Phytochemical Screening and Anti-bacterial activity of *Citrullus colocynthis* (L.) Schrad. from Sindh (Ghotki) Pakistan

Zarbad Shah[1], Alia Gul[2], Tofail Arshad[3], Muhammad Idrees[1], Sumaira Salahuddin Lodhi[4], Nadia Jabeen[5], Syed Majid Rasheed[3], Muhammad Akhlaq[6], Waqar Ali[7], Asma[2], Naila Sardar[8], Syed Hasnain Imad[9], Abdul Basit[10]

ABSTRACT: *Citrullus colocynthis*L. is one of the most important medicinal plants mainly used in folk medicines. It is widely used for the remedy of cancer, tumours, and abdominal disorders. Owing to medicinal importance of *Citrullus colocynthis*, the current work is concentrated to explore itsproximate, metalsanalysis and antibacterial activity. Phytochemical screening of the roots, stem and leaves extracts showed various secondary metabolites such as Alkaloids, Tannins, Saponin, Flavonoids, Terpenoids and Glycosides. Elementalanalysis of the roots, stem and leaves extracts showed variable concentration of eight (08) differentheavy metals such as Iron (Fe), Copper (Cu),Zinc (Zn), Manganese (Mn), Nickel(Ni), Tin (Sb), Cobalt (Co) and Lead (Pb). Various plant parts were tested against pathogenic bacterial strains. Roots and Fruit Ethanolic extract of the plant showed significant anti-bacterial activity upto 19mm against MDR human pathogenic Gram-positive strain*Bacillus subtilis* while it was comparatively effective against *E. coli* and other bacilli with average 17mm zone of inhibition, while levofloxacin was used as standard antibiotics.

Key words:*Citrullus colocynthis*,metals analysis, antibacterial activity, zone of inhibition, *Bacillus subtilis*

I. INTRODUCTION

Plants are efficient source of bioactive compounds having verity of biological activities due to this plants are used throughout the globe. Plants have secondary metabolites like terpenoids, flavonoids, alkaloids, tannins and steroids etc, these compounds are basically responsible for their medicinal activity. Most vital drugs have been screened from customary used medicinal plants [1].

. The role of bioactive medicinal plants in the traditional system of medicine for curing diseases has been previously reported. Presently, with the increasing scientific knowledge and consumer demand, herbal products have been developed as dietary supplements. It has been revealed previously that whatever is taken as food could cause metabolic imbalance subject to the allowed lower and upper limits of trace metals. The heavy metals may be exposed to the soil and ultimately to plants either through the industrialization, polluted water or other environmental contaminants [2].

The presence of heavy metals beyond the allowed upper and lower limits can cause metabolic disturbance. It has been observed that essential (Fe, Cu, Zn etc) and non-essential metals (Pb, Ni, Cd etc) may affect the biochemical processes (metabolism) in the human body. Thus, deficiencies of essential trace metals especially those, which involve in the metabolism of carbohydrates, for example manganese, chromium, potassium, zinc, and magnesium are found in diabetics. Chromium helpful in lowering cholesterol level while triglyceride levels increase insulin sensitivity. Presence of the excess iron in the body can cause oxidative stress, damaging the pancreas and thus imbalances insulin secretion. Manganese has prominent role in energy metabolism. High levels of zinc may increase glycosylation. WHO recommends that those medicinal plants, which constitute the raw materials for the finished products, may be monitored for the presence of heavy metals, antimicrobial activities and other contaminants [3].

Medicinal plants usually used in the form of infusions, herbal extract, decoction and tincture traditionally. Emerging problem of multi-drug resistant is one of the major health concerns in recent years. Therefore production of novel drugs is necessary using natural product like plants to overcome the multidrug resistant microbial strains [1]. Bacteria are single cell microorganism that can act as antigen for human, animals and plants, causes various diseases and infections. These microorganisms spread through blood and saliva. For examples *Staphylococcus aurous* (a gram-positive bacterium) causes necrosis of skin and *Staphylococcus epidermidis* causes nosoc epidermidis causes nosocomial infections [4].

Citrullus colocynthis (CCCT) is one the most important traditional medicinal plant belonging to the family Cucurbitaceae and generally use a local remedy for the treatment of cancer, carcinoma, endothelioma, leukemia, tumours of the liver and spleen. The plant is distributed in Northern Tropical Africa, Atlantic Islands, North-West India, Pakistan.In Pakistan distributed in desert arid sandy soil of Pakistan and extended to from tropical to subtropical areas of Pakistan. *Citrullus colocynthis* is generally referred as the colocynth, bitter apple, bitter cucumber. It is distributed to the Mediterranean basin and Asia and generally desert native viny plant. It is like common watermelon vine, but bears, small, hard fruit with a bitter pulp. The roots, leaves and fruits are used for the treat of a verity of diseases like inflammation of the breasts, amenorrhea, rheumatism, joint pains, externally used in ophthalmia and uterine pains, jaundice, asthma, anthelmintic, antipyretic carminative, tumours, leucoderma, ulcers, asthma, bronchitis,urinary discharge, enlargement of spleen, tuberculosis glands of the neck, dyspepsia, constipation, anaemia's and throat diseases [5].

The extracts of plant have shown provoke insulin-tropic and placid immune-stimulating effects [3&6]. The use of *Citrullus colocynthis* as medicine and for the remedies of many diseases dated back to prehistory and the people of all continents. They also cured infectious diseases with *Citrullus colocynthis*.The *Citrullus colocynthis* plant relates to the family of Cucurbitaceae the species of which are useful against fever, intestinal parasites, hepatic and abdominal diseases [4&7].

The leaves of *Citrullus colocynthis* have considerable antioxidant, anti-microbial, anti-diabetic, regional painkiller, and anti-inflammatory actions. Seeds of *Citrulluscolocynthis* could be a potential source of nutrients especially crude fiber, minerals (Ca, K, Mg and iron), proteins, antioxidant compounds (α-tocopherol) and essential fatty acids (Linoleic and Oleic acids). It could be used commercially to exploit in different nutraceutical and functional food products [5/8]. Comparative study of *Citrullus colocynthis* and *Citrullus vulgaris* reveals that the major seeds are significantly different in protein, fat and biologicalvalues, which identify their genetic differences as different species. However, they are good sources of energy and other food contents, which will enhance proper growth of children and meet the daily amino acid requirements. They can therefore be used to supplement food substances that are deficient in protein, fat or amino acids [6&9].*Citrullus colocynthis*sample analysis shows that they have good nutritional values due to the high content of the major nutrients, carbohydrates, proteins and lipids [7&10]. Previously, the biodiesel capability of *Citrullus Colocynthis* has also been reported owing to its potential characteristics as a biodiesel energy source [8&11]. *Citrullus colocynthis* shows the action like broad spectrum antibiotics against different species of bacteria (*Bacillus subtilis*, *Escherichia coli* etc.) and fungi (*Aspergillus flavus*, *Candida albicans* etc.). The antimicrobial activity of active extracts of *Citrulluscolocynthis* was compared with the standard tetracycline[10&12]. In Serbia, *Citrullus colocynthis*is the most abundant fruit with a common traditional name ″lubenica″. Its seeds/seeds oil have many medicinal applications as well as applied for cooking and frying oras useful products with good nutritional value [13].

The selected plant *Citrullus colocynthis* is of the important desert medicinal plant, therefore the present study was carried out to determine the proximate analysis (Physiochemical analysis), phytochemical screening, Heavy metals and anti-bacterial activity of *Citrullus colocynthis* plant.

II. MATERIAL AND METHODS

Chemicals and Reagents: Sulphuric acid, Ammonia, Sodium hydroxide, Fehling solution, Hydrochloric acid, Copper sulphate, Potassium sulphate, Iron sulphate, Boric acid, Chloroform and Ferric chloride were purchased from Sigma Aldrich mayer's and Dragendroff's reagents were purchased from local market nutrient agar, nutrient broth, Potato dextrose agar were of oxoid company limited. Bacterial and fungus cultures were obtained from Agriculture University of Peshawar, Pakistan. Distilled water used in the study was prepared in laboratory.

Plant Collection: The plant samples of *Citrullus colocynthis* were packed in paper and then collected in Polythene bags from Ghotki region of Sindh, Pakistan. Identification of Plant was caried out in the Department of Botany, Hazara University Mansehra.

Extraction Procedure: 1.0g of fine powder was dissolve in 10ml of organic solvents (N-hexane, dichloromethane, acetone and methanol) in test tubes. After that these tubes were shifted to shaker for shaking the mixture vigorously for 10 minutes at 300 rpm. Solid plant masses are allowed to settle down and supernatant was collected and filtered by using Whitman filter paper No.3. This process was three time repeated for solvent extract combination. Then solvents were removed by using cold air at room temperature. 10 mg/mL plant extract was finally added in to acetone. The dried samples were grinded by electrical grinder and placed in polythene bags for future use.

Proximate Analysis: Proximate analysis of plant sample defines total proteins, carbohydrates, ash, crude fats, crude fibres and moisture reported as the percentage composition of the product. The dried powder samples were employed to determine the proximate composition of the wastes.The AOAC methods were applied for the proximate analysis [14&15].

Determination of Metals: Atomic absorption spectroscopy is a spectro-analytical procedure for the quantitative determination of metal elements in samples. Through this technique over 62 different metals can be analyzed. It works according to the Beer and Lambert Law. In atomic absorption technique usually, flame is used to atomize the sample, using atomic absorption (Perkin Elmer A 900T). The samples were analyzed by Atomic Absorption Spectrometer, model A 900T, made by Perkin Elmer, equipped with Flame and Graphite furnace controlled by a PC having A.A Wind lab software. The flame atomization was used for analysing the samples. Hollow cathode lamps were used as line source. All the analysis was carried out in Advanced Research Lab (ARL) of the Department of Chemistry, Bacha Khan University Charsadda [12].

Digestion of plant samples

0.5 g sample of all parts of plant was taken in a beaker and treated with 1: 3 mixtures of HNO_3 and HCl. This mixture was heated slowly on heating plate for batter dilution. Double distilled water was used for preparation of solution and filtered by What man filter paper. 20ml of solution was stored in glass bottles.

Anti-bacterial Activity

The anti-bacterial activity was determined through agar diffusion method. The agar plates were inoculated with bacterial strain, then filter paper discs contained the test compounds were placed on the agar surface. The petri dishes were incubated at 37 °C for 24 hours. Then the diameters of inhibition growth zones were measured [22].

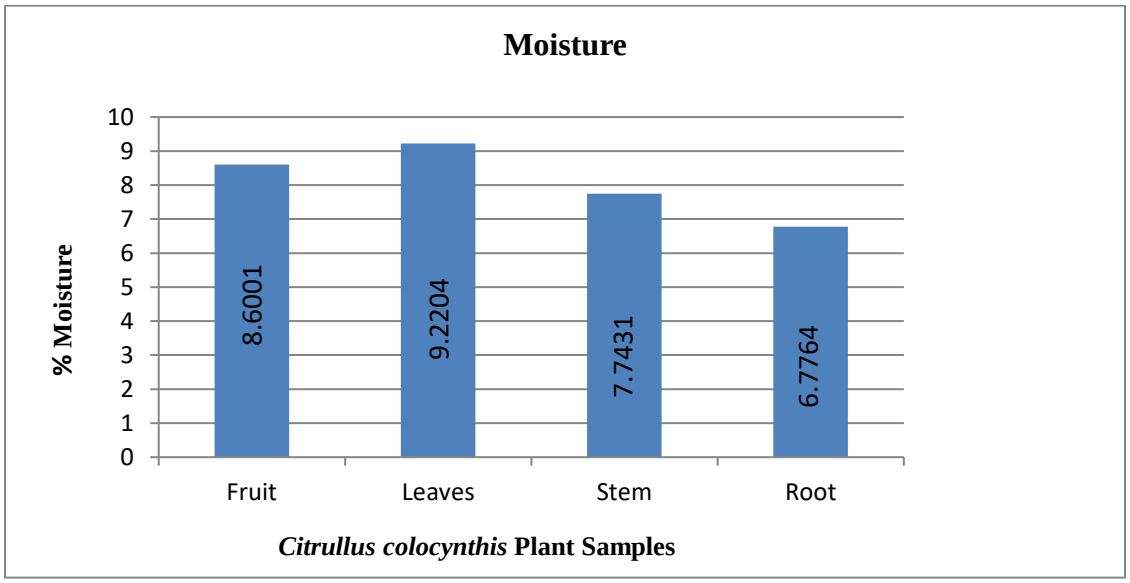
III. RESULTS AND DISCUSSION

Proximate analysis of *Citrullus colocynthis:* Proximate analyses were carried for the determination of the amount of carbohydrate, protein, crude fats, crude fibers, ash, moisture, and energy values of the given samples. The results were expressed in percent. Several physicochemical tests were performed for the determination of mentioned components.

Moisture analysis: The results show that fruit contains 8.60% moisture, leaves 9.22%, stem 7.74% and root 6.77% shown in Fig.1.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	8.9324	8.5923	8.2756	8.6001
Leaves	9.3417	9.2088	9.1109	9.2204
Stem	7.9692	7.7180	7.5421	7.7431
Root	6.9674	6.7331	6.6288	6.7764

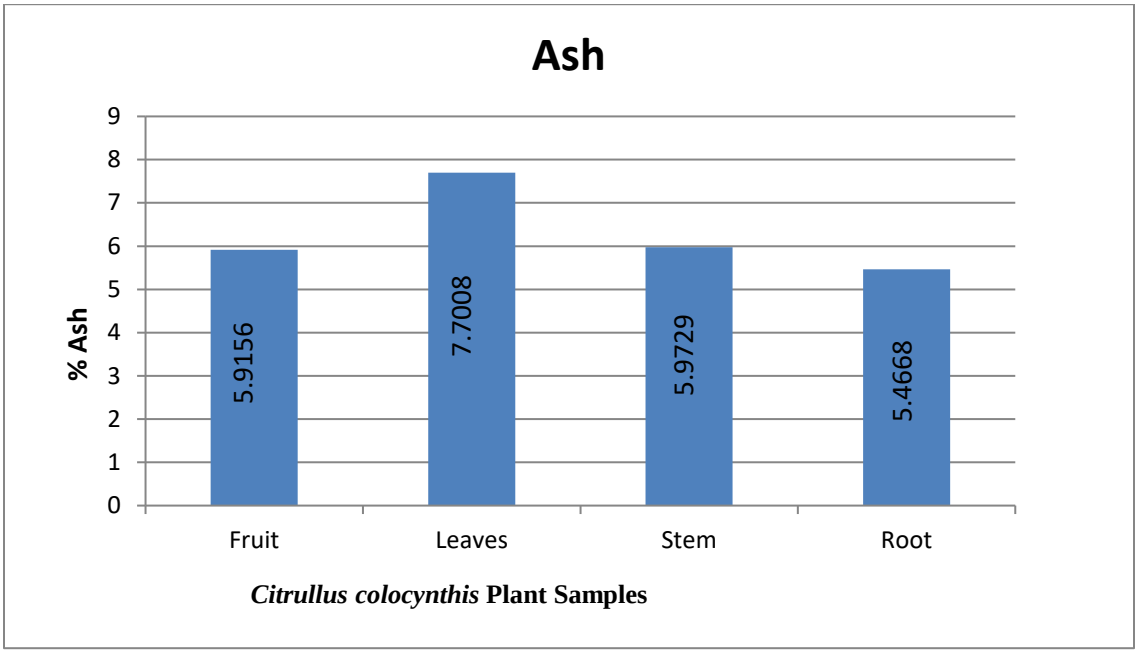
Fig.1: Moisture contents of *Citrullus colocynthis* plant



Ash analysis: The results show that fruit contains 5.91% ash, leaves 7.70%, stem 5.97% and root 5.46% shown in Fig.2.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	5.9890	5.8868	5.8712	5.9156
Leaves	7.7344	7.6881	7.6799	7.7008
Stem	6.1241	5.9279	5.8667	5.9729
Root	5.5327	5.4676	5.4002	5.4668

Fig.2: Ash contents of *Citrullus colocynthis* plant



Crude fats analysis: The results show that fruit contains 8.63% crude fats, leaves 7.36%, stem 5.78% and root 4.27% shown in Fig.3.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	8.7619	8.6049	8.5495	8.6387
Leaves	7.4431	7.3561	7.2887	7.3626
Stem	5.8570	5.7793	5.7114	5.7825
Root	4.3221	4.2703	4.2367	4.2763

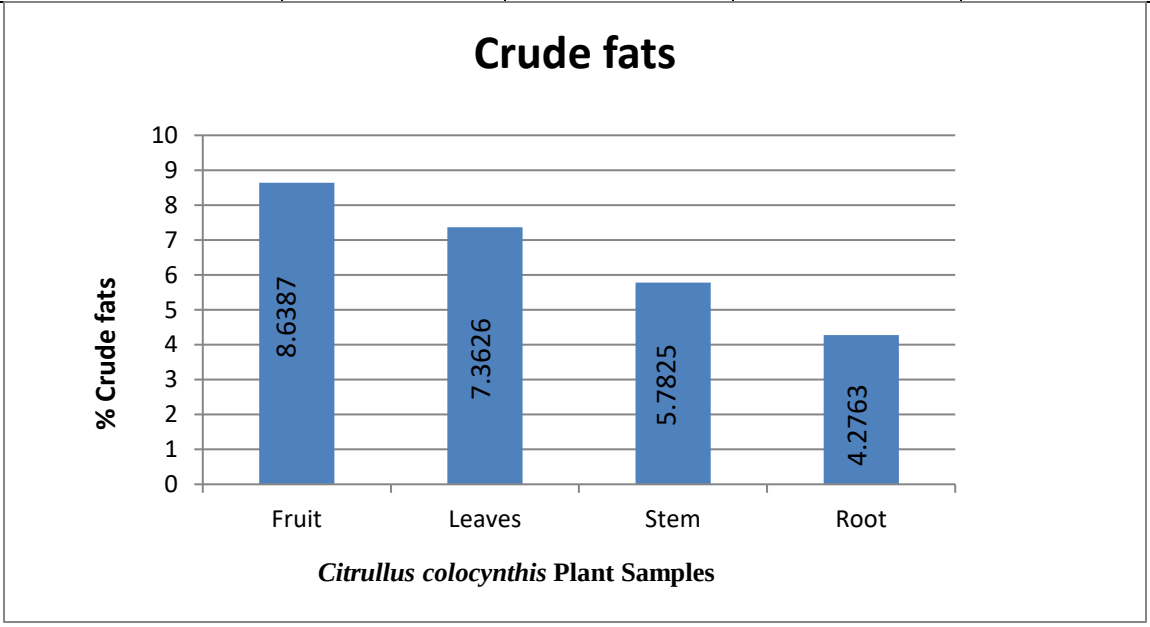
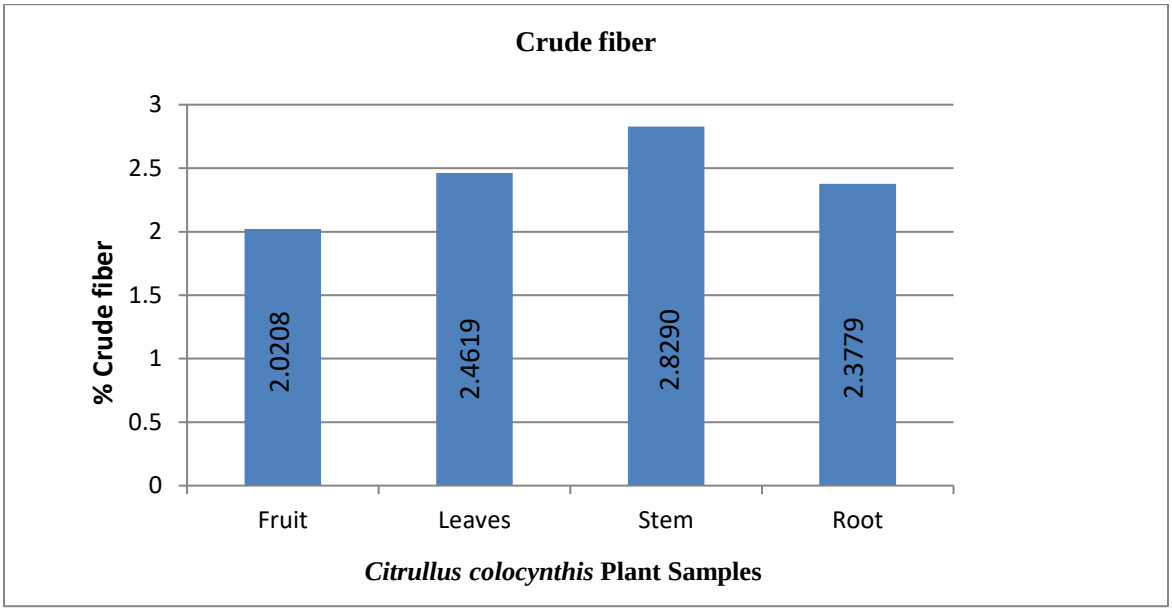


Fig.3: Crude fats contents of *Citrullus colocynthis* plant

Crude FiberAnalysis: The results show that fruit contains 2.02% crude fiber, leaves 2.46%, stem 2.82% and root 2.37% shown in Fig.4.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	2.1933	2.0169	1.8523	2.0208
Leaves	2.8274	2.4365	2.1220	2.4619
Stem	3.0021	2.8847	2.6004	2.8290
Root	2.5867	2.3417	2.2053	2.3779

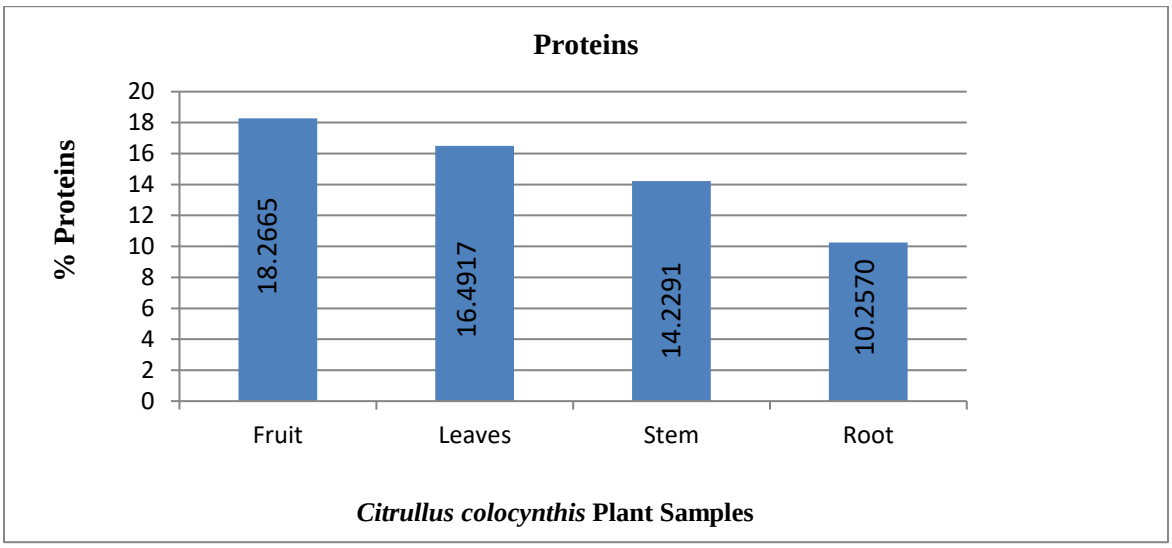
Fig.4: Crude fibre contents of *Citrullus colocynthis* plant



Proteins Analysis: The results show that fruit contains 18.26% proteins, leaves 16.49%, stem 14.22% and root 10.25% shown in Fig.5.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	18.6539	18.2190	17.9267	18.2665
Leaves	16.9803	16.3906	16.1044	16.4917
Stem	14.7087	14.1927	13.7860	14.2291
Root	10.5439	10.2303	9.9968	10.2570

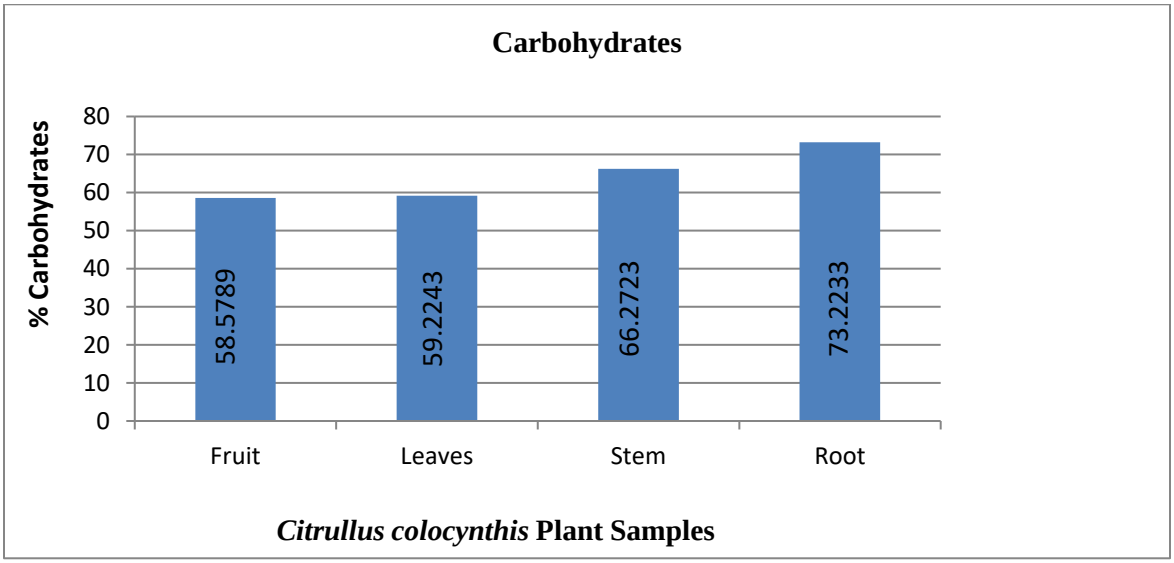
Fig.5: Proteins contents of *Citrullus colocynthis* plant



Carbohydrates Analysis: The results show that fruit contains 58.57% carbohydrates, leaves 59.22%, stem 66.27% and root 73.22% shown in Fig.6.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	57.6628	58.6970	59.3770	58.5789
Leaves	58.5005	59.3564	59.8161	59.2243
Stem	65.3410	66.3821	67.0938	66.2723
Root	72.6339	73.2987	73.7375	73.2233

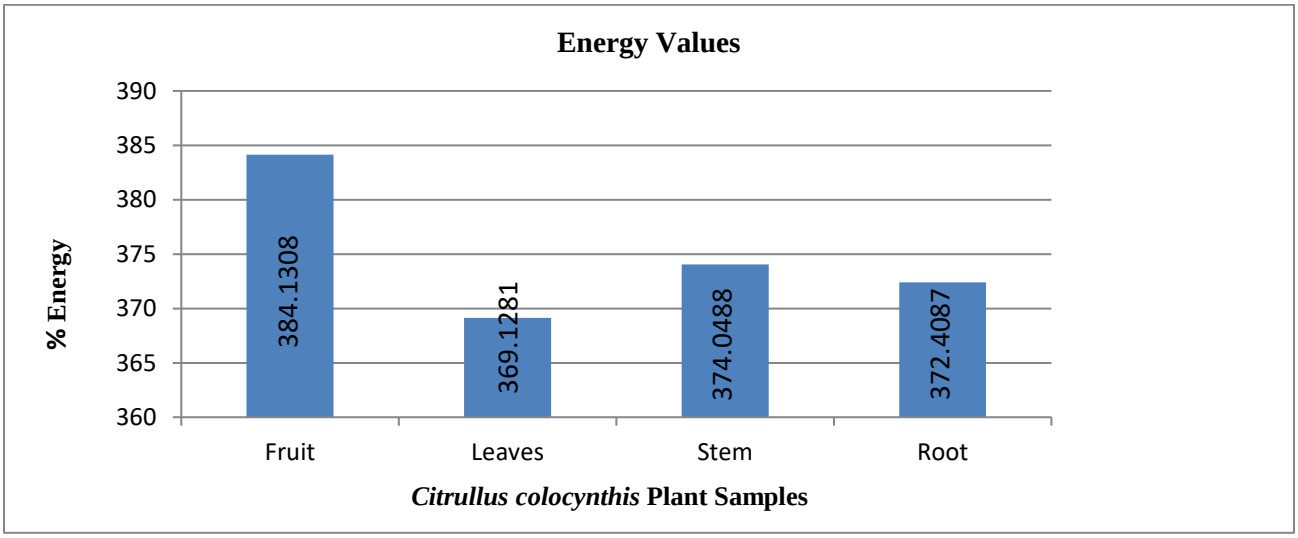
Fig.6: Carbohydrates contents of *Citrullus colocynthis* plant



Energy values: The results show that fruit contains 384.13% energy values, leaves 369.12%, stem 374.04% and root 372.40% shown in Fig.7.

Sample	Reading 1 (%)	Reading 2 (%)	Reading 3 (%)	Mean
Fruit	384.1239	382.1081	386.1603	384.1308
Leaves	368.9111	369.1929	369.2803	369.1281
Stem	372.9118	374.3129	374.9218	374.0488
Root	371.6101	372.5487	373.0675	372.4087

Fig.7: Energy values of *Citrullus colocynthis* plant

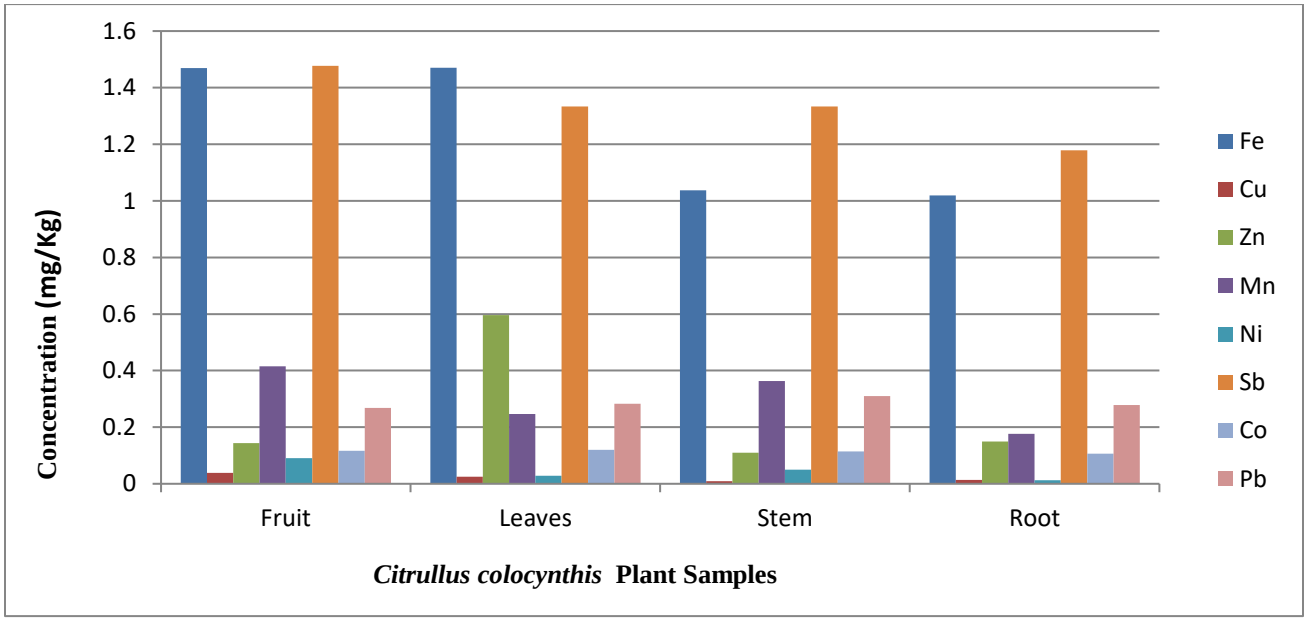


Heavy Metals Analysis: The results show that fruit contains 1.469 mg/Kg Fe, 0.039 mg/Kg Cu, 0.144 mg/Kg Zn, 0.415 mg/Kg Mn, 0.090 mg/Kg Ni, 1.477 mg/Kg Sb, 0.117 mg/Kg Co and 0.268 mg/Kg Pb. The leaves contain 1.470 mg/Kg Fe, 0.025 mg/Kg Cu, 0.596 mg/Kg Zn, 0.247 mg/Kg Mn, 0.028 mg/Kg Ni, 1.333 mg/Kg Sb, 0.120 mg/Kg Co and 0.283 mg/Kg Pb. The stem contains 1.037 mg/Kg Fe, 0.009 mg/Kg Cu, 0.110 mg/Kg Zn, 0.363 mg/Kg Mn, 0.050 mg/Kg Ni, 1.333 mg/Kg Sb, 0.114 mg/Kg Co and 0.310 mg/Kg Pb. The root contains 1.019 mg/Kg Fe, 0.014 mg/Kg Cu, 0.149 mg/Kg Zn, 0.177 mg/Kg Mn, 0.012 mg/Kg Ni, 1.179 mg/Kg Sb, 0.106 mg/Kg Co and 0.278 mg/Kg Pb as shown in Fig.8.

Heavy Metal	Fruit mg/kg	Leaves mg/kg	Stem mg/kg	Root mg/kg
Fe	1.469	1.470	1.037	1.019

Cu	0.039	0.025	0.009	0.014
Zn	0.144	0.596	0.110	0.149
Mn	0.415	0.247	0.363	0.177
Ni	0.090	0.028	0.050	0.012
Sb	1.477	1.333	1.333	1.179
Co	0.117	0.120	0.114	0.106
Pb	0.268	0.283	0.310	0.278

Fig.8: Heavy metals of *Citrullus colocynthis* plant



Anti-bacterial Activity: Human MTDR (multidrug resistant pathogenic bacteria) causing various severe infections such as sepsis, endocarditis, skin infections, food poisoning, bacteremia, endocarditis, pneumonia, and septicaemia in human. Various extract of the selected plant parts was tested against MDR strains. Etthyl alcohol extracts of plant fruits, leaves . stem, and roots were found effective against Gram-positives trains *Bacillus pumilis* and *S. aureus*, while fruit ethanolic extracts showed significant zone (19mm) of inhibition against *Bacillus subtilis* compared to other Gram-Positive strains less than 17mm (average) zone of inhibition.

IV. CONCLUSIONS

The studied medicinal plant *Citrullus colocynthis*analysisindicated that the plant has good nutritional values due to content of major nutrients carbohydrates, proteins, fats and also has high energy values. The plant could be used for therapeutic purpose and many useful drugs can be synthesized for the remedial purpose of different diseases. The plant extracts have a good value of fibres and minerals like iron. The extracts of plants may be

rich in nutrient composition and chemical substances which has a great importance for pharmaceutical industries.The plant extracts showed heavy metals which are due to soil texture. The extracts of plant samples showed a significant value of antibacterial activity. The proximate analyses of *C. colocynthis* gave important information about the chemicals present in the plant. The chemical tests showed the presence of alkaloids, saponins, tannins, flavonoids and terpenoids etc.

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Authors

1Dr.Zarbad Shah, Associate Professor, Department of Chemistry, Bacha Khan University, Charsadda-24420, Khyber Pakhtunkhwa, Pakistan, zshej@hotmail.com,

2. Dr.Alia Gul Lecturer Department of Botany, Hazara University, Mansehra, 21300, Khyber Pakhtunkhwa, Pakistan, *Corresponding author aliagul@hu.edu.pk, , aliagulbotanist@gmail.com, https://orcid.org/0000-0002-5493-5130?lang=en,

3.Tufail Arshad, Department of Agriculture, Bacha Khan University Charsadda, Charsadda-24420, Khyber Pakhtunkhwa, Pakistan, tofailarshad@gmail.com

4.Muhammad Idrees, Department of Chemistry, Bacha Khan University, Charsadda-24420, Khyber Pakhtunkhwa, Pakistan, idrees_chemist@yahoo.com

5.Nadia Jabeen lecturer, Department of Agriculture, Hazara University, Mansehra, 21300, ORCID id 0000-0001-6617-8301, Email nadia_khan909090@yahoo.com

6. Dr.SumeraShaheen,lecturer, Department of Biochemistry, Hazara University, Mansehra,21300, sumaira.bch@gmail.com

7. Syed Majid Rasheed, Department of Agriculture, Bacha Khan University Charsadda, Charsadda-24420, Khyber Pakhtunkhwa, Pakistan, zhej2910@gmail.com,

8.Dr. Muhammad Akhlaq, Associate Prof., Chairman Department of Pharmacy, Hazara University, Mansehra, 21300 akhlaqhayat@hotmail.com

9. Waqar Ali, PhD scholar, Department of Pharmacy, University of Peshawar, Pakistan. Email: aliwaqar241@gmail.com,

10. Asma, Department of Botany, Hazara University, Mansehra,21300, Asma.faheem84@gmail.com

11. Naila Sardar, Post Graduate Resident Pediatrics, Lady Reading Hospital, Peshawar, Khyber Pakhtunkhwa , Pakistan nailasardar70@gmail.com

12: Sayed Hasnain Imad, BS Institute of Chemical Sciences, University of Peshawar, Pakistan, 25000 Email: sayyedhasnain661@gmail.com,

13. Abdul Basit: BS Botany, University of Peshawar, 25000, Email: abdulbasitraza440@gmail.com