International Journal of Phytopharmacology
Research Article
www.onlineijp.com
e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

A STUDY ON HYDROALCOHOLIC EXTRACT OF CITRULLUS COLOCYNTHIS LEAVES: PHARMACOGNOSICAL, PHYTOCHEMICAL AND IN-VITRO ANTI-OXIDANT EVALUATION

Sherisha Bhavani D[1*], Laxmi Prasanna Y [3], Varsha S[3], Nikitha Reddy D[3], Swathi Goud N[3], Naveen G[3], Sumalatha K[2], Nagamani C[1]

[1]Associate Professor, Department of Pharmaceutical Chemistry, Bhaskar Pharmacy College, Hyderabad, India.
[2]Associate Professor, Department of Pharmacognosy, Bhaskar Pharmacy College, Hyderabad, India.
[3]Bhaskar Pharmacy College, Hyderabad, India.

ABSTRACT

The objective of the present work is to study the Pharmacognostical, Phytochemical characterization and in vitro anti-oxidant potential of hydro alcoholic extract of *Citrullus colocynthis*. In developing countries, herbal medicines account for about 80% of primary health care used by the global population. It is believed that this is attributed to the chemical constituents in them which are part of the physiological functions of the living flora, thus they are considered to be more compatible with the human body due to their physiological functions. *Citrullus colocynthis* (L.) Schrad. is a species of cucurbit that belongs to the family of Cucurbitaceae. It is a perennial herbaceous vine that grows up to three meters in length, flowers and has a berry-like fruit. The leaves of *Citrullus colocynthis* was collected in and around Vellore. The leaves powder was analyzed macroscopically, microscopically, physiochemically, and phytochemically by macroscopic, microscopical, and physicochemical methods. A variety of chemical tests were performed on this hydro-alcoholic extract of leaves of *Citrullus colocynthis* to identify flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannins, amino acids, and sterols as per standard procedures. The total phenol content of *Citrullus colocynthis* was determined by the Folin-Ciocalteu colorimetric method. The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts. The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce FeCl3 solution as mentioned. The total phenolic content in the hydro alcoholic extract of C. colocynthis leaves was found to be 64.6 mg/g. The total flavonoid content in the hydro alcoholic extract of *C. colocynthis* leaves was found to be 85.5 mg/g. A hydroalcoholic extract from *Citrullus colocynthis* showed moderate antioxidant activity despite having strong antioxidant properties despite plants' strong antioxidant properties. By absorbing electrons from antioxidants, it neutralizes its free radical nature. There is a scientific foundation for the prospect of applying the leaves of *Citrullus colocynthis* for the treatment of anti-oxidants through the present study. A future scope of work includes identifying the chemical components responsible for these activities and conducting in-vivo pharmacological screenings.

Key words: Citrullus Colocynthis, Herbal Medicine, Anti-Oxidant Activity, Phenolic, Flavonoid Content.

Corresponding Author: **Sherisha Bhavani D** Email: sherisha0110@gmail.com

Access this article online

Home page: www.onlineijp.com

Quick Response code

Received:19.01.2023 **Revised**:17.02.2023 **Accepted**:04.03.2023

INTRODUCTION

In indigenous cultures (such as Black African and Native American cultures) herbal remedies have played a significant role in healing rituals, while other cultures have developed traditional medical systems (such as Siddha, Ayurveda, Unani and Traditional Chinese Medicine) that also incorporated herbal remedies (Ampofo

AJ, *et al.* 2012). The use of plant-based medicines dominated our medicinal practices around two centuries ago, when they dominated most of our medical practices. Since more predictable synthetic drugs became commonly available in the West, the medicinal use of herbs fell into a rapid decline, which resulted in a rapid decline in the use of herbs. In the Middle East and South Asia, traditional Chinese medicine (TCM) and Unani medicine are still widely practiced by a large number of people, including Siddha & Ayurvedic medicines in India, Kampo Medicine in Japan, and Tibetan medicine (TM) in China (Mosihuzzaman M, *et al.* 2008). In developing countries, herbal medicines account for about 80% of primary health care used by the global population. It is believed that this is attributed to the chemical constituents in them which are part of the physiological functions of the living flora, thus they are considered to be more compatible with the human body due to their physiological functions (Partap S, *et al.* 2012). A wide range of herbal formulations have been developed by researchers from several different systems of medicine, with the plants being a promising source of herbal formulations not only among the professionals of various systems of medicine, but also in various scientific communities (Padmawar A, 2011). A rapid increase in the number of herbal drug manufacturers has been caused by the use of herbal drugs as a result of the toxicity and side effects of allopathic medicines. People who have no prescription for herbal drugs have been increasingly consuming them without having to seek a prescription for the past few decades. A total of 120 active compounds have been isolated from the higher plants and are now widely used in modern medicine as a result of their positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Rukangira E. 2000). Of these 120 active compounds, 80 percent demonstrate a positive correlation with their traditional use in modern medicine. One of the major challenges involved in evaluating conflicting toxicological, epidemiological, and other data, and verifying the herbal materials used in the research, is objectively assessing conflicting data. *Citrullus colocynthis* (L.) Schrad. is a species of cucurbit that belongs to the family of Cucurbitaceae. Known as a perennial trailing vine that can grow in desert environments, this plant is native to the Mediterranean Basin, as well as subtropical and tropical Asia. The main reason this plant is cultivated is because it is used in a number of ethnomedical and ethnoveterinary applications (Kamboj A. 2012). It is possible to eat the seeds once they have been cooked. In addition to producing oil, colocynths are also able to convert this oil into biodiesel, making them an attractive source of energy. *Citrullus colocynthis* is a perennial herbaceous vine that grows up to three meters in length, flowers and has a berry-like fruit. An angular and rough texture can be found on the stems of this plant. On 1-7 cm petioles, the stiff leaves are slender and have 3-7 lobes, measuring 5-10 cm long by 2.5-6 cm in diameter, and bearing fruit. This plant has solitary flowers on axillary buds, which appear greenish to bright yellow in color and are monoecious (male and female are distinct), solitary, pentamerous, and borne on axillary buds (Farnsworth NR, Bingel AS.1997). As the fruit ripens, it will turn yellow, with a globular shape, about the size of a small orange, with a green and yellow variegated surface, turning yellow when ripe. Among its uses, *C. colocynthis* is beneficial for treating digestive disorders, pulmonary, skin, and bacterial infections, constipation, edema, cancer, and diabetes. GI disorders like indigestion, gastroenteritis, and intestinal parasites can be treated with the dried pulp of *C. colocynthis. C. colocynthis* plant extracts (from the plant's roots, stems, and leaves), as well as three maturation stages of its fruit and seeds, have been shown to be active against Gram-positive and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Enterococcus faecalis*), but are more effective on newer bacteria. In this method, the minimum inhibitory concentration (MIC) that prevents visible bacterial growth was determined (Nazim Mamedov. 2012). The antioxidant properties of cucurbitacin make it an effective anti-oxidant as well. Free radicals, such as hydroxyl radicals and superoxide anions, can be destroyed with this type of treatment. Peroxidation and oxidation of lipids can also be completely inhibited by the compound (Kinghorn A.D., and Balandrin M.F. 1993). Different in vivo screening models were used to evaluate the anti-inflammatory activity of methanolic extracts of *C. colocynthis* leaves. As a result, it had a powerful anti-inflammatory effect in both acute and subacute phases of inflammation. It inhibited the formation of exudate caused by carrageenan, leukocyte infiltration, and the oedema of the paw caused by different inflammatory drugs at doses of 250 and 500 mg/kg (Harbone J.B and Baxter H.H. 1993). In this study, the Pharmacognostical, Phytochemical, and anti-oxidant potential activities of hydro alcoholic extract of Citrullus colocynthis has been evaluated.

Methodology

The leaves of *Citrullus colocynthis* was collected in and around Vellore. The leaves were authenticated by a botanist. The fresh leaves were used for the macroscopic, microscopic evaluation and for the determination of leaf constants. The leaves were collected, washed and cutted into small pieces and shade dried. The shade dried leaves were powdered, sieved and used for physico chemical analysis and for powder microscopy.

Pharmacognostical study

The organoleptic characteristics such as shape, size, color, odor, taste was determined. Microscopical characters were determined by fixing the plant in FAA solution and graded with alcohol series of 10 percent tert-butyl, according to the standard method.

Powder microscopy were carried out to know about the inclusion and detailed anatomical characters of the materials.

Physicochemical study

Standard guidelines were followed for the physicochemical analysis of powdered plant materials such as foreign organic matter, loss on drying, ash value was studied.

Phytochemical study

Extraction of plant materials

Collected plant powder was extracted with hydro alcohol (Ethanol (70%): Water (30%)) by means of maceration process for 72 hrs, then filter it and concentrated under vacuum. The dried extract was collected and stored in a air tight container.

Preliminary Phytochemical Screening

A variety of chemical tests were performed on this hydro-alcoholic extract of leaves of *Citrullus colocynthis* to identify flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannins, amino acids, and sterols as per standard procedures.

Quantitative Estimation of Phytoconstituents

Determination of total phenolic content

The total phenol content of *Citrullus colocynthis* was determined by the Folin-Ciocalteu colorimetric method. The Folin Ciocalteu reagent is a mixture of phosphomolybdate and phosphor tungstate. The method measures the amount of substance needed to inhibit the oxidation of the reagent. The total phenols were determined by spectrophotometrically at 760nm. Gallic acid at different concentration i, e.20, 40, 60, 80, 100µg/ml was taken and treated in the same manner and the absorbance was measured at 750nm.

Determination of total flavonoid content

The aluminum chloride colorimetric technique was used for estimation of total flavonoid estimation. Aluminum ions form stable complexes with C4 keto group and either to C3 or C5 hydroxyl groups of flavones and flavanols in acidic medium. It also forms acid labile complexes with ortho hydroxyl groups in the A or B rings of flavonoids. These complexes showed a strong absorption at 415nm which is used for the estimation of flavonoids. The amount of flavonoids present can be determined by linear regression analysis. The total flavonoid content was expressed as mg quercetin equivalents /g of extract.

IN-VITRO ANTI OXIDANT ACTIVITY

1. Free radical Scavenging activity using di phenyl picryl hydrazyl (DPPH) free-radical

DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of specific compound or plant extracts. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. A stock solution of 1mg/ml concentration of hydro alcohol extract of *Citrullus colocynthis* was prepared. To 1ml of various concentrations (40 to 200µg/ml) of test samples, 4ml of DPPH was added. Control was prepared without sample in an identical manner. DPPH was replaced by ethanol in case of blank. The reaction was allowed to complete in the dark for about 30min. Then the absorbance was measured at 517nm. Vitamin C was used as standard.

2. Reducing power assay

The reducing power ability of plant extracts was screened by assessing the ability of the test extract to reduce $FeCl_3$ solution as mentioned. 0.2 to 1 ml of hydro alcohol extract of *Citrullus colocynthis* leaves of (1mg/ml) was mixed with 0.75ml phosphate buffer and 0.75ml of 1% potassium ferricyanide [K_3 Fe (CN_6)] and incubated at 50ºC for 20min.0.75ml of 1% trichloro acetic acid was added to the mixture allowed to stand for 10min. The whole mixture was then centrifuged at 3000rpm for 10min. Finally, 1.5ml of the supernatant was removed and mixed with 1.5ml of distilled water and 0.1ml of 0.1%ferric chloride solution and the absorbance measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution.

RESULTS

The fresh leaves were used for the macroscopic, microscopic evaluation and for the determination of leaf constants. The leaves were collected, washed and cutted into small pieces and shade dried. The shade dried leaves were powdered, sieved and used for physico chemical analysis and for powder microscopy.

Macroscopical Studies

Acutely divided, lobes slender, thick and barren. The slanted leaves are alternately positioned on prolonged petioles. Leaves are almost 5- 10 cm in length and have approximately 3- 7 lobes.

Microscopical Studies

The following features have been observed in the transverse section of the leaves of *Citrullus colocynthis.*

The leaf consists of a thick midrib and thin lamina with densely tomentose abaxial surface. The Lamina consist of thick and wide, vertically oblong

epidermal cells with prominent cuticle. The stomata are actinocytic type. The epidermis bears dense non-glandular trichomes. The mesophyll cells consist of adaxial palisade and abaxial spongy parenchyma cells. The palisade cells are single layered with thin vertical, long cylindrical cells and cylindrical, spherical or lobed parenchyma cells in spongy mesophyll tissue. The vascular strand consists of two arc shaped collateral bundles. The venation is densely reticulate. The primary, secondary and tertiary veins are thin, but distinct. The vein- islets are wide and well defined with prominent vein – boundaries. The vein- terminations, when present are long, slender and forked at the tip.

It consists of narrow epidermal layer of thick-walled cylindrical cells and angular thick walled compact paranchymatous ground tissue. On the adaxial end of the midrib the palisade tissue continues, leaving gap of parenchyma cells. There are two Vascular bundles in the midrib. Both bundles are bicollateral. Phloem occurs both on the inner and outer part of the xylem and so that bundles are called bicollateral. The mesophyll tissue consists of adaxial broad zone of palisade cells and lower zone of spongy parenchyma. The palisade is single layered. The spongy parenchyma has about 5 layers of large, lobed cells forming inter cellular air-chambers. Calcium carbonate crystals are often seen deposited in the epidermal cells, especially in the adaxial epidermal cells, beneath the trichomes. The stomata are of anomocytic type of stomata. Parenchyma cells of different size and shapes are seen in the powder.

PHYSICOCHEMICAL PARAMETERS

The powdered drug was evaluated for its physico-chemical parameters like total ash values, acid insoluble ash, water soluble ash and loss on drying, and the results were tabulated below.

QUANTITATIVE ESTIMATION OF PHYTOCONSTITUENTS

The quantitative estimation of phytoconstituents is very essential for identifying and quantifying the phytochemicals present in the medicinal plants which is important for therapeutic action.

1. Determination of total phenolic content

Quantitative estimation of phenol was done by folin ciocalteu method using gallic acid as a standard. Then the absorbance was noted for five different concentrations of standard gallic acid and the extract at 760 nm. The total phenolic content was expressed in terms of GAE. The total phenolic content in the hydro alcoholic extract of *C. colocynthis* leaves was found to be 64.6 mg/g.

2. Determination of total flavonoid content

Quantitative estimation of flavonoid was done by aluminum chloride method using quercetin as a standard. Then the absorbance was noted for five different concentrations of standard gallic acid and the extract at 510 nm. The total flavonoid content was expressed in terms of QAE. The total flavonoid content in the hydro alcoholic extract of *C. colocynthis* leaves was found to be 85.5 mg/g.

***IN-VITRO* ANTI OXIDANT ACTIVITY**

Despite the fact that plants possess strong antioxidant properties, an hydroalcoholic extract from *Citrullus colocynthis* showed moderate antioxidant activity when compared to standard antioxidants despite the fact that plants possess strong antioxidant properties. A stable free radical, it neutralizes its free radical nature with an electron from antioxidants.

Table 1: Physicochemical analysis of the leaves of *C. colocynthis*

S.No	Parameters	Observation
1	Total ash	7.8 %
2	Acid Insoluble ash	0.23%
3	Water soluble ash	3.6%
4	Sulphated ash	9.16%
5	Loss on drying	9 %

Table No: 2 Preliminary phytochemical screening ethanolic extraction for the fruit powder of *Citrullus colocynthis*

S.NO	TEST	Results
1.	**TEST FOR STEROLS**	
	a. Salkowski's test	-
	b. Libermann- burchard's test	-
2.	**TEST FOR ALKALOIDS**	
	a. Mayer's reagent	+
	b. Dragendorff's reagent	+
	c. Hager's reagent	+
	d. Wagner's reagent	+
3.	**TEST FOR FLAVONOIDS**	
	a. Shinoda test	+

	b. Alkali test	+
	c. Acid test	+
4.	**TEST FOR CARBOHYDRATES**	
	a.Molisch's test	+
	b. Benedict's test	+
	c. Fehling's test	+
5.	**TEST FOR GLYCOSIDES**	
	i) Borntrager's test	+
	ii) Modified Borntrager's test	+
6.	**TEST FOR FIXED OIL**	-

Figure No: 1 Macroscopical leaves of leaves of Citrullus colocynthis

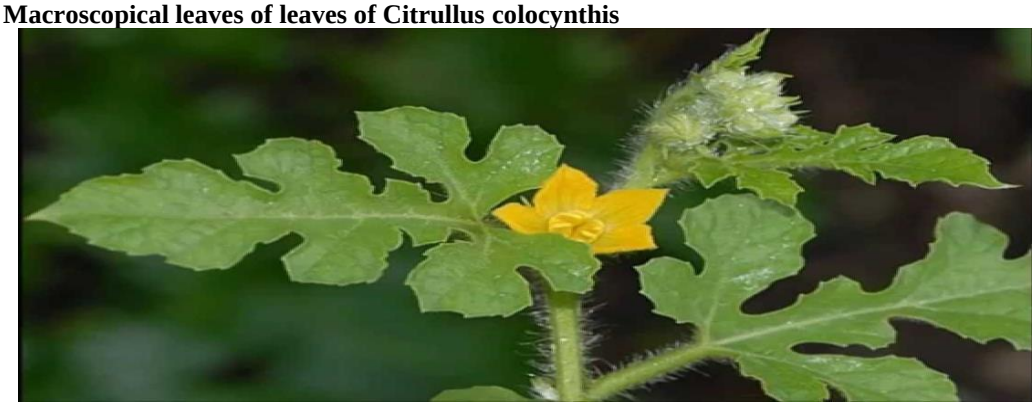
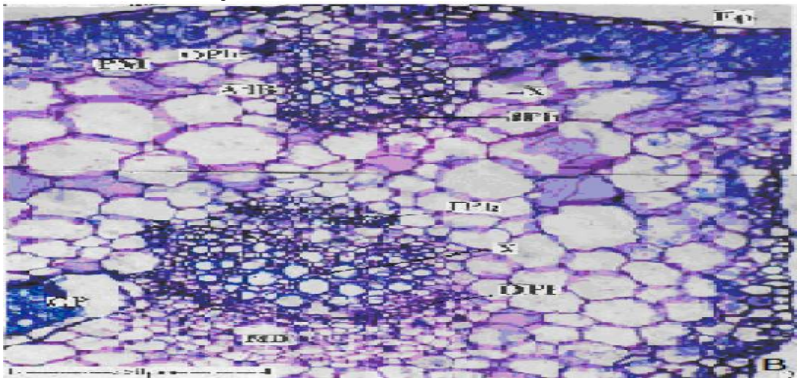
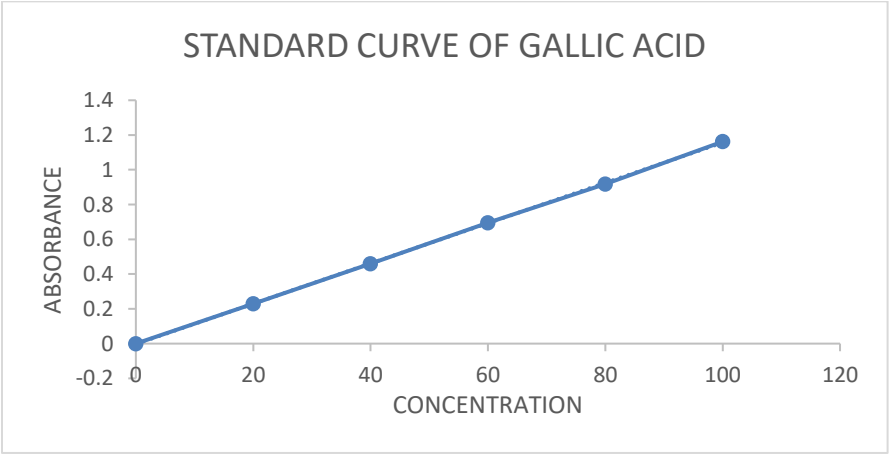


Figure No. 2. Transverse section of C. colocynthis leaf





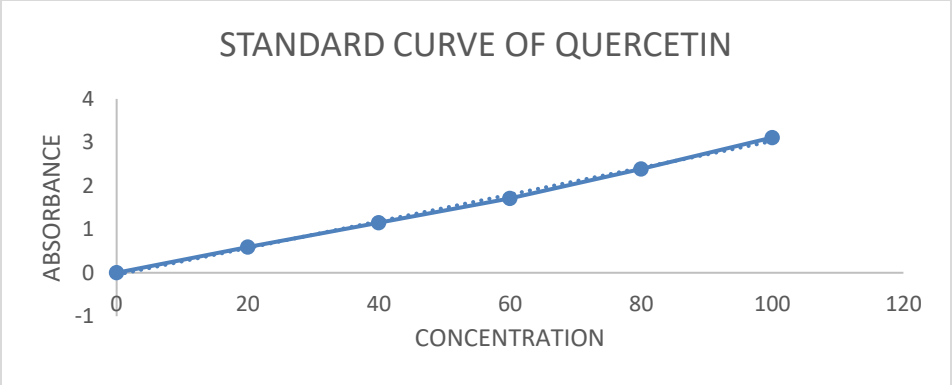


Table No: 3 Percentage inhibition by hydro alcohol extract of *Citrullus colocynthis* and standard ascorbic acid against DPPH at 517nm

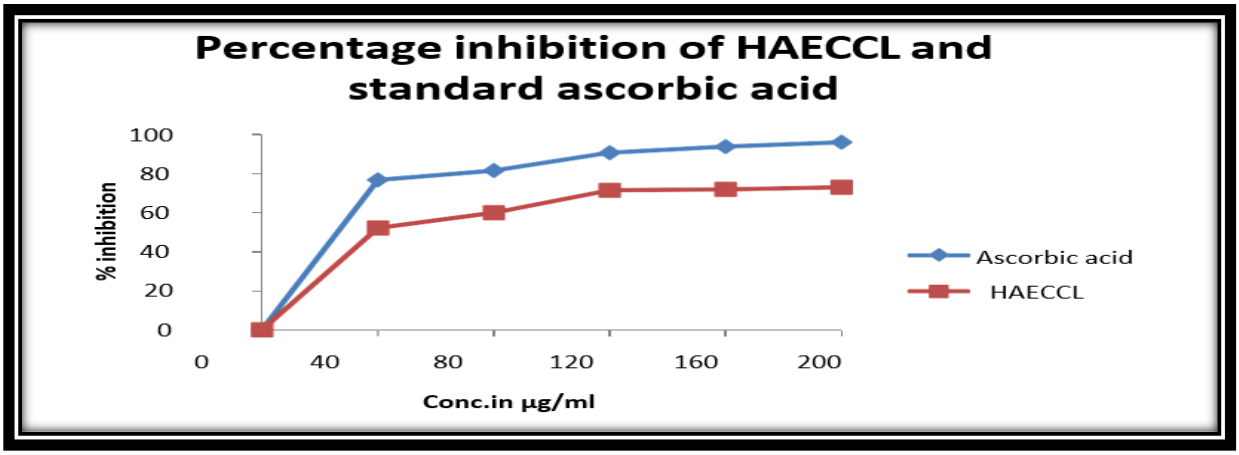
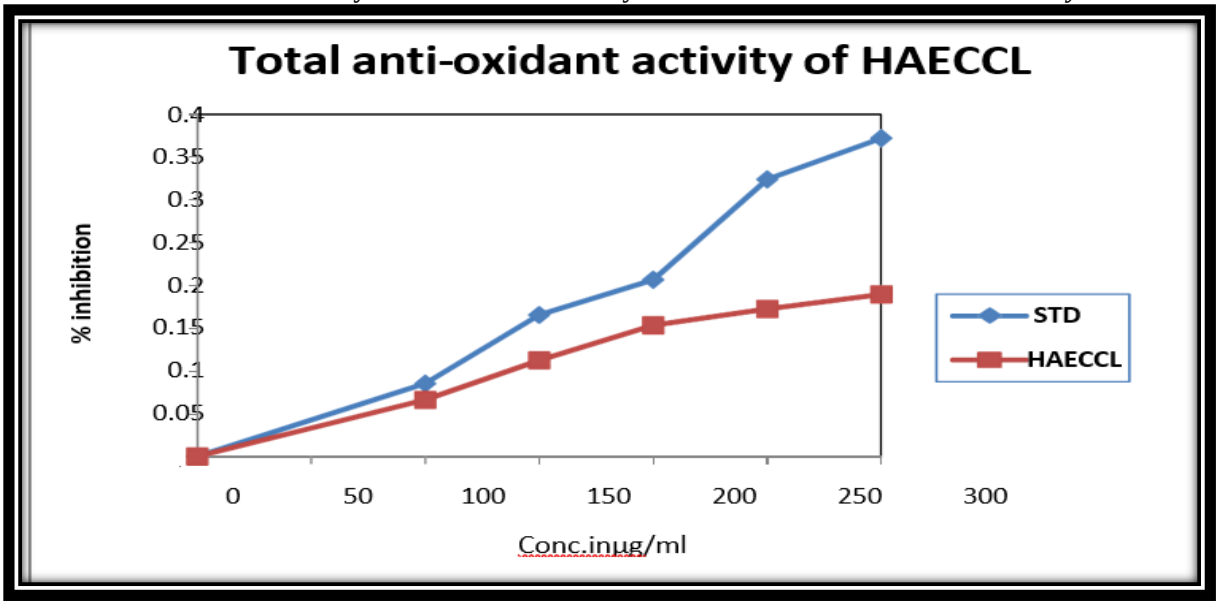


Table No: 4 Total antioxidant activity of ascorbic acid and hydro alcoholic extract of *Citrullus colocynthis*



DISCUSSION

A study on the impact of the hydro alcohol extract of *Citrullus colocynthis* on pharmacognosy, phytochemistry, and pharmacological effects was conducted as part of this dissertation. An analysis of macroscopical and microscopical characteristics, quantitative microscopy, and standardization parameters like ash value, loss on drying has been conducted. In the hydro alcohol extract of *Citrullus colocynthis*, significant concentrations of carbohydrates, alkaloids, flavonoids were found. A screening for in vitro pharmacological activity revealed that *Citrullus colocynthis* hydro alcohol extract showed significant antioxidant activity, as evaluated by free radical scavenging by DPPH assay, reducing power assay.

CONCLUSIONS

Citrullus colocynthis leaves extracts with a hydro alcohol content were evaluated for their anti-oxidant activity, which was found to be significant. Therefore, we can conclude that the hydro alcohol extract of *Citrullus colocynthis* leaves exhibited antioxidative properties. The nature and category of such actions of the more investigation are difficult to determine at the moment before any definite conclusion can be drawn. The possible explanation for the activity may be related to the presence of carbohydrate, alkaloids, flavonoids, and glycosides in the leaves. There is a scientific foundation for the prospect of applying the leaves of *Citrullus colocynthis* for the treatment of anti-oxidants through the present study. A future scope of work includes identifying the chemical components responsible for these activities and conducting in-vivo pharmacological screenings.

REFERENCES

Ampofo AJ, Andoh A, Tetteh W, Bello M. Microbiological Profile of Some Ghanaian Herbal Preparations-Safety Issues and Implications for the Health Professions, *Open Journal of Medical Microbiology*. 2, 2012,121-130.

Mosihuzzaman M, Choudhary MI. Protocols on Safety, Efficacy, Standardization, and Documentation of Herbal Medicine, *Pure Appl. Chem*. 80(10), 2008, 2195–2230.

Partap S, Kumar A, Sharma NK, Jha KK. Luffa Cylindrica: An important medicinal plant, *J. Nat. Prod. Plant Resource* 2 (1), 2012, 127-134.

Padmawar A, Bhadoria U. Phytochemical investigation and comparative evaluation of in vitro free radical scavenging activity of Triphala & Curcumin. *Asian Journal of Pharmacy and Medical Science*. 1(1), 2011, 9-12.

Rukangira E. The African Herbal Industry: Constraints and Challenges, proc: "The natural Products and Cosmeceutcals 2001conference". Africa. 2000, 1-20.

Kamboj A. Analytical Evaluation of Herbal Drugs, *Drug Discovery Research in Pharmacognosy*, 3, 2012, 23-55.

Farnsworth NR, Bingel AS. Problems and prospects of International Journal of Herbal Medicine discovery new drugs from higher plants by pharmacological screening. *Springer Verlag, Berlin*, 1997, 1-22.

Nazim Mamedov. Medicinal plants studies: history, challenges and prospective. Medicinal & Aromatic Thillaivanan S, Samraj K. Challenges, constraints and opportunities in herbal medicines – a Plants 1(8), 2012, 1.

Kinghorn, A.D., and Balandrin, M.F., (1993). Human medical agents from plants, American Chemical Society, San Francisco, USA.

Harbone, J.B., and Baxter, H.H. 1993. Phytochemical Dictionary: A hand Book of Bioactive Compound from plants. Taylor and Francis, Washington, D.C., U.S.A, 237.