor docking for type 2 Diabetes NIDDM. *WJPPS.* **6**(4)1029-1049.
38. Tuhela L, Sims GK and Tuovinen O 1989, *Polymerization of substituted anilines, phenols, and heterocyclic compounds by peroxidase in organic solvents.* The Ohio State University. Columbus, Ohio. Pp. **58**.
39. Yadav PR and Tyagi R 2006, *Lipid Biotechnology*. Discovery Publishing House New Delhi. pp **89**.
40. Yu B, Liu Y, Liu J, Guo X and Tang Z 2022, Integrated analyses of metabolomic profiling and associated gene expression of *Catharanthus roseus* seedling reveal the metabolic alternations of primary metabolites and flavonoids during the apical hook opening phase. *Journal of Plant Growth Regulation* **41**(6) 2093-2107.
41. Zhang S 2023, Recent Advances of Polyphenol Oxidases in Plants. *Molecules* **28**(5) 2158.