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Physico-Chemical Analysis and Phytochemical Characterization of Catharanthus *Roseus* (L.) G. Don in Contaminated Soil and Garden Soil.

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Abstract: The physico-chemical characteristics and phytochemical profiles of *Catharanthus roseus* (L.) G. Don grown in both garden soil and contaminated soil are examined in this study. The purpose of the study is to evaluate how soil pollution affects that plant's growth characteristics, metabolic components, and secondary metabolites. While plant samples were assessed for growth metrics, and phytochemical substances like alkaloids, flavonoids, and phenolics, soil samples were examined for pH, Dissolved solids, Dissolved oxygen and Total hardness and Calcium hardness. The Qualitative test for plant extract of solvent methanol and distilled water is done by Harbone's Qualitative method for detection of secondary metabolites i.e., Alkaloid, Tannin, Phenol, Flavonoid, Saponins and Lignin. The determination of the Total Phenolic Content, Total Tannin Content and Total Flavonoid Content was done by Quantitative analysis with the help of Folin- ciocalteu, Folin- Phenol and Aluminium Chloride methods respectively. Determination of Total Antioxidant Capacity id done by Phosphomolybdenum Assay. The test results of quantitative are all using the concentration of 1 ml of prepare sample extract. The plant extract of solvent methanol and distilled water detects many secondary metabolites like Alkaloid, tannin, phenol, flavonoid, saponins and lignin this all are detect in the phytochemical test by qualitative methods and according to quantitative analysis the pollution affects the phytochemical but the Antioxidant capacity remains same. The findings demonstrate the adaptability of *Catharanthus roseus* (L.) G. Don under challenging circumstances and its potential for application in phytoremediation by showing notable differences in growth and phytochemical composition between plants in contaminated areas and their consequences for environmental health and sustainable agriculture are better understood thanks to this research.

Keywords: Antioxidant, Flavonoids, Phytochemical, Physico-chemical, Phenols.

I. Introduction

Catharanthus roseus (L.) G. Don, is a noteworthy evergreen herb that belongs to the Apocynaceae family of dogbanes. The bloom colors of the two common cultivars of Catharanthus roseus are "Alba" for white flowers and "Roseus" for pink flowers. The plant is often called Madagascar periwinkle because it is indigenous to Madagascar. In the past, a variety of ailments, such as rheumatism, diabetes, menstrual problems, dyspepsia, cancer, hypertension, and skin conditions, were treated using Catharanthus roseus. The plant is rich in bioactive compounds and possesses a broad range of pharmacological properties (Jaleel, et al., 2008). The plant is reportedly cultivated extensively for its alkaloids, which have anticancer properties (Nayak et al., 2007). The main alkaloids found in Catharanthus roseus are used to treat constipation, diabetes, cancer, blood pressure, and asthma. The plant generates two main terpene indole alkaloids with anti-cancer properties: vincristine and vinblastine (Ajaib, et al., 2010). Catharanthus roseus contains significant levels of phenolic and volatile compounds, such as caffeoylquinic acids and flavonol glycosides. These substances are essential to the plant defense system and act as antioxidants against reactive oxygen species (Kabesh, et al., 2015). These compounds also exhibit other anti-inflammatory, antibacterial, anti-allergic, and cardio-protective properties (Kumar, et al., 2015). Catharanthus roseus has medicinal properties, as well as it also possesses properties for phytoremediation. The increasing use of a variety of heavy metals in industry and agricultural has raised serious concern about environmental contamination (Sinhal, et al., 2010). Uncontrolled use of sewage sludge, compost, mining waste, chemical fertilizers, and industrial development all contribute to the accumulation of heavy metals in agriculture areas. The soil is under long-term risk from these metals. Restoring soils polluted with potentially dangerous metals and metalloids is a serious global concern (Shelmerdine, et al., 2009).

II. Materials and Methods

Phytochemical Analysis:

Collection of Samples: *Catharanthus roseus* (L.) G. Don is use for this study and it is collected from two different sites that is, Pirana Landfill Site, AMC Oxygen Park, Science city- Ahmedabad. The soil and water are also collected from the respective sites.

Preparation of Sample Extracts: The plant materials that is, Leaves & Stem, were dried under the sunlight so they get dehydrated. Then the plants part was crushed properly using a mixture and stored for the preparation of extract. Then 10 g of sample powder is dissolved it in solvent like methanol and distilled water respectively. The mixture is allowed to properly

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dissolve in respective solvent. The mixture was separated with the help of filter paper in test-tubes then the liquid extract in a petri- plates was allowed to dry under room temperature. Then the extract is prepared of 0.030 g of dry extract in 30ml of respective solvent.

Qualitative Analysis:

Test for Alkaloids:

Wagner's test: To 1ml of the extract, 1 ml of Wagner's reagent was added in test-tubes. Appearance of brown/reddish color indicates the presence of alkaloids (Raaman, 2006).

Iodine test: To 3 ml of extract, 3-4 drops of iodine solution was added in test-tube. Appearance of blue color indicates the presence of alkaloids (Bhatt, & Dhvani, 2012)

Test for Flavonoids:

Lead acetate test: To 1 ml of extract, few drops of 10% lead acetate was added in test-tube. Appearance of yellow precipitate indicates the presence of flavonoid (De silva, et al., 2017).

Conc. H_2SO_4 test: To 2 ml of extract, 1ml of concentrated H_2SO_4 was added in test-tube. Appearance of orange color indicates the presence of flavonoid (Tyagi, 2017).

Test for Phenol:

Iodine test: To 1 ml of extract, few drops of dil. Iodine solution was added in test-tube. Appearance of red color indicates the presence of phenol (Jagessar, 2017).

Folin-ciocaltue test: To 0.5 ml of extract, 1ml of Folin-ciocaltue was added in test-tube. Appearance of bluish green precipitate indicates the presence of phenol.

Test for Saponins:

Sodium bicarbonate test: To 2 ml of extract, 1 ml of 5% sodium bicarbonate solution was added in test-tube. Appearance of honeycomb like froth indicates the presence of saponins.

Olive oil test: To 2 ml of extract, 2 drops of olive oil was added in test-tubes. Appearance of foam or emulsification indicates the presence of saponins (Dahanayake, et al., 2019).

Test for Lignin:

Labatt test: To 2 ml of extract, few drops of Gallic acid was added in test-tube. Appearance of olive green color indicates the presence of lignin (Bhatt, & Dhyani, 2012).

Test for Tannin:

Lead acetate test: To 2 ml of extract, 1ml of 10% lead acetate solution was added in test-tube. Appearance of white or brownish precipitates indicates presence of Tannin (Sheel, et al., 2014).

Quantitative Analysis:

Determination of Total Phenolic Content (TPC):

The Phenolic phytochemicals are naturally occurring plant compounds that include a hydroxyl (-OH) group attached to an aromatic benzene ring. These materials are necessary for plants to grow, procreate, and defend themselves against environmental stressors like UV radiation, diseases, and herbivores. Folin-Ciocalteu method was used for the determination of TPC. Gallic acid was used as a standard for TPC. 1 ml of extracted was followed by the addition of 3 ml of distilled water, then 0.5 ml of Folin-Ciocalteu was added and then the mixture was allowed for incubation at room temperature for 3 minutes. After incubation, 2 ml of Na_2CO_3 was added, followed by heating the test-tubes in water bath for a minute. With the help of a spectrophotometer, at the wavelength of 650 nm, the absorbance of sample was calculated Using a standard calibration curve ($y = 0.457x + 0.1206$, $R^2 = 0.9987$), the Gallic acid equivalent (GAE) per gram was used to express the total phenol content in the plant extract. (Malik, & Singh, 1980).

Determination of Total Flavonoid Content (TFC):

Flavonoids are a large class of polyphenolic compounds found in plants. The various colors of fruits, vegetables, and flowers are caused by these secondary metabolites. Flavonoids, well-known antibacterial, anti-inflammatory, and antioxidant properties make them essential for human health as well as plant defense. $AlCl_3$ method was used for the determination of TFC. Quercetin was used as a standard for TFC. 1 ml of extract is followed by the addition of 0.3 ml of 5% $NaNo_2$ and the test-tube were let to incubate for 5 minutes at room temperature. After that, addition of 0.3 ml of 10% $AlCl_3$ is done and again test-tube were let to incubate for 6 minutes at room temperature. Now, 3.3 ml of Distilled water is added followed by the addition of 2 ml of 1M

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NaOH. With the help of spectrophotometer, at the wavelength of 510 nm, the absorbance of sample was calculated Using a standard calibration curve ($y = 0.581x + 0.0333$, $R^2 = 0.9989$), the Quercetin equivalent (QE) per gram was used to express the total Flavonoid in the plant extract (Zhishen, et al., 1999).

Determination of Total Tannin Content (TTC):

Tannins are a type of polyphenolic compound found in plants. They can bind to proteins, alkaloids, and other biomolecules as astringent secondary metabolites. Folin-phenol method was used for the determination of TTC. Tannic acid was used as a standard for TTC. 1 ml of extract is followed by the addition of 7.5 ml distilled water and 0.4 ml of Folin-phenol reagent. Then 1 ml of 35% Na_2Co_3 was added and at last, volume is made of 10 ml was done in the test-tubes. Now the test-tubes were allowed to incubate for 30 minutes at room temperature. With the help of a spectrophotometer, at the wavelength of 725 nm, the absorbance of sample was calculated. Using a standard calibration curve ($y = 0.3463x + 0.1179$, $R^2 = 0.9989$), the tannin acid equivalent (TAE) per gram was used to express the total tannin in the plant extract (Lahare, et al., 2021).

Total Antioxidant Capacity- Phosphomolybdenum Assay (PMA):

Phosphomolybdenum assay method was used for the total antioxidant capacity. Ascorbic acid was used as a standard for PMA. Concentration of 0.2 ml of extract was used for the test followed by the addition of 2 ml of phosphomolybdenum reagent. Then the test-tubes were allowed for incubate at 90^o C for 90 minutes in water bath and then allowed them to cool down at room temperature. With the help of a spectrophotometer, at the wavelength of 765 nm, the absorbance of sample is calculated. Using a standard calibration curve ($y = 0.558x + 0.258$, $R^2 = 0.9943$), the Ascorbic acid equivalent (AAE) per gram was used to express the total Antioxidant capacity in the plant extract (Prieto, et al., 1999).

Physico-chemical Tests of Soil and Water:

pH test

The pH of sample was calculated using digital pH meter. For water directly measure the pH with the help of pH meter. For soil, 1 part of soil is mixed with 2 parts of distilled water e.g., 10 g of soil is mixed with 20 ml of distilled water and mixed well then the mixture was allowed to settled down and the pH was measured of extracted water.

Total Dissolved Solids Test (TDS)

The TDS of samples was calculated with the help of TDS meter. For water directly measure the TDS with TDS meter. For soil, 10 g of soil is mixed in 20 ml of distilled water, the mixture is allowed to settle and then the TDS value is measured.

Total Hardness Test

For soil, 10 gm of soil was added to 50 ml of distilled water and then extracted water is used for the total hardness test. In a flask of 250 ml, to 10 ml of sample water, 2-3 drops of Eriochrome Black-T indicator was added, which turns red if hardness is present. Then 1-2 ml of ammonium buffer solution was added to flask. The burette is filled with EDTA. Start titrating the solution against EDTA. The color will change from red to blue (Indalkar, et al., 2023).

Total hardness = (Eq weight of $CaCo_3$ x N of EDTA x Vol of EDTA used)/ (Vol of water sample) x 1000

Calcium Hardness Test

For soil take 10 g of soil in 50 ml of distilled water and mix properly and the extracted water is used for the calcium hardness test. To 10 ml of sample water was added to the flask followed by the addition of 2-3 drops of EB-T indicator and then 2 ml of ammonium buffer solution to maintain pH of the solution. The burette is filled with EDTA and start titrating. The color will change from red to blue.

Total hardness = (Eq weight of Ca^{+3} x N of EDTA x Vol of EDTA used)/ (Vol of water sample) × 1000

Dissolved Oxygen Test (Winkler Titration Method)

For soil mix 10 g of soil to 50 ml of distilled water and mix properly, then the extracted water is used for the dissolved oxygen test. In a BOD bottle 1 ml of 48% Manganese sulfate solution was added to water sample. The bottle was closed and the precipitate form of manganese dioxide. Then 1 ml of alkaline iodide solution was added followed by the addition of 1 ml of con. sulfuric acid. The burette is filled with 0.02 N of sodium thiosulfate. In a conical flask of 250 ml, 10 ml of solution from BOD bottle was taken and titrated against sodium thiosulfate. When yellow color appeared, a few drops of starch indicator were added, the color changed to blue. The titration continued till blue color disappear (Bruckner, 2011).

Dissolve Oxygen = (Vol of sodium thiosulfate used x N of sodium thiosulfate x weight of oxygen)/ (Vol of water sample) × 1000

III. Results and Discussion

Phytochemical Test Results:

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Qualitative analysis:

Plants naturally contain chemical molecules called phytochemicals that give them their color, flavor, and resistance to disease. In addition to being essential for the growth, defense, and reproduction of plants, these substances have major positive effects on both human and animal health when ingested. Several classes of phytochemicals are distinguished, such as phenolic acids, alkaloids, flavonoids, Saponins, and Lignin.

The table below shows the results of phytochemicals present in different parts of plant from different sites.

Table 1: Phytochemical screening of plant extract

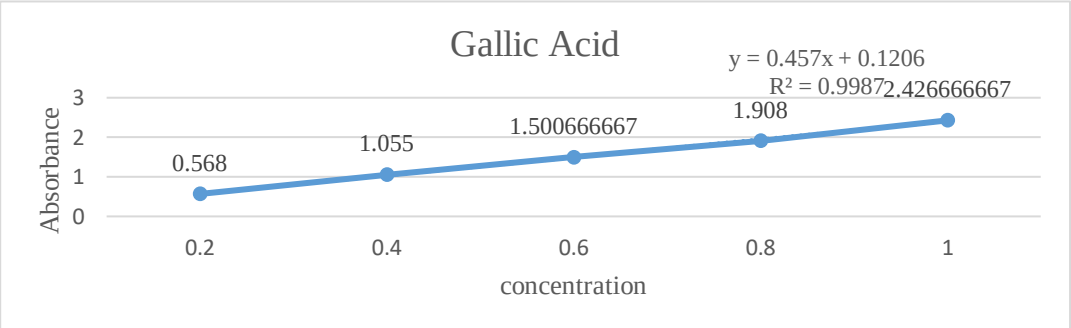
Sr. No	Phyto-chemicals	Solvent	Plant Samples			
			Stem (Garden site)	Leaf (Garden site)	Stem (Dump site)	Leaf (Dump site)
1	Alkaloids	Methanol	+	+	+	+
		D.W	-	-	+	-
2	Flavonoids	Methanol	+	-	-	-
		D.W	+	+	+	+
3	Phenols	Methanol	+	-	+	-
		D.W	+	-	+	-
4	Tannin	Methanol	-	-	-	-
		D.W	+	+	+	+
5	Saponins	Methanol	+	+	+	+
		D.W	+	+	+	+
6	Lignin	Methanol	+	+	+	+
		D.W	-	+	-	+

The '+' sign indicates the presence and '-' sign indicates the absence of secondary metabolites in the sample plant extract.

Discussion: Phytochemical screening of plant extracts shows the presence of Alkaloids, Flavonoids, Tannin, Phenols, Saponins, and Lignin. Alkaloid, Saponins and lignin were detected in methanol extracts whereas flavonoids and tannins were majorly detected in distilled water extracts. Flavonoids were not detected in either of stem extracts. Research published by R. Lahare, et al., 2021 also reported the presence of saponins, tannin, flavonoid, alkaloid, and phenolic compounds in *Catharanthus roseus*.

Quantitative Analysis

Total Phenolic Content (TPC) The possible antioxidant activity of plant phenolics found in plants has drawn a lot of interest (Dziedzic & Hudson, 1983). Plants contain phenolic compounds in large quantities (Li, Smith, & Hossain, 2006). These compounds have drawn a lot of interest because of their antioxidant properties and capacity to scavenge free radicals, which may have positive effects on human health (Govindarajan, Singh, & Rawat, 2007). Total phenol content (TPC) was calculated and expressed as mg GAE/g dry sample after comparison with standard gallic acid.



Graph 1: Standard for TPC

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Table 2: Results for TPC (Methanol)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/GAE
1	Stem (Garden site)	Methanol	1 ml	0.2296	23.86±0.009
2	Leaf (Garden site)		1 ml	2.7056	56.566±0.037
3	Stem (Dump site)		1 ml	2.322	48.185±0.009
4	Leaf (Dump site)		1 ml	0.231	24.15±0.047

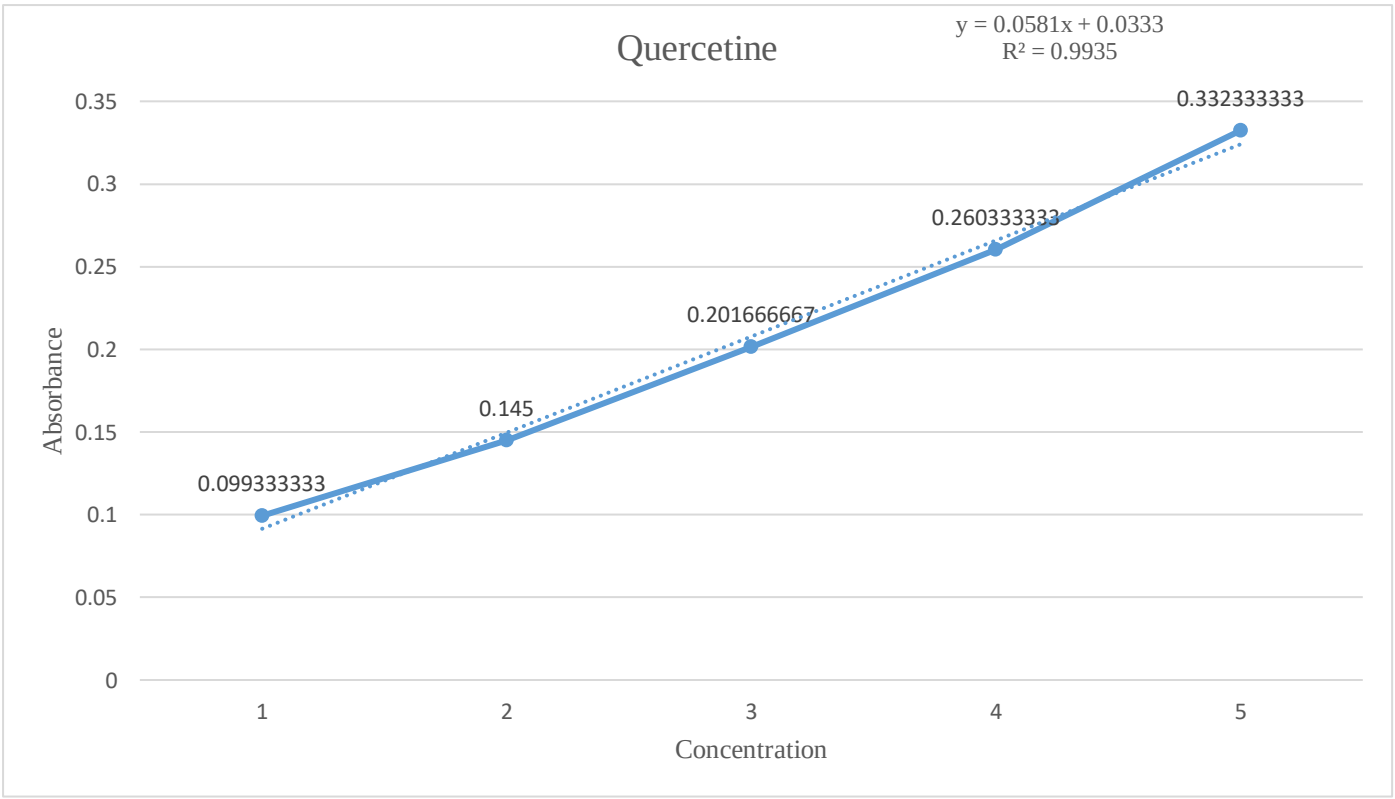
Table 3: Results for TPC (Distilled Water)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/GAE
1	Stem(Garden site)	Distilled Water	1 ml	0.3246	44.65±0.013
2	Leaf(Garden site)		1 ml	0.0453	44.53±0.018
3	Stem (Dump site)		1 ml	0.4421	44.21±0.009
4	Leaf (Dump site)		1 ml	1.0563	10.56±0.019

Discussion: The fresh parts of plant that is Stem and Leaf shows higher phenolic content with value of 23.86±0.009 mg/GAE and 56.566±0.037 mg/GAE in methanol and 44.65±0.013 mg/GAE, and 44.53±0.018 mg/GAE in distilled water respectively, as compared to the dump site plant part. R. Lahare, et al., 2021 reported that Stem contains higher phenolic content than leaves. So based on the comparison, the polluted soil affects the plant which lowers the concentration of Phenolic content.

Total Flavonoid Content (TFC)

Flavonoids are physiologically active phytochemicals that are ubiquitous in the plant kingdom which are being employed in many herbal remedies for many years now. They are a necessary component of our regular diet. They mostly gather in the plant portions that can be eaten. Total Flavonoid content (TFC) was calculated and expressed as mg QE/g dry sample after comparison with standard Quercetine (Mathesius, 2018).



Graph 2: Standard for TFC

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Table 4: Results for TFC of plant Extract (Methanol)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/QE
1	Stem(Garden site)	Methanol	1 ml	0.234	34.54±0.014
2	Leaf (Garden site)		1 ml	0.2456	36.55±0.020
3	Stem (Dump site)		1 ml	0.1936	27.601±0.013
4	Leaf (Dump site)		1 ml	0.2003	28.749±0.0165

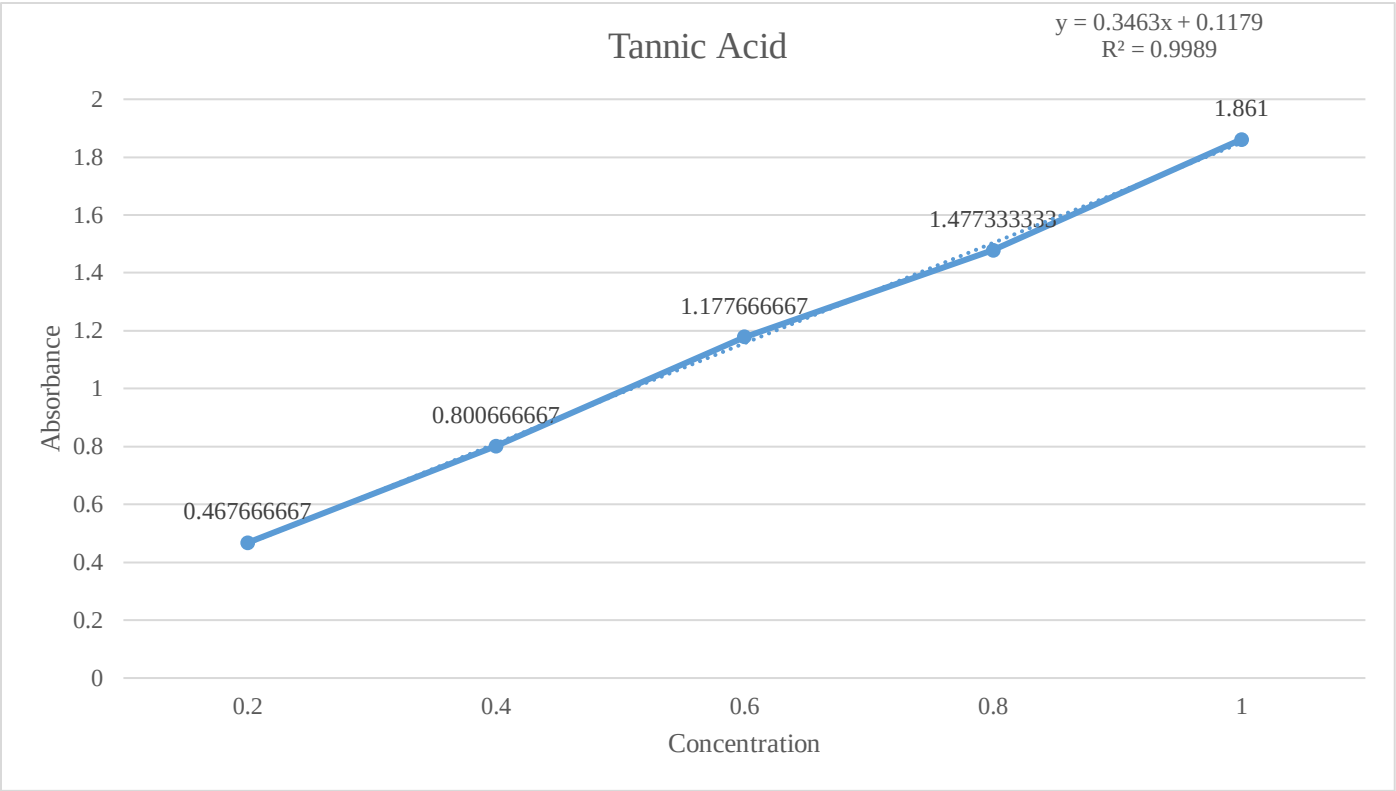
Table 5: Results for TFC of Plant Extract (Distilled Water)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/QE
1	Stem (Garden site)	Distilled Water	1 ml	0.123	15.438±0.004
2	Leaf (Garden site)		1 ml	0.105	12.34±0.003
3	Stem (Dump site)		1 ml	0.1103	13.258±0.005
4	Leaf (Dump site)		1 ml	0.128	16.299±0.001

Discussion: The fresh parts of plant that is stem and leaf shows the higher flavonoid content with value of 34.54±0.0144 mg/QE, and 36.55±0.020 mg/QE in methanol and 15.438±0.004 mg/QE, 12.34±0.003 mg/QE in distilled water respectively, compared to the dump site plant part. R. Lahare, et al., 2021 reported that leaf contains higher amount of flavonoid. According to the values obtained, the polluted soil affects the plant phytochemical especially Flavonoid which results in lower concentration.

Total Tannin Content (TTC)

Tannins help plants fight against diseases and herbivores by preventing feeding and preventing the growth of microorganisms. A varied class of compounds found in plants, tannins have both noteworthy sensory qualities and possible health benefits. Understanding their characteristics is crucial from a nutritional and culinary standpoint, as their presence varies greatly amongst plant species and food products. Total Tannin content (TTC) was calculated and expressed as mg TAE/g dry sample after comparison with standard tannic acid (Robbins, et al., 1987).



Graph 3: Standard for TTC

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Table 6: Results for TTC of Plant Extract (Methanol)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/TAE
1	Stem (Garden site)	Methanol	1 ml	1.9066	51.653±0.246
2	Leaf (Garden site)		1 ml	1.4433	38.27±0.367
3	Stem (Dump site)		1 ml	2.021	54.955±0.220
4	Leaf (Dump site)		1 ml	2.511	69.124±0.167

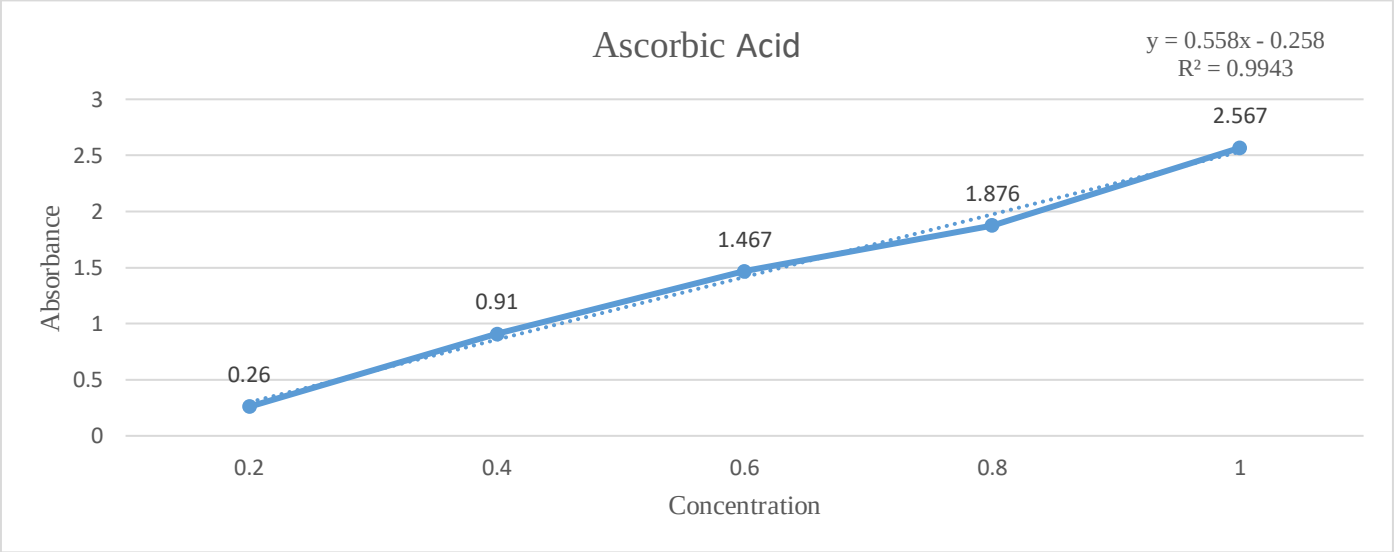
Table 7: Results for TTC of Plant Extract (Distilled Water)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/TAE
1	Stem (Garden site)	Distilled Water	1 ml	0.1956	22.45±0.0136
2	Leaf (Garden site)		1 ml	0.265	42.47±0.008
3	Stem (Dump site)		1 ml	0.1793	17.73±0.008
4	Leaf (Dump site)		1 ml	0.184	19.08±0.0135

Discussion: The dump site plant parts that is stem and leaf have shown higher tannin content with the value 54.955±0.220 mg/TAE, and 69.124±0.167 mg/TAE in methanol and 17.73±0.008 mg/TAE, and 19.08±0.0135 mg/TAE in distilled water respectively, compare to fresh plant parts. According to R. Lahare, et al., 2021, leaf shows higher concentration of tannin as compare to other parts. From the values obtained, the tannin present in the plant does not affect by polluted soil.

Total Antioxidant Capacity

Antioxidant capacity in plant phytochemicals refers to the ability of numerous non-nutritive chemical compounds produced by plants to protect cells from harm caused by free radicals. These unstable molecules, known as free radicals, can harm lipids, proteins, and DNA in cells, which can lead to aging and a number of chronic illnesses (Ahmad, & Umar, 2011).



Graph 4: Standard for PMA

Table 8: Results for PMA of Plant Extract (Methanol)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/AAE
1	Stem (Garden site)	Methanol	0.2 ml	0.1506	73.23±0.0005
2	Leaf (Garden site)		0.2 ml	0.405	11.88±0.002
3	Stem (Dump site)		0.2 ml	0.1996	82.01±0.008
4	Leaf (Dump site)		0.2 ml	0.255	9.19±0.013

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Table 9: Results for PMA of Plant Extract (Distilled Water)

No.	Plant Sample	Solvent	Concentration	Absorbance	mg/AAE
1	Stem (Garden site)	Distilled Water	0.2 ml	0.124	68.45±0.008
2	Leaf (Garden site)		0.2 ml	0.158	74.61±0.002
3	Stem (Dump site)		0.2 ml	0.132	69.89±0.005
4	Leaf (Dump site)		0.2 ml	0.1676	76.28±0.001

Discussion: The total antioxidant capacity results shows that in methanol fresh stem has 73.23±0.0005 mg/AAE, for fresh leaf 11.881±0.0026 mg/AAE, for dump stem 82.01±0.0086 mg/AAE, and for dump leaf 91.93±0.0135 mg/AAE. In distilled water for fresh stem 68.45±0.008 mg/AAE, for fresh leaf 74.61±0.0020 mg/AAE, for dump stem 69.89±0.0051, and for dump leaf 76.28±0.0011 mg/AAE. The results show that the antioxidant capacity of both plant extracts was found to be similar. According to the results, the antioxidant properties of plant is not affected by the polluted soil as it shows same concentration.

Physico-Chemical Test Results:

The quality of soil and water, as well as their capacity to sustain biodiversity, promote plant development, and supply clean water for a range of applications, are largely determined by their physico-chemical characteristics. These attributes encompass a variety of chemical and physical traits that affect human activity and the environment.

Table 10: Results for Physico-chemical tests of soil and water

No.	Sample	pH	TDS	Total Hardness	Calcium Hardness	Dissolved Oxygen
1	Water (Garden site)	6.19 pH	171 ppm	4513 ppm	122 ppm	238 ppm
2	Water (Dump site)	5.77 pH	292 ppm	4546 ppm	530 ppm	383 ppm
3	Soil (Garden site)	6.48 pH	002 ppm	2066 ppm	632 ppm	362 ppm
4	Soil (Dump site)	7.89 pH	007 ppm	2993 ppm	581 ppm	462 ppm

Discussion: According to pH results fresh site water is less acidic than dump site water and fresh site soil is slightly acidic and dump site soil is slightly basic. According to TDS results, Dump site water and soil has higher dissolved solids compare to fresh site soil and water.

The total hardness results show that the dump site of soil and water has higher total hardness than the fresh site of soil and water. According to calcium hardness test, dump site water and fresh site soil has more calcium hardness compare to fresh site water and dump site soil respectively. Dissolved oxygen results shows that the dump site of water and soil has higher dissolved oxygen than the fresh site of soil and water.

Conclusion

Many secondary metabolites, including alkaloids, tannins, phenols, flavonoids, saponins, and lignins, are detected by the plant extract of solvent methanol and distilled water. According to the TPC and TFC data, the plant parts from the fresh site have higher concentration of Flavonoids and phenol respectively, than the dump site plant parts. According to TTC data, plant parts from dump site have a higher tannin content than plant parts from fresh sites. The antioxidant capacity of both site plants is the same, according to the results of the phosphomolebdenum assay for total antioxidant capacity.

The physico-chemical test of soil and water reveals that dump site water and soil exhibit higher levels of acidity, total dissolved solids, Dissolved oxygen, and total hardness compare to fresh site soil and water. Calcium hardness of fresh site soil and dump site water is higher than dump site soil and fresh site water.

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