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NUTRITIONAL EVALUATION OF EXTRACT OF THE RHEUM PALMATUM (RHUBARB) IN RAW & STEAMED SAMPLE: A COMPARAITIVE STUDY

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ABSTRACT:

Rhubarb as a vegetable contains many of the same nutrients found in other vegetables although mostly in lesser amounts. Rhubarb plays a significant role as food. It is low to moderate source of vitamins and minerals and varieties for most of the common nutrients. Rhubarb is important for its nutrients which complement its usefulness as a diet food because of its high water and fiber content. Its photo protective and antioxidant effects made it used in skin & sunscreen products. Protein, fluoride, iron (II) and iron (III) was determined by spectrophotometry and manganese was determined by complexometric titration. The results of the above contents were compared with the values obtained from the literature. The results were almost in aggrement with the literature. Rhubarb raw sample contains 0.95 g and steamed sample contains 0.345 g of protein, hence 36% has been lost on steaming. It was found that rhubarb contains 0.139 mg of fluoride and steamed contains 0.046 mg of fluoride. Hence 33% has been lost on steaming. Raw sample contains 0.238 mg, whereas steamed rhubarb contains 0.015 mg of Fe (II) loosing 66% on steaming. It was found that rhubarb contains 0.238 mg of Fe (III) and steamed contains 0.043 mg loosing 60% on steaming. Raw rhubarb contains 20.6 mg of manganese and steamed sample contains 5.0 mg, revealing 24.3% has been lost on steaming. Interesting result has been found in the case of manganese 20.6 mg has been found but from literature only 0.196 mg has been reported. So better to use raw rhubarb in the form of salad, than using steamed rhubarb. Hence the above investigation was carried in order to explore the importance of nutritional composition of rhubarb and in order to depict that there is some percentage of loss in steamed sample.

Keywords: Rhubarb, Nutrient, Protein, Spectrophotometry, Antioxidants, Raw, Steamed.

INTRODUCTION:

Rhubarb is a perennial herb grown for its attractive succulent rose red, edible leafy stalks. This is a cool season plants in Siberia and popular in many regions of Europe and in North America as "PIE" plant. In its natural habitat, the plant expands over the ground surface as a larger spread. Rhubarb is a strange-looking plant with a very interesting history and it is

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widely considered as a vegetable but in America it is considered as a fruit. Scientifically they are herbaceous perennials with leaves growing off the top of a thick rhizome. Rheum palmatum or Rheum officinale is a large leafy perennial with hallows stalks that may reach 10 feet in height. The root rhizome is the part used medicinally [1]. Rhubarb is thick and branching with a brown exterior and a yellow interior. Garden rhubarb typically grows to about three feet and has reddish to purple stems. It contains similar active ingredients but is much less potent [2]. Rhubarb is most often used to make jellies, jams, cakes, muffins and other desserts. It can also be used as a sauce to serve with meats and fish. Rhubarb also known as **''pie plant"** often considered a fruit but actually a vegetable (leaf stem). To harvest the plants leaf stalks from the plant were pulled and trim off the leaf blades. The edible part of the plant is stem only. Leaves are not recommended because they are poisonous, impairing hemostasis, causing Nausea & vomiting. In addition they contain large amount of oxalic acid and can't be eaten [3].

Chinese herbalists have relied on rhubarb rhizomes (roots) for 1000 years. The rhizome contains powerful "Anthraquinones" that acts as stimulant, laxatives & tannins that act as astringent (A lotion used for skin). The Chinese also uses rhubarb to treat "Gastric ulcer". Chronicle renal failure & pregnancy induced hypertension [4]. The current practice of using rhubarb to treat cancer lacks the support of controlled clinical trials. Rhubarb root can cause severe diarrhea & abdominal cramps. Rhubarb exhibit both antimicrobial and antiviral. Anthraquinone extracts were viricidal against HSVI, Measles, Polio, influenza virus [5]. The antibacterial effects of rhubarb are believed to be due to its inhibition of enzymes in the mitochondrial electron transport system. A series of 157 adults suffering from "Gonorrhea" was treated with Chinese rhubarb tablets, they had reported cure rate of 66% but diagnostic criteria & co-therapies were not reported [6]. Rhubarb is traditionally used against tooth ache in china. Vitamin-K promotes Osteotrophic activity meaning that it stimulates bone growth and the repair. Combined with rich amount of calcium and other minerals found in rhubarb [7], the vegetable as a whole is a major player in "Bone protection. Rhubarb is a good source of "Beta Carotene" and other polyphenol compounds like Lutein and Zia xanthine [8] which acts in a similar way to Vitamin-A, protecting the skin and eyes from the effects of free radicals. These phenolic compounds have been connected to preventing oral and the lung cancer [9]. The most important vitamin present in rhubarb is Vitamin –K, plays a very significant role in brain and neuronal health. It prevents the oxidation of brain cells and stimulates cognitive activity [10], thereby helping to delay or even prevent the onset of Alzheimer's disease. Rhubarb is extremely low in fat and cholesterol, the vegetables poses no threats to cardiovascular health [11]. Furthermore, the impressive amounts of antioxidants in rhubarb ensure that free radicals don't cause heart disease. Rather it is referred to as low caloric vegetable. The high amount of dietary fiber found in rhubarb help a healthy digestive system by making the bowel movements are smooth and regular [12]. By easing constipation and other digestive tissues, it can prevent a wide range of more serious "Gastrointestinal" disorders, including bloating, cramping and even colorectal cancer [13].

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Rhubarb also drawing attention towards it as it contains active chemical compounds such as anthroids [14, 15] especially anthraquinone glycosides, rhein (Sennosides A and B), aloe-emodin, Physcion. Many workers including Seto [16] and Zwaving [17] have worked on these chemicals for preparation of crude drug and the comparison of these components in processed rhubarb. Murata et. al. [18] has made comparison of the contents of sennosides A, B in rhubarb. Rhubarb exhibit photo protective and antioxidant effect. Inhibitory action of Tyrosine kinase and Tyrosinase activities and TNF-α and α-MSH production in human melanocytes [19]. Exposure to UV radiation causes various form of acute and chronic skin damage including immune suppression, inflammation, premature aging and photo damage [20]. Furthermore it induces the generation of reactive oxygen species, pro inflammatory cytokines and melanocytes stimulating hormone (MSH) and increase Tyrosinase activity [21]. The effects of rheum R Haponticum rhizome extract on Tyrosinase kinase activity, and on interleukin1α tumour, necrosis factors and α-MSH production in human epidermal melanocytes were evaluated under UV stimulated and non-stimulated conditions [22]. Antioxidants were evaluated by lipid peroxidation and 1,1 Diphenyl 2-Picryl-Hydrazyl (DPPH) assays, while anti- Tyrosinase activity was evaluated by the mushroom. Tyrosinase method Rheum-rhapontieum.L.Rhizome extract showed in-vitro antioxidant properties against lipid peroxidation free radical scavenging and antityrosinase activities and inhibited the production of 1L-1α, TNF-α, α-MSH and Tyrosinase kinase activity in melanocytes subjected to UV radiation [23]. These results support the inclusion of Rheum rhaponticum L rhizome extract into cosmetic, sunscreen and skin care products for the prevention or reduction of photo damage [24]. Studies also have revealed aqueous extract from rhubarb plant inhibits Adenosine deaminase activity in cancerous and non cancerous human gastric and colon tissues. Investigation of possible effects of rhubarb extract on adenosine (ADA) deaminases activity [25] in cancerous & non-cancerous human gastric & colon tissue's has been made to obtain information about possible mechanism of anticancer action [26] of rhubarb on cancerous and non-cancerous human gastric and colon tissues, tissues removed from patients by surgical operation were used in the studies. The extract were prepared in distilled water before and after treatment with extracts ADA activities in the tissue's homogenates were measured [27, 28]**.** ADA activity was found to be higher in gastric tissue but no difference were found between ADA activities of cancerous and non- cancerous tissues. Results suggests that aqueous extract from rhubarb inhibits ADA activities in both gastric and colon tissues significantly [29]. It is suggested that in addition to other proposed mechanism, accumulated adenosine due to the inhibition of ADA enzyme might also play a part in the anti- cancerous properties of the rhubarb. Since the Rhubarb species are exhibiting photo protective and antioxidant effect, Tyrosinase activities, Adenosine deaminase activities, anticancerous activites, the present investigation has been undertaken to explore the health benefits of raw as well as steamed rhubarb sample. There are two main varieties of rhubarb. They are red petioles and green petioles. Red petioles have Canada red, Victoria, Cherry red, Crimson red, Valentine and Mac Donald variety. In this study Mac Donald variety was used as it was available in the local market.

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MATERIALS AND METHODS:

Chemicals

General chemicals and solvents used were of analytical grade obtained from RANKEM. They are casein, Bovine Serum albumin (BSA), Folin's reagent, sodium hydroxide, sodium carbonate, copper sulphate, Ferrous Ammonium Sulphate (FAS), thiocyanate, 1,10-phenanthrolein, acetic acid, sodium acetate. Cerium (IV) sulphate, Zinc Sulphate, Ethylene Diamine Tetra Acetate (EDTA), ammonium chloride, potassium dihydrogen phosphate, Molybdate, sodium sulphite, sodium hydrogen sulphite, sodium chloride, potassium chloride etc.

Collection of the extract from Rhubarb:

One Kilogram of fresh green ***Rheum palmatum*** (rhubarb) Mac Donald variety was obtained from the super market. The leaves were cut off and 0.5 kg of young stem of raw rhubarb was grinded with distilled water from which 250 mL of the extract was obtained through filtration and centrifugation. Similarly 250 mL of extract was obtained from 0.5 kg by steam, for 10 mins at 100 ^{o}C. The collected extract was stored in freezer and then the frozen extract was used for further analysis.

Protein Content of the rhubarb extract

Protein content was carried out by Lowry's method [30] as described below. It is the most commonly used method for determination of protein in cell free extracts because of its high sensitivity and quantities as low as 20 µg of protein can be measured. The peptide bonds in poly peptide chain react with copper sulphate in an alkaline medium to give a blue colored complex. In addition, tyrosine and tryptophan residues of protein cause reduction of the phosphomolybdate and phosphotungstate components of the Folin-Ciocalteau reagent to give bluish products which contribute towards enhancing the sensitivity of this method. However, several compounds like EDTA, Tris, Carbohydrates, thiol reagents, phenols etc., interfere with the color development and it should be ensured that such substances are not present in sample preparation. The experiment was conducted by adding a series of 0.1, 0.2, 0.3, 0.4, 0.5 mL of BSA was taken in 25 mL calibration flask. In the similar way samples raw and steamed preparation of about 0.1 and 0.2 mL was taken in the calibration flask and 1mL of NaOH was added to all flasks. To this 5 mL of alkaline copper sulphate solution was added and allowed to stand for 10 minutes. Then add 0.5 mL of Folin's solution and stirred well and allowed for 10 minutes. The absorbance was measured at 660nm. The graph was plotted as absorbance versus concentration of BSA. From the standard curve the amount of protein in the sample was determined.

Determination of Iron (II) by spectrophotometry:

Iron (II) forms a stable, highly colored complex with 1, 10 – phenanthrolein:

$Fe^{2+} + 3\,(ph)H^{+} \rightarrow Fe\,(ph)_3^{2+} + 3H^{+}$

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Iron (III) does not form the complex. For this analysis to be successful, all of the iron must be reduced from Fe (III) to Fe (II). In this experiment [31], the reduction is carried out with hydroxylamine hydrochloride, while the solution is buffered with sodium acetate. Calibration curve was prepared by pipetting 0.4, 0.8, 1.2, 1.6 and 2.0 mL of 50 ppm solution into a series of 10 mL volumetric flask, so as to get the concentration of solution 2, 4, 6, 8 and 10 ppm respectively. Add 2 mL of 1,10-phenanthrolein and 3 mL of buffer in each of the flask. The absorbance of the solution was measured at 510 nm. A calibration curve was plotted with the concentration in ppm on x-axis and absorbance on y-axis. Similarly extract samples were also treated with 2 mL of 1,10 – phenanthrolein and 3 mL of buffer. The absorbance of the samples solution was recorded and concentration was determined with a calibration curve.

Determination of iron (III) by spectrophotometry

Ferric ion reacts with thiocyanate to give a series of intensely red coloured compound which remains in true solution. Ferrous ion does not react, depending upon the thiocyanate concentration series of complex are formed as $[Fe(SCN)_4]^-$ where n = 1,2,3 ….6 at low thiocyanate concentration, the predominant colored species is $[Fe(SCN)]^{2+}$**.**

$Fe^{3+} + SCN^- \rightarrow [Fe(SCN)]^{2+}$

At 0.1 M thiocyanate concentration it is largely the complex formed is **(**$Fe(SCN)_2$**)+,** at very high thiocyanate concentration, the complex formed is **[**$Fe(SCN)_6$**]**$^{3-}$, in the spectrophotometric determination a large excess of thiocyanate should be used, since this increases the intensity and also stability of the color. Strong acid should be present to suppress the hydrolysis.

$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+$

H_2SO_4 is not recommended because sulphate ion have a certain tendency to form a complex with Ferric ions, Silver, Copper, Nickel, Titanium, Uranium, Molybdenum, Mercury, Zinc, Cadmium, Bismuth interfere, mercurous and stannous salt if present must be converted into mercuric and stannic salt otherwise the color is destroyed. Phosphate, arsenate, fluorides, oxalates interfere, since they forms fairly stable complex with ferric ion the influence of phosphate and arsenates can be reduced by use of concentrated acids. In to a series of 25 mL volumetric flask 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 up to 5ml of ammonium ferric (lll) sulphate solution were added using micro burette. In another flask samples raw and steamed were taken, 2.5 mL of 20 % KSCN solution and 3 mL of HCl were added into each flask and allowed to stand for few minutes, then solutions were made up to the mark using distilled water and shaken well, the absorbance were read at 480. The absorbance of the sample was recorded and concentration was determined with a calibration curve.

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Determination of Manganese Using Eriochrome Black T as an indicator by complexometric titration

About 5ml of the sample extract solution was pipetted into a clean conical flask, add 0.5 g of hydroxyl ammonium chloride (to prevent oxidation), warmed and diluted to 10 mL with boiled distilled water (if the solution in acidic, neutralized with dilute sodium hydroxide solution). About 3 mL of triethanol amine added to keep the manganese in solution and it was subsequently made alkaline by adding 2 mL of buffer solution (pH=10) and three drops of EBT indicator was added. Titrated with standard 0.05M EDTA until the color changes from wine red to blue. The amount of manganese preset in the sample extracts were calculated using the relation, **1mL of 1M EDTA ≡ 27.47 mg of Mn.**

Determination of fluoride in rhubarb by spectrophotometric method:

Fluoride occurs in all natural water supplies and in chemical wastes from industries. If present in small concentration up to 2 ppm are generally considered to be beneficial in water. Excessive fluorides in drinking water may cause dental fluorosis which results in discoloration of enamel, crippling of teeth in children in severe cases bone fluorosis (or) crippling effects are observed in the concentration of fluorides exceeds 2 mg /Liter. Fluoride stock solution was prepared by weighing accurately about 0.221 g of sodium fluoride crystals into 100 mL volumetric flask and dissolved in distilled water, made up to the mark and shaken well, this solution corresponds to 1000 ppm, this solution was diluted to get 50 ppm of working solution [31]. To the 10 mL sample extract 1 drop of $NaAsO_2$ (5g /Liter) was added to remove residual chloride ions and 5ml of acid–Zirconyl Alizarin reagent (300 mg $ZrOCl_2.8H_2O$/50mL+70 mg Alizarin red-S/50 mL+800 mL of 1.5N HCl-1.2N H_2SO_4 made up to 1 Liter) was added. Mixed thoroughly and compared the samples and standards after an hour. In to a series of 25 mL volumetric flask 1.0, 2.0, 3.0, 4.0 and 5.0 mL of standard fluoride solution was added using micro burette. In other flasks samples raw and steamed were taken, 1 drop of $NaAsO_2$ (5g /Liter) was added to remove residual chloride ions and 5ml of acid–Zirconyl Alizarin reagent (300 mg $ZrOCl_2.8H_2O$/50mL+70 mg Alizarin red-S/50 mL+800 mL of 1.5N HCl-1.2N H_2SO_4 made up to 1 Liter) was added to all the flasks and allowed to stand for an hour, then solutions were made up to the mark using distilled water and shaken well, the absorbance were read at 505 nm. The absorbance of the sample was recorded and concentration was determined with a calibration curve.

RESULTS AND DISCUSSION:

Rhubarb plays a significant role as food. As a vegetable, Rhubarb contains many of the same nutrients found in other vegetables although mostly in lesser amounts. Rhubarb is a low to moderate source of vitamins and minerals and varieties for most of the common nutrients. This is directly related to the proportion of dark green leaves in the edible portion. Rhubarb is important for its nutrients which complement its usefulness as a diet food because of its high water and fiber content.

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In the present study the 1kg of Rhubarb young stem was collected from market, from that 0.5 kg of rhubarb young raw stem was grinded and extract was collected. Another 0.5 kg of rhubarb stem was boiled in pressure cooker for about 10 mins, and cooled and grinded, the extract was collected. Both extracts were analyzed for protein, Fe (II), Fe (III), manganese, fluoride. The values obtained from the literature were compared with results obtained by the above methods. The values were almost same. Protein was determined by spectrophotometry shown in table 1, it was found that raw contains 0.95 g and boiled contains 0.345 g. Hence 36% has been lost on steaming. Iron (II) was determined by spectrophotometry as shown in table 2, it was found that rhubarb contains 0.128 mg, whereas steamed rhubarb contain 0.043 mg of Fe (II). Hence 66% has been lost on steaming. Iron (III) was determined by spectrophotometry as shown in table 3. It was found that rhubarb contains 0.110 mg of Fe (III) and boiled contains 0.049 mg. Hence 60% has been lost on steaming.

Table 1. Protein content of raw and steamed rhubarb sample

Sl. No	Protein solution (std)	Conc. µg	Alkaline CuSO4 solution mL	Foling's reagent	Incubation time (mins)	Absorbance at 660 nm
1	0.1	100	5	0.5	5	0.160
2	0.2	200	5	0.5	5	0.301
3	0.3	300	5	0.5	5	0.492
4	0.4	400	5	0.5	5	0.694
5	0.5	500	5	0.5	5	0.850
6	Sample (raw)	-	5	0.5	5	0.353
7	Sample (steamed)	-	5	0.5	5	0.178

Manganese was determined by complexometric titration, raw rhubarb contains 20.6 mg of manganese and boiled rhubarb contains 5.0 mg. Hence 24.3% has been lost on steaming. Flouride was determined by spectrophotometry as shown in Fig 1, it was found that rhubarb contains 0.139 mg of fluoride and boiled contains 0.046 mg of fluoride. Hence 33% has been lost on steaming (Table 4). Hence it is convenient to include it in daily diet in raw form only as salads for the daily supplements of nutrients as it has low calories also.

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Table 2. Detremination of Fe II by 1,10 phenanthrolein be spectrophotometric method

Sl. No	Vol. of Fe (II) in mL	Conc. of Fe (II) in ppm	Volume of 1,10 phenanthrolein added in mL	Volume of buffer added in mL	Absorbance
1	0.4	2	2.0	3.0	0.201
2	0.8	4	2.0	3.0	0.412
3	1.2	6	2.0	3.0	0.631
4	1.6	8	2.0	3.0	0.854
5	2.0	10	2.0	3.0	1.123
6	Sample (raw)	-	2.0	3.0	1.060
7	Sample (steamed)	-	2.0	3.0	0.616

Table 3. Determination of Fe (lll) by thiocyanate method spectrophotometrically

Sl. No	Volume of Fe (lll) in mL	Concentration in ppm	Volume of KSCN added in mL	Volume of HCl added in mL	Absorbance At 480nm
1	0.5	1	1.5	3	0.0755
2	1.0	2	1.5	3	0.085
3	1.5	3	1.5	3	0.105
4	2.0	4	1.5	3	0.205
5	2.5	5	1.5	3	0.236
6	3.0	6	1.5	3	0.333
7	3.5	7	1.5	3	0.4012
8	Sample (raw)	-	1.5	3	0.359
9	Sample (steamed)	-	1.5	3	0.105

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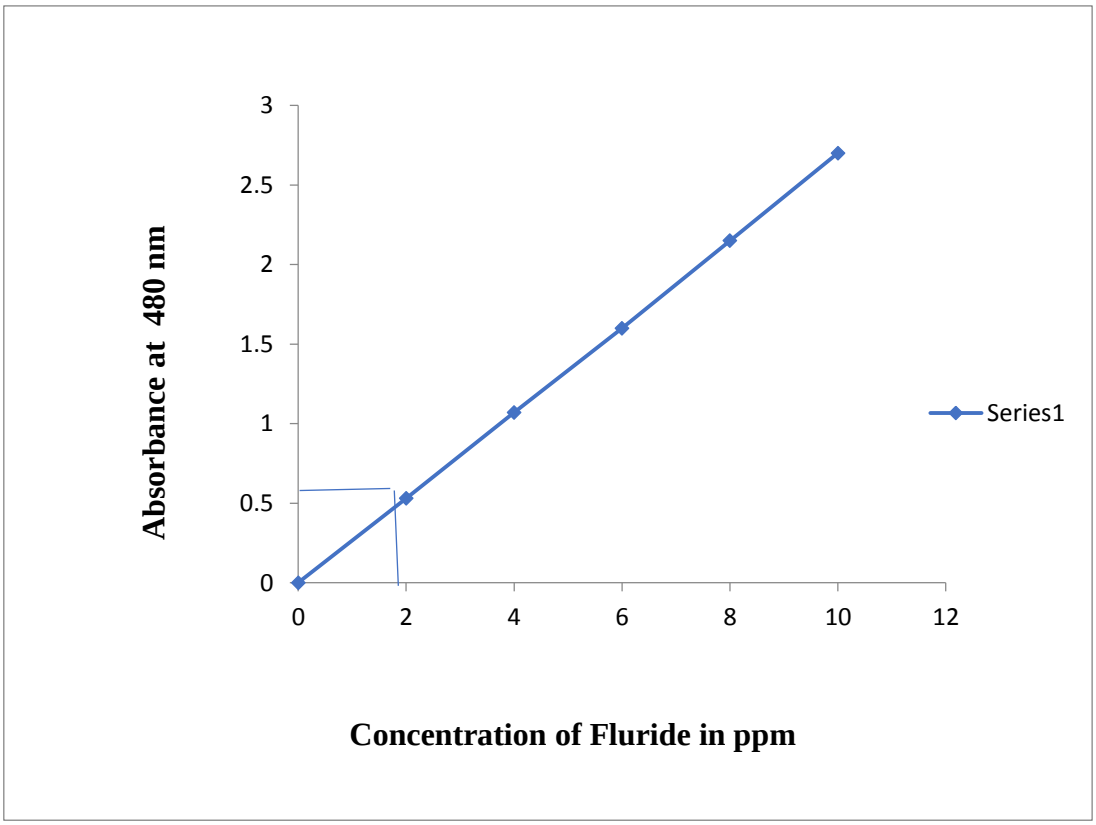

Figure 1. Determination of Fluoride by spectrophotometry

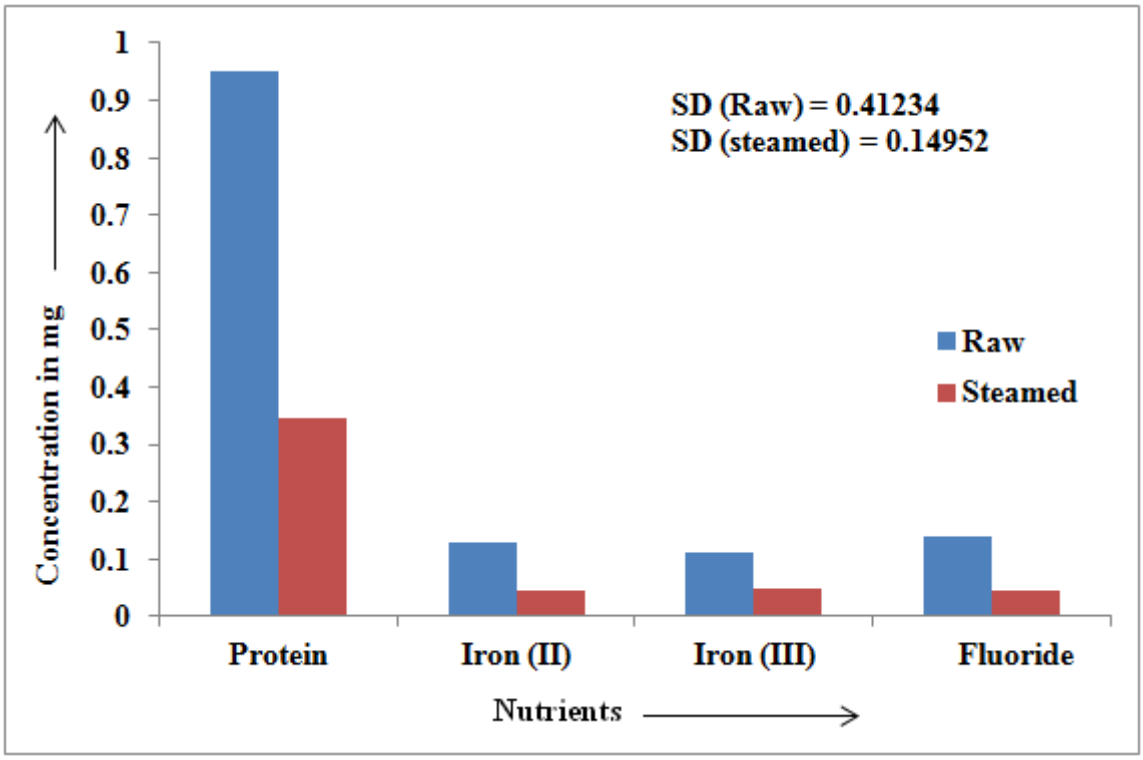

Figure 2. Graph depicting loss of nutrients in steamed extract

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Table 4. Comparison of Biochemical Components in Raw and Steamed Rhubarb Extract

Sl. No	Nutrients	raw rhubarb/100 g (literature)	raw rhubarb/100 g (from experiment)	steamed/100 g	% of loss in steaming
1	Protein	0.90	0.95 g	0.345 g	36%
2	Iron (II)	0.11	0.128 mg	0.043 mg	66%
3	Iron (III)	0.11	0.110 mg	0.049 mg	60%
4	Manganese	0.196	20.6 mg	5.0 mg	24.3%
5	Fluoride	Not reported	0.139 mg	0.046 mg	33%

The results obtained in the raw sample determined by the above methods are almost tallying with the results obtained in the literature (Table 4). Figure 2 depicts the loss of nutrients in mg concentration per 100 g of steamed sample. Interesting result has been found in the case of manganese 20.6 mg has been found but from literature only 0.196 mg has been reported.

CONCLUSION:

In the present investigation the extract was collected from 0.5 kg of young stem of Rhubarb (*Rheum palmatum*) raw as well as steamed extract. Extract collected from young stem was analyzed for biochemical composition such as Protein, Fe (III), Fe (II), manganese, fluoride. In steamed sample protein, manganese and fluoride has been lost less than 40 %, but iron (II) and iron (III) has been lost about less than 70 %.

Rhubarb contains only 21 calories on 100g of rhubarb. It is rich in precious nutrients like proteins, fluoride, iron and manganese. It is also a very rich source of many vital phyto-nutrients, vitamins, minerals and health benefiting anti-oxidants. Compared to other leafy vegetables like pak choi, lettuce plant & Broccoli, it has got more importance because of its high nutrients values for manganese, fluoride, protein, iron. Rich iron content present in rhubarb is very helpful in blood circulation. Rhubarb contains rich proteins helps in maintaining low body weight maintenance. Its photo protective and antioxidant effects used in skin & sunscreen products. Its ADA inhibition activities might also play a part in the anti-cancerous activity. Hence the above investigation was carried in order to explore the importance of nutritional composition of Rhubarb and in order to exhibit that there is some percentage of loss in steamed sample, hence it is better to use raw rhubarb in salads than using it in steamed form.

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List of abbreviations:

HSVI – Human Herpes Simplex Virus Type I

TNF-α – Tumor Necrosis Factor alpha

MSH – Melanocytes Stimulating Hormone

DPPH – Diphenyl 2-Picryl-Hydrazyl

ADA – Adenosine Deaminases Activity

FAS – Ferrous Ammonium Sulphate

EDTA – Ethylene Diamine Tetra Acetate

BSA – Bovine Serum Albumin

SCN – Thiocyanate

EBT – Eriochrome Black T

Ph – Phenanthrolein

$[Fe(SCN)]^{2+}$ – Ferric thiocyanate

Chemicals Molecular Formulae:

KOH – Potassium hydroxide

HNO_3 – Nitric acid

HCl – Hydrochloric acid

H_2SO_4 – Sulphuric acid

$Fe(OH)_3$ – Ferric hydroxide

Ce (IV) – Cerium IV sulphate

$CuSO_4$ – Copper sulphate

$ZnSO_4.7H_2O$ – Zincsulphate heptahydrate

$NaAsO_2$ – Sodium arsenite

$ZrOCl_2.8H_2O$ – Zirconyl chloride octahydrate

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