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THE QUANTITATIVE ANALYSIS OF METABOLITES FROM SELECTED CHENOPODIUM SPECIES

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ABSTRACT: *Chenopodium album* L., *Chenopodium murale* L., and *Chenopodium giganteum* L. are annual herbs with ethno-medicinal value. The present study investigates the primary and secondary metabolites quantification of *Chenopodium* species. The quantitative estimation of bioactive molecules from the root, stem, and leaves showed the presence of proteins, carbohydrates, starch, phenols, and flavonoids. However, these metabolites vary in root, stem, and leaves. The result obtained indicates that *Chenopodium album* leaves contain the highest amount of protein (9.515 ± 0.119 mg/gdw equivalent of bovine serum albumin), *Chenopodium murale* root contains the highest amount of carbohydrates (39.798 ± 0.105 mg/gdw equivalent of glucose) and starch (19.294 ± 0.183 mg/gdw equivalent of glucose), *Chenopodium giganteum* leaves contains the highest amount of flavonoids (12.397 ± 0.121 mg/gdw equivalent of quercetin), *Chenopodium murale* leaves contains the highest amount of phenol (4.882 ± 0.056 mg/gdw equivalent of gallic acid), among the other species. The data were analyzed by one-way ANOVA. $p<0.05$ and F-statistical value > F-critical value were considered the minimum significance level.

INTRODUCTION: Since prehistoric times, people have recognized and utilized the healing properties of certain plants, commonly referred to as medicinal herbs, in traditional medicine. Plants produce a multitude of chemical compounds for different reasons such as protecting themselves from insects, fungi, diseases, and herbivorous animals [1]. During the COVID-19 pandemic, the market for dietary supplements and nutraceuticals experienced a growth of 5% [2]. Non-industrialized societies often rely on medicinal plants due to their availability and affordability compared to modern medicines.

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Traditional medicine lacks regulation in many countries, but the WHO has established a network to encourage safe and reasonable usage [3]. Plants contain a variety of polyphenols that serve different purposes, such as protecting against diseases and predators. For centuries, plants containing hormone-like substances called phytoestrogens have been used to treat various gynaecological disorders, including menstrual, fertility, and menopausal problems. These plants also contain astringent tannins [4].

Numerous synthetic and semi-synthetic drugs are altered versions of alkaloids, to improve or alter the main impact of the drug while minimizing any undesired side effects. One such example is naloxone, which acts as an opioid receptor antagonist and is a derivative of thebaine, a component found in opium [5]. In animals, neurotransmitters like serotonin, dopamine, and histamine are crucial alkaloid-related substances.

Alkaloids are capable of regulating the growth of plants [6]. Organisms produce small molecules through metabolic pathways, known as secondary metabolism, which are not essential for their growth or reproduction [7]. Plants utilize secondary metabolites for various purposes, including promoting growth and development, enhancing innate immunity, adapting to environmental stressors, and facilitating critical defenses mechanisms and signalling processes [8, 9]. Medicinal plants contain bioactive compounds such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids that can interact with the body and produce physiological effects [10]. Young leaves of the *Chenopodium album* display excellent taste. Notably, high levels of proteins and ashes were identified, alongside a rich concentration of essential nutrients such as fat, calcium, iron, zinc, ascorbate, and carotenes [11]. *Chenopodium* grows in various climates and is a great source of protein, minerals, and vitamin C. People in different regions with tropical climates, like India and South America, cultivate many wild species of *Chenopodium* for human and livestock consumption. These are annual weeds that grow in orchards, fallow lands, gutters, corrals, and other places. They are usually collected in the winter and spring, cooked like spinach, and their seeds are used to make bread. However, these plants contain several anti-nutritional and toxic substances, such as nitrate, oxalate, and saponins, making them difficult to use for their nutritional benefits [12]. *Chenopodium album* L. is a white, mealy herb that is annual and many-branched. It can grow up to 20-200 cm tall and stands erect **Fig. 1A** [13]. *Chenopodium murale* L. is a medicinal plant found in the United States, Brazil, Mexico, Argentina Republic, Puerto Rico, Barbados, and India. *Chenopodium murale* L. grows in waste areas, on garbage, on the side of the road, and in crops **Fig. 1B** [14]. This plant belongs to the Chenopodiaceae family, comprising 102 genera and 1400 species of annual and perennial herbs and shrubs worldwide [15]. The *Chenopodium murale* L. is an erect, yearly growing plant that can reach up to 60 cm in height. Its leaves are ovate, with angular sides and lobes. They are sharp teeth, with a wedge-shaped base, and can be stalk, either long or short, measuring between 2-8 x 1-6 cm in breadth [16].

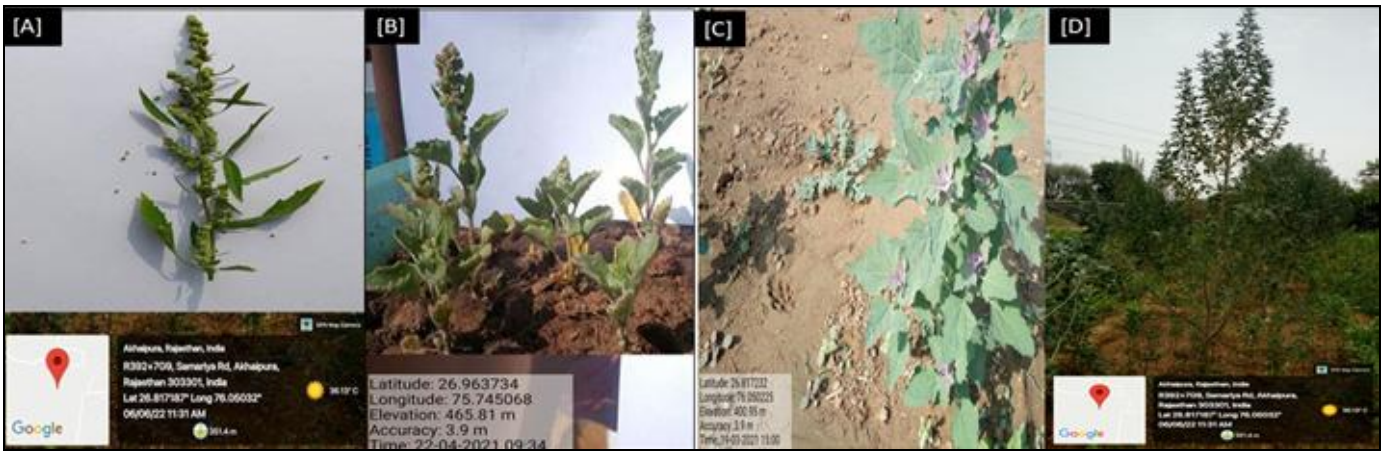

FIG. 1: [A] *CHENOPODIUM ALBUM L.* [B] *CHENOPODIUM MURALE L.* [C] *CHENOPODIUM GIGANTEUM L. GROWTH PHASE,* [D] *CHENOPODIUM GIGANTEUM L.* OLD STAGE)

Tree spinach, scientifically named *Chenopodium giganteum L.*, is a tall and multi-branched shrub that grows up to 3 meters high. Its stem can have a diameter of 5 cm at the base and is an annual plant. The leaves of *Chenopodium giganteum* start magenta and hairy when they are young, but turn green, as they get older. The shape of the leaves can be rhombic to ovate, and they can have a surface area of up to 20 x 16 cm. The plant's inflorescence consists of terminal panicles that contain hermaphrodite flowers. The flowers' pollination is solely reliant on the wind **Fig. 1C, 1D** [17]. This study aims to conduct a phytochemical analysis, including isolation, qualitative assessment, and quantification, on specific species of *Chenopodium.*

MATERIALS AND METHOD:

Sample Collection and Authentication: *Chenopodium* species (*Chenopodium album* L.,

Chenopodium giganteum L., and *Chenopodium murale* L.) were collected from Bassi (Jaipur), Rajasthan, and authenticated by The Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India. The plant parts were hand-harvested, cleaned, dried, ground, and stored in an airtight container.

Preparation of Extracts:

Total Protein: Homogenized 1g of dried plant powder with 10 mL of 10% TCA for 30 min help of a magnetic stirrer and then keep it at 4 °C for 24 hours. Centrifuge it for 5 min at 10,000 rpm. After the centrifugation, carefully pour out the liquid part (supernatant) and put it in a separate test tube. Then, mix the solid part (pellet) with 10 mL of 5% TCA. Hot on water bath at 80 °C for 30 min and cold in freeze. Centrifuge it for 5 min at 10,000 rpm. Separate the supernatant and the pellet dissolved in 10 mL of 5% TCA. The pellet was washed with distilled water and centrifuged for 5 min at 10,000 rpm. The supernatant was discarded and the residue dried in a hot oven at 30 °C. The dried residue was dissolved in 10 mL of 1N NaOH and left at room temperature for 48 hours. Filtrate and make the volume 25 mL with 1N NaOH. All extracts were stored in the freezer until used [18].

Total Carbohydrates: Take 100 g of the dried powder of each sample and glucose standard in a boiling test tube. To each boiling test tube add 5 mL of prepared solution of 2.5 N HCl. Each test tube was boiled in a 100°C water bath for 2-3 hours, then cooled to room temperature. Sodium carbonate was added to each sample until the effervescence ceased.The mixture was separated by centrifugation or filtration to obtain the supernatant. Each sample was then, brought up to a total volume of 100 mL using distilled water [19].

Total Starch: Mix 500 mg dried powder in 80% ethanol with the help of a magnetic stirrer heat it at 20 °C for 30 min, and left 24 hours at room temperature. After 24 hours, each sample was centrifuged at 10,000 rpm for 5 min and dried with the help of a hot incubator at 30 °C. The residue was added to 5 mL distilled water, 6.5 mL of 52% perchloric acid, and cold in freeze overnight at 4 °C. All samples were centrifuged and collected supernatants. Fresh perchloric acid should be used to repeat the extraction four times. Centrifuged and pooled the supernatants and made up to 100 mL with distilled water and stored in the freeze until used [20].

Total Phenol: Dried samples (500 mg each) were extracted in 5 mL of 70% methanol in a shaker at 25°C for 12 hours. with non-stop shaking and sonicated at 50 Hz for 15 min. The extracts were kept at ambient temperature overnight. The extracts were centrifuged at 3,000 rpm for 5 min, and the supernatant was collected and dried in an oven at 30 °C. Each extract was diluted with 10 mL of 70 % methanol and kept at 4°C until used.

Total Flavonoids: 200 mg dry powder dissolved in 10 mL of 80% acetone and left overnight. The sample was centrifuged at 10,000 rpm for 5 min. All sample supernatants were collected and stored in the freeze until used.

Chemicals and Reagents:

Preparation of Reagents for Protein Estimation: Reagent-I: 48 mL A(2% Na_2CO_3 in 0.1 N NaOH: 2g Na_2CO_3 and 0.1g NaOH dissolved in 100 mL distilled water.**)** + 1 mL B (1% Na-K Tartrate in H_2O: 1g Na-K Tartrate dissolved in 100 mL distilled water.) + 1 mL C (0.5% $CuSO_4.5H_2O$ in H_2O:500 mg Cupric Sulphate Pentahydrate mixed in 100 mL distilled water.) Reagent-II: 1 part of Folin-Phenol reagent and 2 parts distilled water mix well.

Preparation of Anthrone Reagent for Carbohydrates and Starch Estimation: Anthrone reagent was prepared before starting the experiment. 200 mg anthrone powder was mixed in freeze-cooled 95% H_2SO_4. After shaking, it was kept at 4°C for 40 min. All chemicals were used in analytical grade.

Quantitative Determination of Metabolites:

Estimation of Total Protein: The total protein content of *Chenopodium* species extracts was determined according to the method of Lowry *et al*., 1956 [21]. Bovine serum albumin was used as a reference standard for plotting the calibration curve. Using a micropipette, working standard solutions of 50, 100, 150, 200, 250, 300, 350, and 400 µg/mL were created from the prepared bovine serum albumin stock (1 mg/1mL) in separate tubes and 0.2 mL aliquots of each extract for analysis.

Prepare a blank solution by adding 1 mL of water. Add 5 mL of the reagent-I and incubate for 10 min at room temperature. After 10 min 0.2 mL of the reagent-II was mixed, vortex well, and left at room temperature at 30 min. The reaction mixture turns light to a dark blue colour whose absorbance is measured at 750 nm against the reagent blank using a UV-1800 (Shimadzu) spectrophotometer. The total protein content was expressed as mg/g dry-weight equivalent of bovine serum albumin.

Estimation of Total Carbohydrates: The total carbohydrate contents were determined bythe phenol sulphuric acid method [22]. Using a micropipette, working standard solutions of 10, 20, 40, 60, 80, and 100µg/mL were created from the prepared glucose stock (100 mg/100 mL) in separate tubes and 0.2 mL aliquots of each extract for analysis and prepared a blank with 1 mL of water. For each test tube, add 1 mL of phenol solution and shake thoroughly. Add 96% concentrated sulfuric acid to each test tube and vortex. After 10 min, the test tube was placed in a water bath, heated at 25°C to 30°C for 20 min and cooled in ice rapidly. The yellow-to-orange colour produced is proportional to the total carbohydrate amount measured by the UV-1800 Shimadzuspectrophotometer at 490 nm. Through graphical, absorbance vs. concentration, the calibration curve was created. The total carbohydrate content was expressed as mg/g dry-weight equivalent of glucose.

Estimation of Total Starch: The total starch content was determined by the an throne reagent method[23]. Using a micropipette, working standard solutions of 10, 20, 40, 60, 80, and 100µg/mL were created from the prepared glucose stock (1 mg/mL) in separate tubes and 0.2 mL aliquot of each extract for analysis.

Prepared a blank with 1 mL of water. Add 4 mL of an throne reagent solution to each test tube and vortex well.Left room temperature for 6 min and cool in ice rapidly. The light-to-dark green colour produced is proportional to the amount of total starch present and measured by aUV-1800 Shimadzuspectrophotometer at 630 nm. The calibration curve created absorbance vs. concentration. The total starch content was expressed as mg/gdw equivalent of glucose.

Estimation of Total Phenol: Total Phenol content in the methanol extracts quantified with slight modification [24, 25]. Prepare the 70 % methanol stock solution of gallic acid with a concentration of mg per mL. The working standard solution of gallic acid (25, 50, 100, 150, 200, 250 and 300 µg/mL) was prepared. 0.5 mL methanol extract and the working standard solution of gallic acid diluted with 25 mL distilled water. Adding 1 mL of folin-ciocalteu reagent and the mixture allowed on stand for 5 min at room temperature. The mixture was added to 2 mL of 7.5% sodium carbonate and a boiling water bath for 1 min. The mixture was incubated dark for 15 min at room temperature. Absorbance was measured against the blank at 650 nm of the blue-coloured solution with a UV-1800 Shimadzu spectrophotometer. Total phenol content calculated as mg of gallic acid equivalent (GAE) per gram of dry weight. The experiments were run three times.

Estimation of Total Flavonoid: Total flavonoid content was determined by the aluminium chloride method [26]. Take 1 mL aliquot from a stored sample. A stock solution of 1 mg/mL quercetin was prepared in 80% acetone. From this stock, 20, 50, 100, 150, 200, 250, 300, 350, and 400 µl samples were taken into distinct test tubes and made up the volume of 1mL with appropriate dilution. All the test tubes were mixed with 2 mL distilled water. Add 0.5 mL sodium nitrite, and after for 5 min 0.5 mL aluminium chloride hexahydrate and vortex. Left room temperature for 5 min and add 2 mL of 1M sodium hydroxide. All samples were incubated for 15 min in dark conditions at room temperature. The light yellow to orange colour produced is directly proportional to the amount of flavonoid. The absorbance was measured at 510 nm against a blank sample, using a UV-1800 Shimadzu spectrophotometer. The calibration curve was created as absorbance vs. concentration. The content of total flavonoid was calculated as mean ± SD (n = 3) and expressed as mg/gdw of quercetin equivalents.

Statistical Analysis: All statistical analyses were performed in triplicate. Analysis of data performed using Microsoft Excel and Word 2016. The data were scrutinized by one-way ANOVA followed by Microsoft Excel Single Factor Test. $p < 0.05$ and F-

statistical value > F-critical value were considered the minimum significance level.

RESULTS AND DISCUSSION:

Estimation of Total Protein: To determine the contents of total protein in different species of *Chenopodium*, bovine serum albumin standard was used. The described method was implemented quantitatively to evaluate total protein contents in *Chenopodium* species. The highest total protein content was found in *Chenopodium album* leaves (9.515 ± 0.119 mg/gdw equivalent of bovine serum albumin) as compared to other *Chenopodium* species roots, stems, and leaves. The F-critical value for α = 0.05 is 9.552. Since, the observed F-statistical value is 29.897, which is greater than the F-critical value of 9.552, we can reject the null hypothesis. Additionally, the p-value (0.01) is less than 0.05. Therefore, we have enough evidence to conclude that the test is significant **Table 1** and **Fig. 2.**

TABLE 1: TOTAL PROTEIN CONTENT IN SOME SELECTED *CHENOPODIUM* SPECIES

***Chenopodium* Species**	**Total protein content in mg/gdw equivalent of bovine serum albumin**		
	Root	**Stem**	**Leaves**
Chenopodium album	4.903 ± 0.108	4.392 ± 0.143	9.515 ± 0.119
Chenopodium murale	6.377 ± 0.096	6.066 ± 0.146	8.018 ± 0.145
Chenopodium giganteum	6.685 ± 0.093	5.442 ± 0.086	7.892 ± 0.212

All data expressed mean and standard deviation as triplicates. All analyses were carried out in triplicate for each extract. The data were scrutinized by one-way ANOVA followed by Microsoft Excel Single Factor Test (F-statistical value= 29.897, p-value= 0.01 and F-critical value= 9.552). $p < 0.05$ and F-statistical value > F-critical value were considered the minimum significance level.

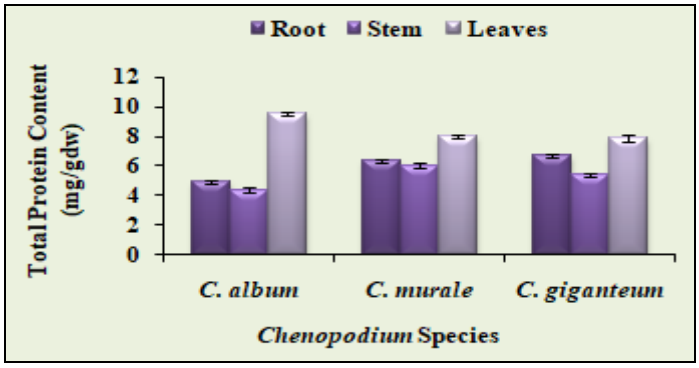

FIG. 2: BAR SHOWING TOTAL PROTEIN CONTENTS OF *CHENOPODIUM* SPECIES. ALL DATA EXPRESSED MEAN AND STANDARD DEVIATION AS TRIPLICATES. ALL ANALYSES WERE CARRIED OUT IN TRIPLICATE FOR EACH EXTRACT. THE DATA WERE SCRUTINIZED BY ONE-WAY ANOVA FOLLOWED BY MICROSOFT EXCEL SINGLE FACTOR TEST (F-STATISTICAL VALUE= 29.897, P- VALUE= 0.01 AND F-CRITICAL VALUE= 9.552). P < 0.05 WAS CONSIDERED THE MINIMUM LEVEL OF SIGNIFICANCE. *C* = *CHENOPODIUM.*

Determination of BSA calibration curve: A calibration curve was plotted by using the absorbance (750 nm, UV-1800 Shimadzu) and concentrations (50, 100, 150, 200, 250, 300, 350, and 400 µg/mL).

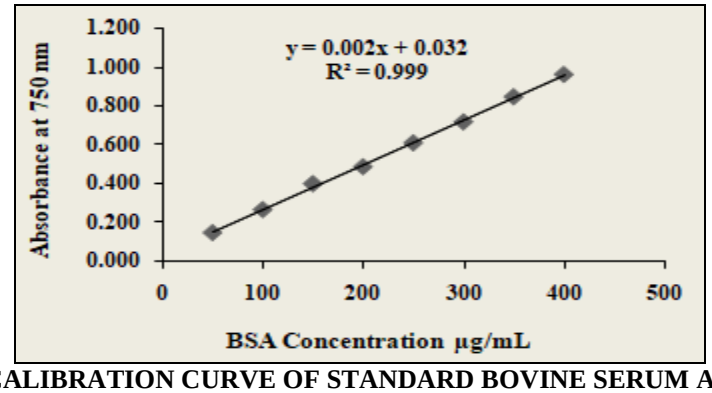

FIG. 3: CALIBRATION CURVE OF STANDARD BOVINE SERUM ALBUMIN

The linear regression analysis found the equation y = 0.0023x + 0.0323 and R^2 with a coefficient of 0.999. Where x represents the total protein content in the extracts and y indicates the absorption at 750 nm (UV-1800 Shimadzu). As a result, the estimated straight line explained the experimental data. The total protein content of the extracts was calculated and expressed as mg bovine serum albumin equivalents (BSAE) per gram of sample in dry weight (mg/g). The Lowery method involves two chemical reactions. Firstly, copper ions are reduced under 'alkaline conditions' and form a complex with peptide bonds. Secondly, the copper peptide bond complex reduces the 'folin ciocalteu reagent', resulting in a colour change of the solution to blue. This colour change can be detected at an absorption of 750 nm using a UV-1800 Shimadzu spectrophotometer **Fig. 3** & **4.**

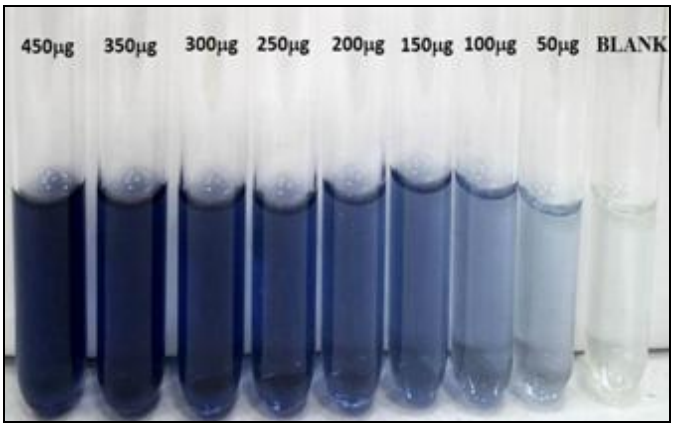

FIG. 4: SHOWING THE RESULT OF STANDARD WORKING SOLUTION WITH DIFFERENT CONCENTRATIONS OF BOVINE SERUM ALBUMIN COMPARED TO BLANK WITH THE INTENSITY OF BLUE COLOUR

Estimation of Total Carbohydrates: To determine the contents of total carbohydrates in different species of *Chenopodium*, a glucose standard was used. The described method was implemented quantitatively to evaluate total carbohydrate contents in *Chenopodium* species. The highest total carbohydrates were found in *Chenopodium murale* root (39.798 ± 0.105 mg/gdw) and the minimum *Chenopodium album* leaves (17.137 ± 0.029 mg/gdw among the *Chenopodium* species. The F-critical value for α = 0.05 is 9.552. Since the observed F-statistical value is 12.271, which is greater than the F-critical value of 9.552, we can reject the null hypothesis. Additionally, the p-value (0.035) is less than 0.05. Therefore, we have enough evidence to conclude that the test is significant **Table 2** and **Fig. 5.**

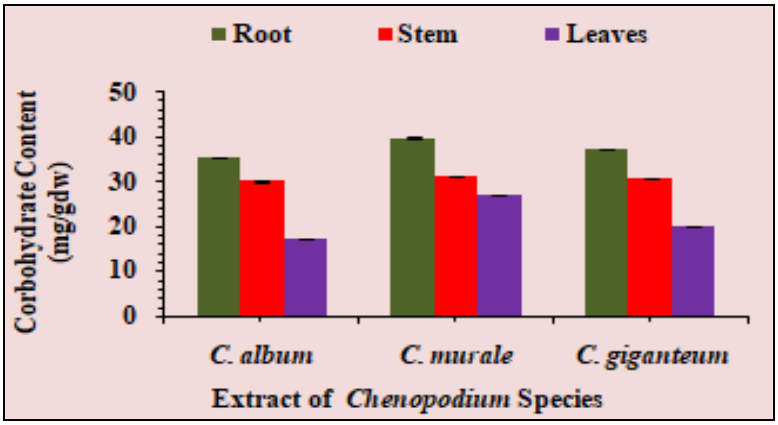

FIG. 5: TOTAL CARBOHYDRATE CONTENT IN EXTRACTS OF SELECTED *CHENOPODIUM* SPECIES. ALL DATA EXPRESSED MEAN AND STANDARD DEVIATION AS TRIPLICATES. ALL ANALYSES WERE CARRIED OUT IN TRIPLICATE FOR EACH EXTRACT. THE DATA WERE SCRUTINIZED BY ONE-WAY ANOVA FOLLOWED BY MICROSOFT EXCEL SINGLE FACTOR TEST (F-STATISTICAL VALUE= 12.271, P- VALUE= 0.035, AND F-CRITICAL VALUE= 9.552). P < 0.05 AND F-STATISTICAL VALUE > F-CRITICAL VALUE WERE CONSIDERED THE MINIMUM SIGNIFICANCE LEVEL

TABLE 2: TOTAL CARBOHYDRATE CONTENT IN SELECTED *CHENOPODIUM* SPECIES

***Chenopodium* Species**	**Carbohydrate Content mg/gdw Equivalent of Glucose**		
	Root	**Stem**	**Leaves**
Chenopodium album	35.471 ± 0.081	30.178 ± 0.078	17.137 ± 0.029
Chenopodium murale	39.798 ± 0.105	31.319 ± 0.047	27.020 ±0.065
Chenopodium giganteum	37.342 ± 0.169	30.909 ± 0.128	20.003 ±0.011

All data expressed mean and standard deviation as triplicates. All analyses were carried out in triplicate for each extract. The data were scrutinized by one-way ANOVA followed by Microsoft Excel Single Factor Test (F-statistical value= 12.271, p-value= 0.035, and F-critical value= 9.552). $p < 0.05$ and F-statistical value > F-critical value were considered the minimum significance level.

Determination of Glucose Calibration Ccurve: By using the appropriate dilutions and the absorbance and concentrations (µg/mL), a calibration curve was created. After doing a regression analysis, Abs = 0.0114x -0.0447 was obtained as the result.

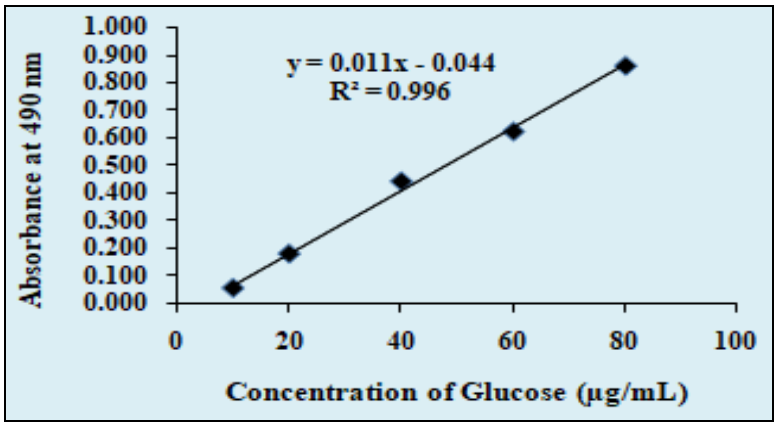

FIG. 6: CALIBRATION CURVE OF STANDARD GLUCOSE

The coefficient of determination for standard curves was found R^2 =0.9966. Where x represents the glucose content in the extracts and y indicates the absorption at 490 nm. As a result, the estimated straight line explained the experimental data. The total carbohydrate content was calculated and expressed as mg glucose equivalents (GE) per gram of sample in dry weight(mg/gdw) using the appropriate formula. Hydroxymethyl furfural is formed on dehydration of carbohydrates in a hot and acidic medium according to the phenol sulphuric acid method. Hydroxymethyl furfural reacts with phenol to form a yellow-to-orange-coloured product. The change in colour at different concentrations of carbohydrates is detected at an absorption of 490 nm with the help of a UV-1800 Shimadzu spectrophotometer **Fig. 6** & **7.**

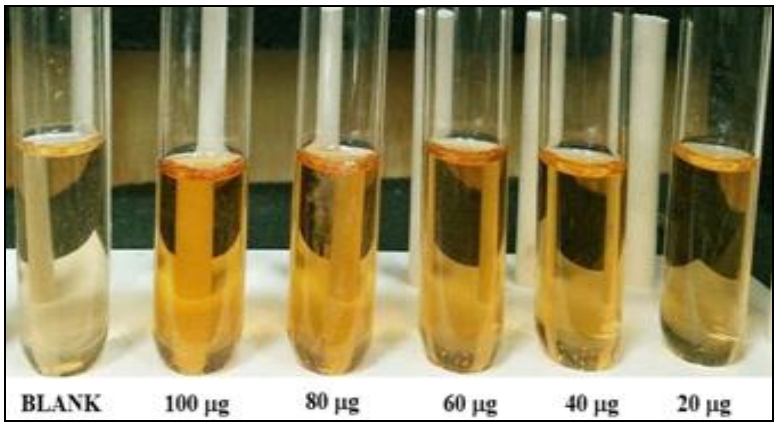

FIG. 7: SHOWING THE RESULT OF STANDARD WORKING SOLUTION WITH DIFFERENT CONCENTRATIONS OF GLUCOSE COMPARED TO BLANK WITH THE INTENSITY OF YELLOW TO ORANGE COLOUR

Estimation of Total Starch Content: The described method (The Anthrone Reagent Method) was implemented quantitatively to evaluate total starch contents in *Chenopodium* species. This study showed that the total starch contents were found in the root (18.945 ± 0.069mg/gdw), stem (15.025 ± 0.038 mg/gdw), and leaves (14.779 ± 0.045 mg/gdw) of *Chenopodium album*.

The total starch contents were found in the root (19.294 ± 0.183 mg/gdw), stem (16.588 ± 0.125 mg/gdw), and leaves (12.374 ± 0.162 mg/gdw) of *Chenopodium murale*. The total starch contents were found in the root (18.326 ± 0.125 mg/gdw), stem (15.866 ± 0.109 mg/gdw), and leaves (12.802 ± 0.075 mg/gdw) of *Chenopodium giganteum*. The result revealed that the roots of *Chenopodium album* contain the highest amount of starch (18.945 ± 0.069mg/gdw) compared to other species.

The F-critical value for α = 0.05 is 9.552. Since the observed F-statistical value is 71.432, which is greater than the F-critical value of 9.552, we can reject the null hypothesis. Additionally, the p-value (0.001) is less than 0.05. Therefore, we have enough evidence to conclude that the test is significant **Table 3** & **8.**

TABLE 3: TOTAL STARCH CONTENT IN SELECTED *CHENOPODIUM* SPECIES

***Chenopodium* Species**	**Total Starch Content mg/gdw Equivalents of Glucose**		
	Root	**Stem**	**Leaves**
Chenopodium album	18.945 ± 0.069	15.025 ± 0.038	14.779 ± 0.045
Chenopodium murale	19.294 ± 0.183	16.588 ± 0.125	12.374 ± 0.162
Chenopodium giganteum	18.326 ± 0.125	15.866 ± 0.109	12.802 ± 0.075

Total starch content was indicated as mean and standard deviation forms. All analyses were carried out in triplicate for each extract. The data were scrutinized by one-way ANOVA followed by Microsoft Excel Single Factor Test (F-statistical value= 71.432, p- value= 0.001, and F-critical value= 9.552). $p < 0.05$ and F-statistical value > F-critical value were considered the minimum significance level.

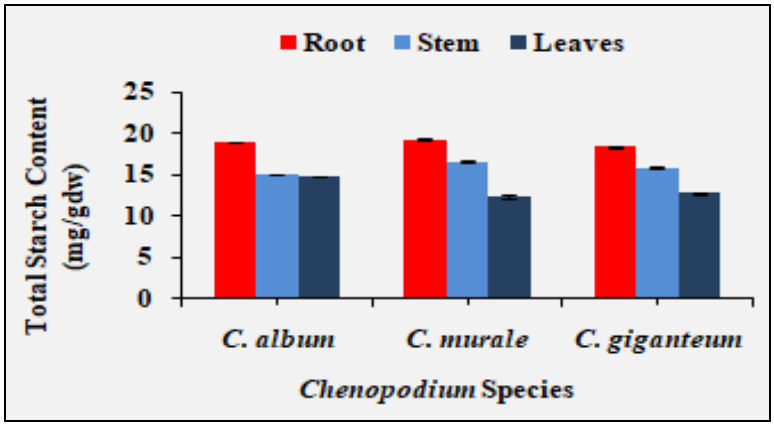

FIG. 8: THE TOTAL STARCH CONTENT IN SELECTED *CHENOPODIUM* SPECIES. ALL ANALYSES WERE CARRIED OUT IN TRIPLICATE FOR EACH EXTRACT. THE DATA WERE SCRUTINIZED BY ONE-WAY ANOVA FOLLOWED BY MICROSOFT EXCEL SINGLE FACTOR TEST (F-STATISTICAL VALUE= 71.432, P-VALUE= 0.001, AND F-CRITICAL VALUE= 9.552). P <0.05 AND F-STATISTICAL VALUE > F-CRITICAL VALUE WERE CONSIDERED THE MINIMUM SIGNIFICANCE LEVEL

Determination of Glucose Calibration Curve: A calibration curve was plotted by using the absorbance (630 nm) and concentrations (10, 20, 40, 60, 80, and 100 µg/mL).

The linear regression analysis found the equation y = 0.0084x - 0.0157 and R^2 with a coefficient of 0.997. Where x represents the total starch content in the extracts and y indicates the absorption at 630 nm. As a result, the estimated straight line explained the experimental data.

After treating the sample with 80% ethanol to get rid of the sugars, perchloric acid is used to get rid of the starch. Starch is hydrolyzed to glucose and dehydrated to Hydroxymethyl Furfural in a hot, acidic solution. Combined with anthrone, this chemical produces a light to dark green-coloured compound. This colour change can be detected at an absorption of 630 nm using a UV-1800 Shimadzu spectrophotometer **Fig. 9** & **10.**

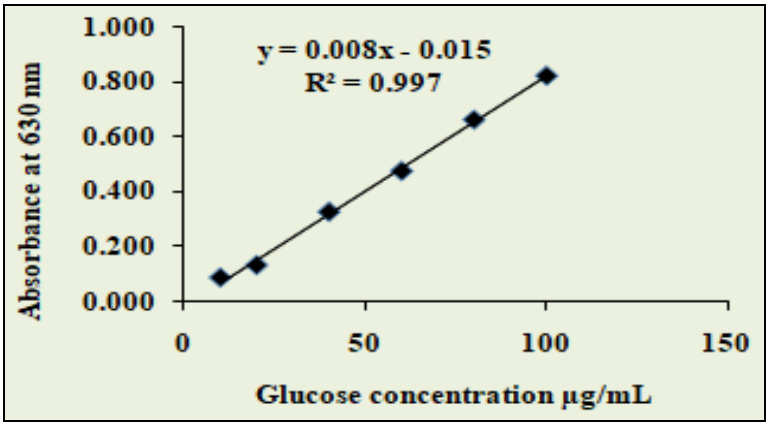

FIG. 9: CALIBRATION CURVE OF STANDARD GLUCOSE

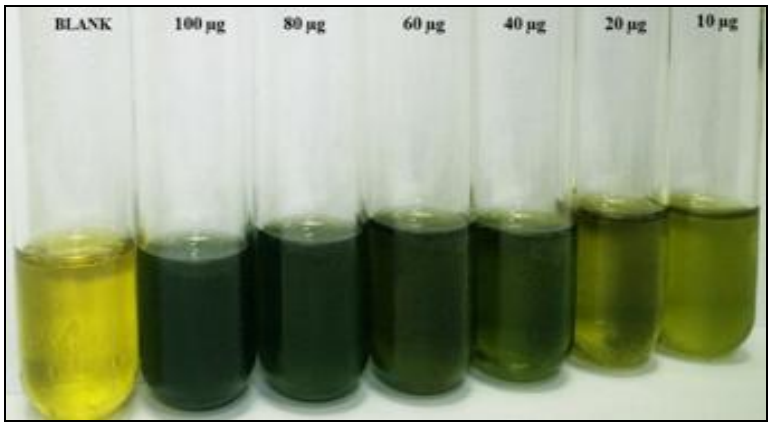

FIG. 10: SHOWING THE RESULT OF STANDARD WORKING SOLUTION WITH DIFFERENT CONCENTRATIONS OF GLUCOSE COMPARED TO BLANK WITH THE INTENSITY OF LIGHT TO DARK GREEN COLOUR

Estimation of Total Phenol Contents: The described method (Folin-Ciocalteu reagent method) was implemented quantitatively to evaluate total phenol contents in *Chenopodium* species. This study showed that the total phenol contents were found in the root (1.001 ± 0.029 mg/gdw), stem (1.684 ± 0.023 mg/gdw), and leaves (2.821 ± 0.052 mg/gdw) of *Chenopodium album*. The total phenol contents were found in the root (1.564 ± 0.039 mg/gdw), stem (2.227 ± 0.060 mg/gdw), and leaves (4.882 ± 0.056 mg/gdw) of *Chenopodium murale*. The total phenol contents were found in the root (0.916 ± 0.026 mg/gdw), stem (1.502 ± 0.037 mg/gdw), and leaves (4.520 ± 0.059 mg/gdw) of *Chenopodium giganteum*. The result revealed that the leaves of *Chenopodium murale* contain the highest amount of total phenol (4.882 ± 0.056 mg/gdw) compared to other species. The F-critical value for $\alpha = 0.05$ is 9.552. Since the observed F-statistical value is 37.892, which is greater than the F-critical value of 9.552, we can reject the null hypothesis. Additionally, the p-value (0.01) is less than 0.05. Therefore, we have enough evidence to conclude that the test is significant **Table 4** & **Fig. 11**.

TABLE 4: QUANTIFICATION OF TOTAL PHENOL IN SELECTED *CHENOPODIUM* SPECIES

***Chenopodium* Species**	**Phenolic content mg/gdw equivalent of gallic acid**		
	Root	**Stem**	**Leaves**
Chenopodium album	1.001 ± 0.029	1.684 ± 0.023	2.821 ± 0.052
Chenopodium murale	1.564 ± 0.039	2.227 ± 0.060	4.882 ± 0.056
Chenopodium giganteum	0.916 ± 0.026	1.502 ± 0.037	4.520 ± 0.059

Expressed as values mean ± SD (Standard deviation) triplicate calculated. All analyses were carried out in triplicate for each extract. The data were scrutinized by one-way ANOVA followed by Microsoft Excel Single Factor Test (F-statistical value= 37.892, p- value= 0.01 and F-critical value= 9.552). $p < 0.05$ and F-statistical value > F-critical value were considered the minimum significance level.

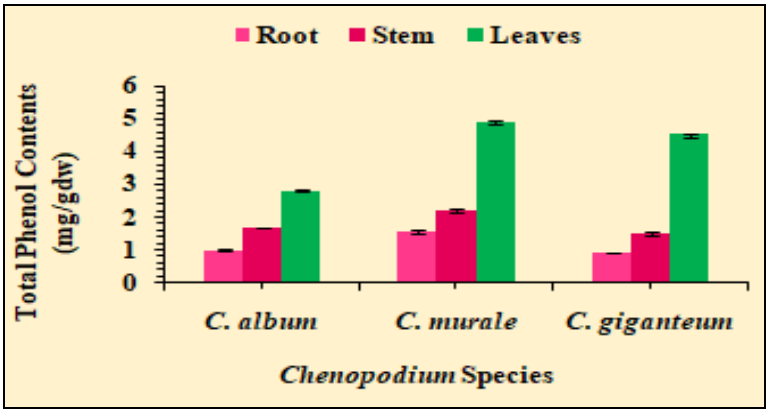

FIG. 11: BAR DIAGRAM SHOWING TOTAL PHENOL CONTENT OF *CHENOPODIUM* SPECIES. EXPRESSED AS VALUES MEAN ± SD TRIPLICATE CALCULATED. ALL ANALYSES WERE CARRIED OUT IN TRIPLICATE FOR EACH EXTRACT. THE DATA WERE SCRUTINIZED BY ONE-WAY ANOVA FOLLOWED BY MICROSOFT EXCEL SINGLE FACTOR TEST (F-STATISTICAL VALUE= 37.892, P- VALUE= 0.01, AND F-CRITICAL VALUE= 9.552). P < 0.05 AND F-STATISTICAL VALUE > F-CRITICAL VALUE WERE CONSIDERED THE MINIMUM SIGNIFICANCE LEVEL

Calibration Curve for Total Phenol Contents: A calibration curve was plotted by using the absorbance (650 nm, UV-1800 Shimadzu) and concentrations (25, 50, 100, 150, 200, 250, and 300 µg/mL).

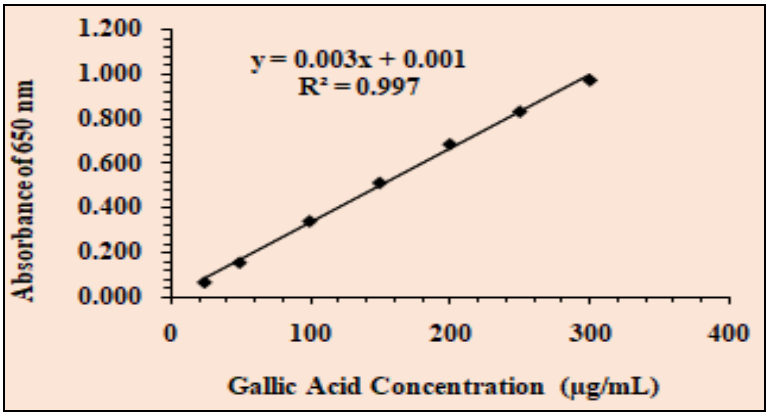

FIG. 12: CALIBRATION GRAPH FOR QUANTIFICATION OF TOTAL PHENOL CONTENT

Total Phenol content was calculated as mg of gallic acid equivalent (GAE) per gram of dry extract (standard curve equation: y= 0.0033x + 0.0012, R^2 = 0.9976). The experiment was run three times **Fig. 12** & **13.**

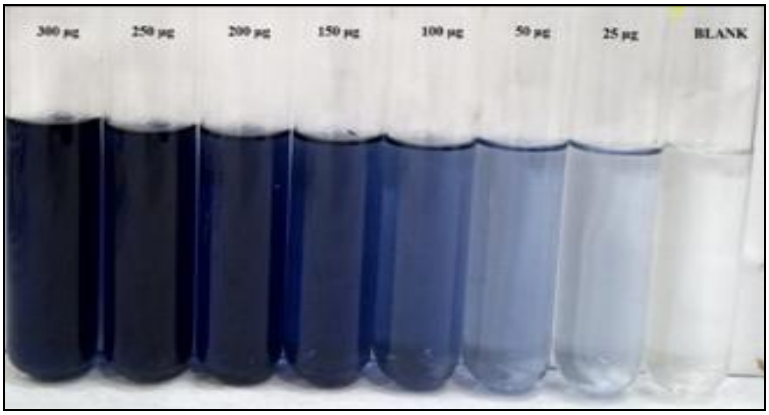

FIG. 13: SHOWING THE RESULT OF STANDARD WORKING SOLUTION WITH DIFFERENT CONCENTRATIONS OF GALLIC ACID COMPARED TO BLANK WITH THE INTENSITY OF LIGHT TO DARK BLUE COLOUR

Estimation of Total Flavonoid Contents: The described method (aluminium chloride method) was implemented quantitatively to evaluate total flavonoid contents in *Chenopodium* species. This study showed that the total flavonoid content was found in the root (1.716 ± 0.040 mg/gdw), stem (2.445 ± 0.025 mg/gdw), and leaves (5.522 ± 0.005 mg/gdw) of *Chenopodium album*. The total flavonoid content was found in the root (2.022 ± 0.011 mg/gdw), stem (3.876 ± 0.022 mg/gdw), and leaves (9.980 ± 0.124 mg/gdw) of *Chenopodium murale*. The total flavonoid content was found in the root (2.327 ± 0.004 mg/gdw), stem (2.890 ± 0.004 mg/gdw), and leaves (12.397 ± 0.121 mg/gdw) of *Chenopodium giganteum*. The F-critical value for α = 0.05 is 9.552. Since the observed F-statistical value is 41.591, which is greater than the F-critical value of 9.552, we can reject the null hypothesis. Additionally, the p-value (0.01) is less than 0.05. Therefore, we have enough evidence to conclude that the test is significant **Table 5** and **Fig. 14.**

TABLE 5: QUANTITATIVE ANALYSIS OF TOTAL FLAVONOIDS CONTENT IN SELECTED *CHENOPODIUM* SPECIES

***Chenopodium* Species**	**Flavonoid Content mg/gdw equivalent of quercetin**		
	Root	**Stem**	**Leaves**
Chenopodium album	1.716 ± 0.040	2.445 ± 0.025	5.522 ± 0.005
Chenopodium murale	2.022 ± 0.011	3.876 ± 0.022	9.980 ± 0.124
Chenopodium giganteum	2.327 ± 0.004	2.890 ± 0.004	12.397 ± 0.121

All data are expressed as mean ± SD of three samples. For α = 0.05, the F-critical value is 9.552. Since the observed F-statistical = 41.591 is greater than the F-critical value of 9.552. p-value (0.01) is less than 0.05, we reject the null hypothesis. There is evidence that the test is significant.

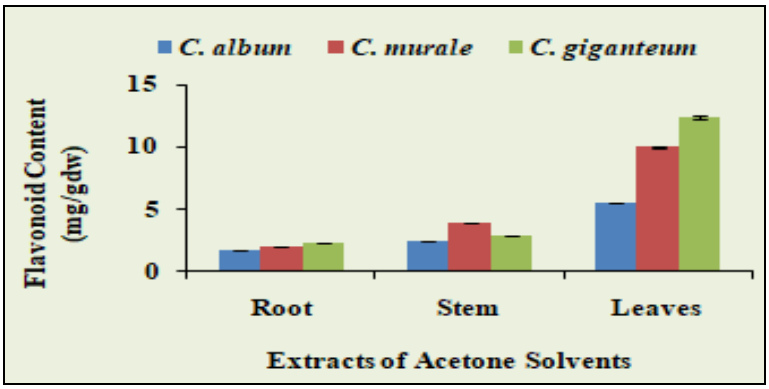

FIG. 14: THE BAR DIAGRAM SHOWS THE TOTAL FLAVONOID CONTENT IN *CHENOPODIUM* SPECIES. FOR A = 0.05, THE F-CRITICAL VALUE IS 9.552. SINCE THE OBSERVED F-STATISTICAL = 41.591 IS GREATER THAN THE F-CRITICAL VALUE OF 9.552. P-VALUE (0.01) IS LESS THAN 0.05, WE REJECT THE NULL HYPOTHESIS. THERE IS EVIDENCE THAT THE TEST IS SIGNIFICANT

Calibration Curve for Quantification of Total Flavonoid Content: A calibration curve was plotted by using the absorbance (510 nm, UV-1800 Shimadzu) and concentrations (25, 50, 100, 150, 200, 250, 300, 350, and 400 µg/mL). Total flavonoid content was calculated as mg of quercetin equivalent (QE) per gram of dry extract (standard curve equation: y= 0.0024x – 0.0267, R2 = 0.9973). The experiment was run three times.

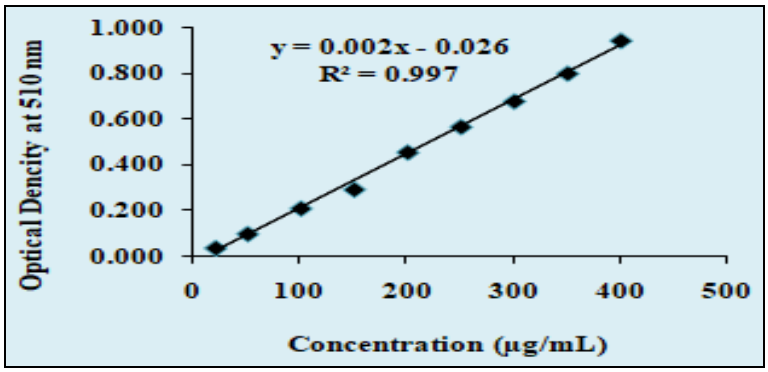

FIG. 15: STANDARD CURVE OF QUERCETIN

The optical density of all the standards was taken into consideration to produce the calibration curve once the maximum of the coloured solution (510 nm, UV-1800 Shimadzu, light yellow to orange solution) was determined. Absorbance versus concentration was plotted to create the calibration curve **Fig. 15** & **16.**

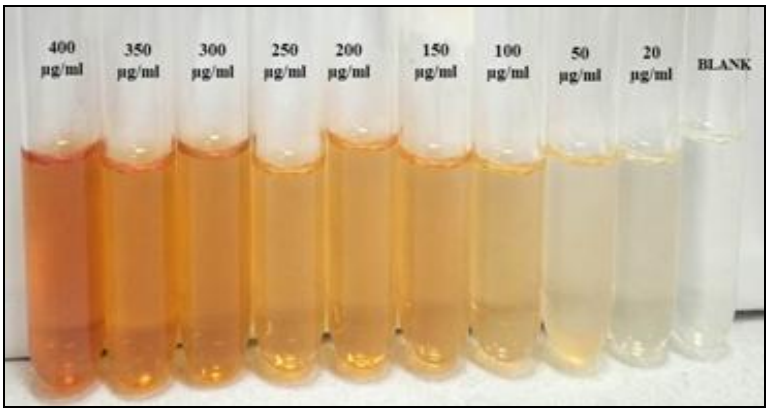

FIG. 16: SHOWING THE RESULT OF STANDARD WORKING SOLUTION WITH DIFFERENT CONCENTRATIONS OF QUERCETIN COMPARED TO BLANK WITH THE INTENSITY OF YELLOW TO ORANGE COLOUR

The current work provides evidence of the presence of metabolites in the root, stem, and leaves extracts of *Chenopodium* species. The present finding indicates that *Chenopodium* species good source of proteins, carbohydrates, lipids, saponins, starch, phenols, flavonoids, terpenoids, and steroids. *Chenopodium* species have been traditionally utilized in various disorders like anthelmintic against round and hookworms, laxatives, antiscorbutic, blood purifiers, abdominal pain, improving appetite, eye disorders, piles, throat troubles, and diseases of blood. *Chenopodium* species' secondary and primary metabolites play a crucial role in pharmaceuticals.

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