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# Phyto-Pharmacognostic, Antioxidant and Acetylcholinesterase Inhibitory Properties of *Citrullus colocynthis* Fruits

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## Abstract

**Background**: Alzheimer's disease (AD) is a major global health burden. Fruits containing phenolic compounds have shown substantial promise against pathological changes (e.g. oxidative stress and cholinergic deficit) of AD. *Citrullus colocynthis* (bitter apple) is a berry type fruit used extensively in traditional systems of medicine. This is rich in polyphenols but has not been investigated for its potential against AD. Moreover, its pharmacognostic study is not documented. **Objective:** This study involved evaluation of phyto-pharmacognostic parameters of *C. colocynthis* fruits followed by *in-vitro* appraisal of antioxidant and acetylcholinesterase inhibitory activities of the fruit extracts. **Methods:** The phyto-pharmacognostic study of fruits was done as per IP and WHO guidelines. Various extracts were prepared and assessed for antioxidant activity by determining the ability of the extracts to scavenge 2,2-diphenyl-1-picryl hydrazyl (DPPH). Anti-cholinesterase activity was determined using the Ellman's colorimetric method. The total phenols and flavonoids (TPC and TFC) content were determined. **Results**: Methanol extract showed the most significant acetylcholinesterase inhibitory activity ($IC_{50}$ value 10.46 ± 2.10 mg/ml); while remarkable antioxidant activity was shown by fruit extracts in the following order: aqueous extract> methanol extract> chloroform extract. Methanol extract had the highest TFC. **Conclusion:** The detailed pharmacognostic records generated shall be useful for fruit authentication in future. Methanol extract of *C. colocynthis* has significant anti-cholinesterase as well as anti-oxidant activity, probably due to the high TFC. This is first such report on these activities of *C. colocynthis*. Detailed phytochemical and pharmacological investigation is required to develop this as an effective therapy for the treatment of AD.

**Keywords**
Alzheimer's disease, Anti-cholinesterase, Antioxidant, *Citrullus colocynthis*, Phyto-pharmacognostic study.

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## INTRODUCTION

Alzheimer's disease (AD) accounts for more than 80% cases of dementia in aged population worldwide. It is a chronic progressive neurological degenerative disorder of central nervous system (CNS) [1,2] characterized by cognitive impairments and behavioural disorders [3,4]. Moreover, neuropsychiatric symptoms such as anxiety, sleep disturbances, wandering, agitation, aggression, delusions or hallucinations are also recognised as major components of AD that are expressed to varying degrees throughout the disease [5]. The pathological features that occur in CNS of AD afflicted patients are extracellular deposition of amyloid plaques, formation of intra neuronal neurofibrillary tangles, oxidative stress, neuro-inflammation and neurotransmitter disturbances [6]. A consistent neuropathological occurrence associated with memory loss is a cholinergic deficit, which has been correlated with the severity of AD [7,8]. Therefore, attempts to restore cholinergic function by inhibiting acetylcholine hydrolysis by acetylcholinesterase, through the use of AChE inhibitors (AChEIs) has been accepted as the most effective treatment strategy against AD [9-11]. Commercially available AChEI such as tacrine, donepezil, galantamine and rivastigmine have demonstrated improvement in cognitive, functional and behavioural symptoms of AD. But, considering the drawbacks of synthetic AChEIs which include gastrointestinal disturbances, moderate effectiveness, high cost and short half-life [12,13], there is general call for exploring new AChEI especially from natural products with higher efficacy, less side effects and minimal environmental threat.

Oxidative damage due to presence of reactive oxygen species has also been suggested to play a key pathogenic role in AD progression [14]. To impede the risk associated with synthetic drugs, natural antioxidants are being investigated which may consequently help in preventing aging and neurodegenerative diseases including AD [15,16]. Increasing number of studies have demonstrated that consumption of fruits and vegetables rich in polyphenolic antioxidants may help to reduce or to block neuronal death occurring in the pathophysiology of AD [17]. To date, several different varieties of berry fruits rich in diverse polyphenolic antioxidant compounds such as anthocyanin's, Gallic acid, tannins, caffeic acid, resveratrol, kaempferol, quercetin, myricetin etc have been explored and found to be useful in preventing age-related and pathological degenerative processes in AD [18-21]. Thus, plant extracts containing phenolic compounds may yield compounds for the management of dementia of Alzheimer's type.

*Citrullus colocynthis* (Tumba or Bitter apple, belonging to family cucurbitaceae) is native to dry areas of North America. It is found throughout the desert areas of India, Pakistan, Africa, Australia and other European countries [22]. Traditionally, whole plant of *C. colocynthis* is used in gut disorders such as indigestion, dysentery, gastroenteritis and also used in common cold, cough, toothache and wounds [23] while fruits are used as bitter, pungent, cooling, purgative, anthelmintic, antipyretic, carminative and used to cure various diseases such as diabetes, leprosy, asthma, bronchitis, jaundice, constipation, tumors, ascites, leucoderma, ulcers, urinary discharges, enlargement of spleen, dyspepsia, anaemia, throat diseases, elephantiasis, joints pain [24,25]. Moreover, roots are found to be useful in jaundice, ascites, urinary diseases, rheumatism and given in abdominal enlargements and in cough and asthmatic attack of children. A poultice of root useful in breast inflammation [24]. Seeds used to treat diabetes while leaves used to treat jaundice and asthma [26]. Previous phytochemical investigations have shown the presence of various therapeutically active principles traceable for the treatment of various disorders. The chief active phytoconstituents isolated from the plant are curcubitacins, phenolic compounds and flavonoids along with glycosides, terpenoids and alkaloids [27-29]. Pharmacological reports showed that it exhibits analgesic, anti-inflammatory, anti-allergic, anti-alopecia, anti-microbial, anti-cancer, anti-diabetic, anti-fertility, anti-helmintic, anti-oxidant, anti-ulcer, hepato-protective, hypolipidemic activities [30-32].

To the best of our knowledge, so far no work has been carried out to explore the potential of *C. colocynthis* against AD. Thus, the present study was designed to evaluate *in-vitro* antioxidant activity and anti-cholinesterase activity of *C. colocynthis* fruit by DPPH scavenging assay and ellman's spectrophotometric method respectively. In addition, pharmacognostical standardization of *C. colocynthis* fruit was carried out to establish its macro-microscopical standards and physico-chemical parameters for its identification, authentication, purity and quality control. Preliminary phytochemical investigation was carried out to identify the nature of medicinally important phytoconstituents.

## MATERIALS AND METHODS

### Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH), Acetylcholinesterase Enzyme EE (VI-S type), Acetylthiocholine iodide, 5, 5′-dithio-bis-(2-nitrobenzoic acid (DTNB), tacrine, quercetin, Gallic acid, ascorbic acid was purchased from Sigma (St Louis MO, USA). All other chemicals used in this study were of analytical grade.

### Plant Material

The fresh fruits from healthy plant of *C. colocynthis* selected for our study were collected from the agriculture fields of Durjanpur Village, near Hisar, Haryana, India in October, 2015 and authenticated by S.K Srivastava, Scientist E, Botanical Survey of India, Dehradun (Reference No-BSI/NRC/Tech/2017-18/117209 dated 8/5/2017). A voucher specimen of the plant was deposited in the herbarium of same department for further reference. The collected fruits were cut into small pieces after the removal of its hard woody shell, then shade dried and pulverized with mechanical pulverizer for size reduction.

### Organoleptic evaluation

Fresh fruits of *C. colocynthis* were observed to study the organoleptic features such as colour, odour, taste, size, shape, surface, texture *etc.* [33,34].

### Microscopic evaluation

For the microscopic studies, thin transverse sections of *C. colocynthis* fresh fruit cut by sharp blade were observed under microscope after staining with suitable dye and powder microscopy was also performed to determine the diagnostic microscopical characters of the fruit.

### Physical evaluation

The physical parameters such as foreign organic matter, loss on drying, ash values and extractive values (by cold maceration method) were determined for *C. colocynthis* fruits according to standard methods [34,35]. Each parameter was determined in triplicate.

### Preparation of extracts

Air dried coarse powder of *C. colocynthis* fruit was sequentially extracted with petroleum ether, chloroform, methanol and water by using soxhlet apparatus. The colour, consistency and percentage yield of all obtained extracts were calculated and kept in desiccators till for further use.

The extraction yield was expressed as follows:

$$\text{Percentage yield} = \frac{\text{Weight of dry extract (g)}}{\text{Weight of sample used for extraction (g)}} \times 100$$

### Preliminary phytochemical screening

Preliminary phytochemical screening of prepared fruit extracts was performed by qualitative chemical examination to detect the nature of phytoconstituent like alkaloid, glycosides, carbohydrates, tannins, flavonoids, terpenoids, steroids, saponins, phenols and amino-acids present in extracts [36].

### Standardization of Extract

The prepared extracts were standardized by determining total phenolic and flavonoid content.

*Determination of total phenolic content (TPC)*

Total phenolic content of *C. colocynthis* prepared fruit extracts was determined by using Folin–Ciocalteu spectrophotometric method [37]. The reaction mixture was prepared by mixing 0.5 ml of plant extract (10 mg/10ml) with 2.5 ml of Folin–Ciocalteu reagent (10%) and 2 ml of 7.5% sodium carbonate. TPC was determined after 45 minutes of incubation at room temperature by measuring the absorbance of the samples in UV/Vis spectrophotometer at 765 nm. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2 ml of 7.5% of $Na_2CO_3$. The total phenolic content was determined from the linear equation of a standard curve prepared with Gallic acid. The results were expressed as milligram Gallic acid equivalent (GAE)/g of dried extract. All determinations were performed in triplicate (*n=3*).

*Determination of total flavonoid content (TFC)*

Aluminium chloride colorimetric method was used to determine total flavonoid content of *C. colocynthis* extracts as described by Lin [38]. Different dilutions of each sample (0.5 ml) was taken in test tube and mixed with 1.5 ml methanol (95%), 0.1 ml of aluminium chloride (10% w/v), 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. Mixture was then incubated at room temperature for 30 min and absorbance of the mixture was noted at 415 nm using UV/Vis Spectrophotometer. The amount of aluminium chloride (10%) was swapped by the same amount of distilled water in sample blank. Total flavonoid content was calculated from the standard calibration curve of quercetin and expressed as milligram quercetin equivalents (mg QE)/g of air dried extract. All measurements were done in triplicate.

### *In-vitro* antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity of different prepared fruit extracts was determined by using DPPH radical scavenging assay according to previously reported method with slight modifications [39]. The principle of DPPH assay is based upon the reduction of stable free radical DPPH in the presence of hydrogen donating antioxidant due to the formation of diphenyl picryl hydrazine, resulting in the colour change of reaction mixture from purple to yellow and as consequence the absorbance decreases. The intensity of yellow colour indicates the scavenge potential of antioxidant compounds or extracts in terms of hydrogen donating ability. Briefly, 1.5 ml of DPPH solution (0.1mmol/L in methanol) was incubated with different concentrations of the *C. colocyntnhis* extracts. The reaction mixture was shaken and incubated in the dark for 30 minutes at room temperature and the absorbance was measured at 517 nm against the corresponding blank solution of methanol. The percentage inhibition of free DPPH radical by the samples was calculated based on control reading by following formula:

**DPPH scavenging activity (%) = $[(A_c-A_t)/A_c]$ X 100**

Where, $A_c$ is the absorbance of the control (without sample) and $A_t$ is the absorbance of the sample. Ascorbic acid was used as positive control. The concentration of extract providing 50% inhibition ($IC_{50}$) was calculated by plotting the inhibition degrees against the sample concentrations. The test was carried out in triplicate and the $IC_{50}$ values were reported as means±standard deviation (SD).

***In-vitro* anti-cholinesterase assay (Ellman'smethod)**

*In-vitro* anti-cholinesterase activity of various plant extracts was assessed by modified Ellman's colorimetric method [40]. The acetylcholinesterase enzyme hydrolyses the substrate acetylthiocholine iodide resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. In each well of 96-well micro-titre plate, 260 µl of Phosphate buffer (0.1 M, pH 8), 20 µl extract, 10 µl of AChE (0.1 U/ml) and 10 µl of DTNB (10 mM) were added and the reaction mixture was incubated at room temperature for 30 minutes. Then, 10 µl of acetylthiocholine iodide solution (75 mM) was added to test tube and then absorbance was read at 412 nm after 15 minutes. The percentage of inhibition was calculated in comparison to control (extract absent) by using formula:

**% inhibition=1- ($A_{sample}$/ $A_{control}$) X 100**

Where, $A_{sample}$ = absorbance of the test extract, $A_{control}$ = absorbance of the control. Tacrine was used as standard cholinesterase inhibitor and concentration of extract providing 50% inhibition ($IC_{50}$) was calculated by plotting the inhibition degrees against the sample concentrations. The test was carried out in triplicate, and the $IC_{50}$ values were reported as means±standard deviation (SD).

**Stastical analysis**

All the experiments were carried out in triplicate and the data were expressed as the mean ± standard error and subjected to one-way analysis of Variance (ANOVA) followed by post-hoc Tukey's Multiple range test. Differences were considered significant at $p<0.05$.

## RESULTS AND DISCUSSION

**Organoleptic evaluation**

Organoleptic evaluation is the quickest and simplest way to determine the identity of crude drugs. The various parameters observed for fresh fruits of *C. colocynthis* (Figure 1) are presented in Table 1 which provides important information on its identification and authentication. All the parameters were found to comply with the standards given in Siddha Pharmacopoeia [33]. The half cut section of whole fruit showed (Figure 2) woody epicarp, mesocarp and fleshy endocarp which is divided into 10-16 locules containing 10 or more yellowish brown, oblong, slightly compressed seeds covered with hard white hair arranged on partial placentation.

**Microscopical Evaluation**

The transverse section of fruit shell (Figure 3) showed Epicarp: 3-4 layers of parenchyma cells followed by 4-5 layers of highly thickened loosened sclerenchyma cells; Mesocarp: numerous stone cells with single layer of medullary rays; Endocarp: irregularly arranged large size parenchyma cells with smaller vascular bundles at periphery and larger one towards the interior side. The T.S of seed showed seed coat with thick walled palisade cells having the vertical strips of thickening on the anticlinal walls while testa containing thick walled sclreids cells. Endosperm and cotyledon made up of parenchyma cells with aleurone grains and fixed oil (Figure 4). Powder microscopy of *C. colocynthis* fruit revealed the presence of typical microscopic features such as spiral vessels, aleurone grains with peltate glands, epidermis, Pitted parenchyma cells, prism shaped Ca-oxalate crystals (Figure 5).

**Physical Evaluation**

Physical evaluation of crude drug helps in preliminary assessment of quality and purity of crude drug by determining the moisture present in crude drug which may deteriorate its quality by activating the microbial growth, thus it is inevitable component of crude drug. Additionally, determination of total ash value and acid insoluble ash value helps to evaluate the adulteration of crude drug with inorganic and earthy sand material while extractive value help to determine the nature as well as amount of active phtoconstituents present in crude drug. Various physical parameters such as foreign organic matter, loss on drying, ash values, extractives values were determined and results of physical evaluation are presented in (Table 2) provide simple and reliable standards which could be useful for the proper identification and determination of quality of crude raw material.

**Preparation of extract**

Despite the abundant literature on extraction solvents and techniques for the isolation of polyphenols from different herbal sources, there is little information available on the effect of extraction conditions on the polyphenol content and anti-oxidant activity of *C. colocynthis* fruit. Therefore, in the present study, sequential exhaustive extraction was employed with a view to separate constituents on the basis of polarity. The percentage w/w yield, colour and consistency of different prepared extracts, were noted and results are shown in Table 3. It was observed that aqueous extraction produced maximum yield (20.19% w/w), whereas chloroform extraction yielded only 3.48% w/w.

**Preliminary Phytochemical screening**

Different prepared extracts of proposed fruits were subjected to various qualitative chemical tests to determine the nature of phytoconstituents present in the fruit. Results of phytochemical screening of different extracts of *C.colocynthis* are discussed in Table (4) and the result was found to be similar with the result as discussed in previous earlier report [41].

**Standardization of extract**

The consumption of fruits and vegetables rich in polyphenolic compounds is largely associated with lowering risk of degenerative diseases caused by oxidative stress [19]. Phenolic compounds and flavonoids contributed significantly to total antioxidant activity of medicinal plants [42,43]. Hence, prepared extracts were standardized by determining total phenolic and flavonoids content. Total phenolic content and total flavonoid content in different extracts used in the study was calculated and results are expressed as mg GAE/g and mg QE/g of air dried extract respectively (Table 5). It is well known that complexity of phenolic compounds in plant matrices makes its extraction difficult [44]. Numerous studies have shown that polyphenols are generally insoluble in organic solvents but the solubility of polyphenols increases with the addition of water to organic solvents due to weakening of hydrogen bonds in aqueous solutions [45-46]. In accordance with literature, the highest phenolic and flavonoids values were recorded in aqueous and methanol extracts.

***in-vitro* antioxidant activity (DPPH assay)**

According to the literature, polyphenols including tannins, flavonoids and proanthocyanidins have been well known to acts as primary antioxidant or free radical scavenger [47-48]. Several reports have shown the positive correlation between antioxidant activity and polyphenols content in plants [49]. The *in-vitro* antioxidant activity on prepared extracts was evaluated by determining their ability to scavenge DPPH radical comparable to standard ascorbic acid (Table 6). In earlier published report, methanolic fruit extract of *C. Colocynthis* showed scavenging effect on the DPPH radical 88.0 ± 2.7% ($p<0.005$), at a concentration of 2500 µg/ml compared with ascorbic acid, butylated hydroxyl anisole and α-tocopherol at 50 µg/ml concentration [50]. The result of present study revealed that aqueous extract which hold the maximum amount of phenolic compounds exhibited the most significant antioxidant activity as compared to other extracts with lowest $IC_{50}$ value 186.85±2.19 µg/ml followed by methanol fruit extract.

***in-vitro* anti-cholinesterase activity (Ellman's assay)**

Since their introduction into clinical practice, AChEIs have been proven as successful standard clinical strategy for the symptomatic treatment of AD [51]. Dietary approaches, in particular polyphenol-enriched diets are drawing attention as a marker tool for the prevention and delay of AD progression due to their safety profile, cheap and easily availability [52-54]. The results of anti-cholinesterase activity evaluated by ellman's assay demonstrated that methanol extracts showed most significant anti-cholinesterase activity in dose dependant manner as compared to other extracts with lowest $IC_{50}$ value 10.46 ± 2.10 mg/ml comparable with standard drug tacrine. The $IC_{50}$ values for different extracts in anti-cholinesterase assay are shown in Table 6.

The qualitative and quantitative analytical data document that flavonoids present in *C. colocynthis* extracts may probably responsible for the significant anti-cholinesterase activity.

**Correlations between total phenolic and flavonoid content in *C. colocynthis* fruit extracts with their antioxidant and anticholinesterase properties**

Coefficient of Pearson correlation (r) was determined between TPC and TFC of fruit extracts with antioxidant and anticholinesterase properties (Table: 7). A significant negative correlation shows that as the content of constituents (TPC and TFC) increases, there is decrease in the $IC_{50}$ value in DPPH and Elman's assay. Thus, showing that TPC and TFC are contributing to the antioxidant and cholinesterase inhibitory activities of the plant [55]. From the results, higher phenolic content contributed to the antioxidant property of fruit extracts as determined by DPPH assay as significant and negative correlation was found between TPC and DPPH assay ($r= -0.89$, $P<0.05$). TFC of fruit extracts showed significantly negative correlation with their anticholinesterase activity ($r= -0.85$, $P<0.01$). Thus, it can be predicted that flavonoid present in the extracts may be main contributor for anticholinesterase activity.

**Figure 1: Fruits of *C. colocynthis***

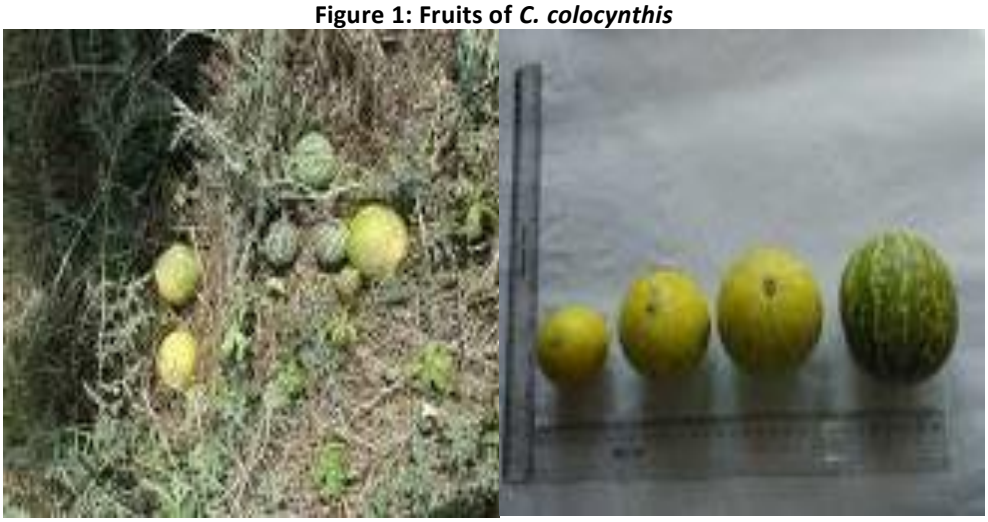


**Figure 2: Transverse half cut section *C.colocynthis* fruit**

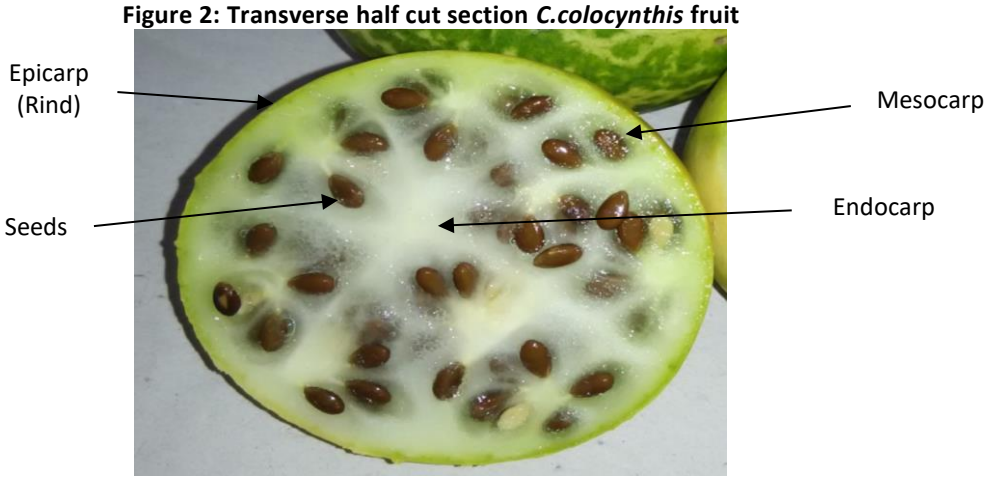


**Figure 3: Transverse section of *C. colocynthis* shell (40X)**

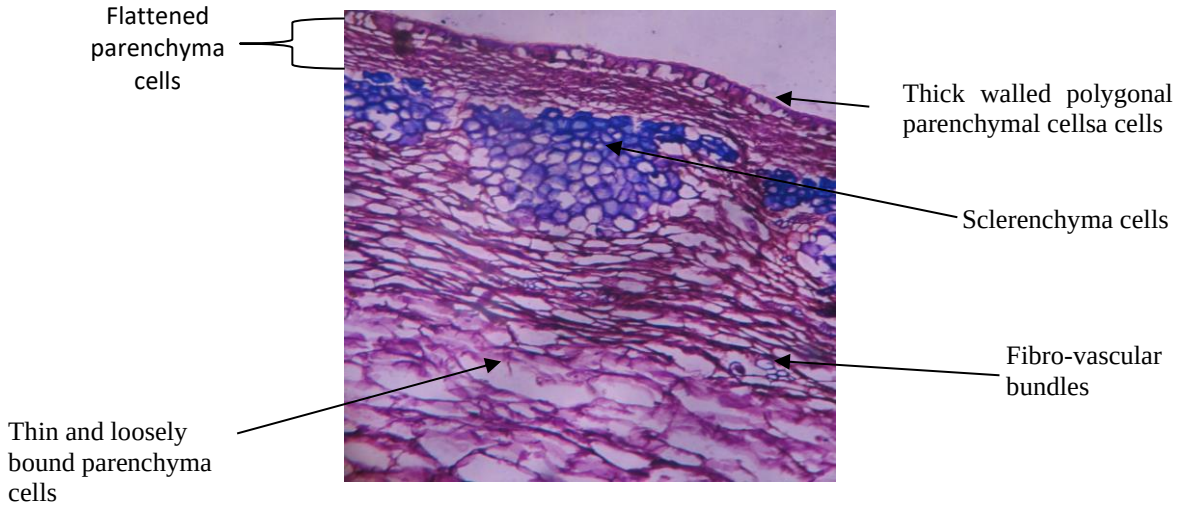



**Figure 4: Transverse section of *C. colocynthis* seed (40X)**

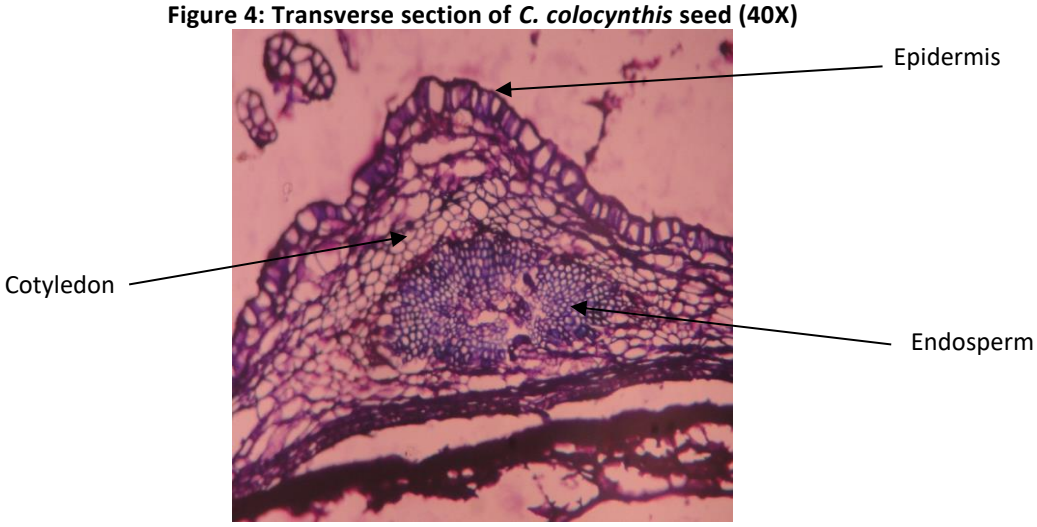
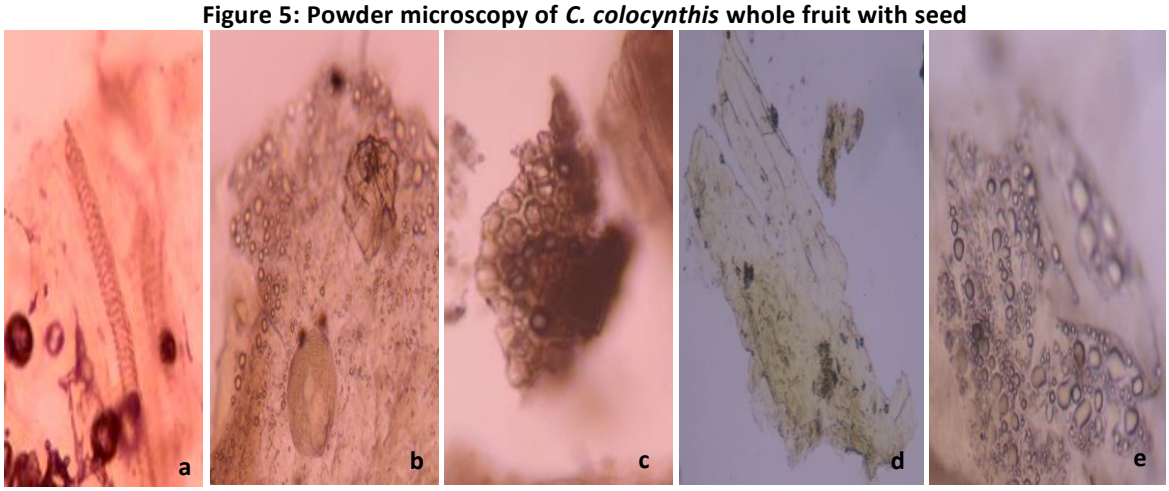



**Figure 5: Powder microscopy of *C. colocynthis* whole fruit with seed**




**Spiral vessels** | **Aleurone grains with peltate gland** | **Epidermis** | **Pitted Parenchyma** | **Ca-oxalate Crystals**

**Table 1: Results of organoleptic parameters for *C. colocynthis* fresh fruits**

| Sr. No | Characters | Observation |
|---|---|---|
| 1. | Colour | Yellow to light green |
| 2. | Size | 3-7.5 cm in diameter |
| 3. | Shape | Oval to globular |
| 4. | Surface | Hard rind with yellow lines |
| 5. | Texture | Smooth |
| 5. | Odour | Characteristic |
| 6. | Taste | Very Bitter |

**Table 2: Results of physico-chemical parameters of *C. colocynthis* fruits**

| Sr. No | Parameters | Results Mean$^{n*}$±SD |
|---|---|---|
| 1. | Foreign Organic Matter | Nil |
| 2. | Loss on Drying | 7.65± 0.57 |
| 3. | Extractive Values | |
| | Petroleum ether soluble | 7.21±0.58 |
| | Alcohol soluble | 8.47±0.45 |
| | Water soluble | 28.65±0.87 |
| 4. | Ash Values | |
| | Total Ash | 12.43±0.80 |
| | Acid insoluble | 6.22±0.40 |
| | Water soluble | 2.54±0.25 |

***n=3**

**Table 3: Results of various parameters observed for *C. colocynthis* fruit extarct**

| Sr. No | Extract | Yield (%w/w) | Colour (Under visible light) | Odour | Consistency |
|---|---|---|---|---|---|
| 1. | Pet-ether | 10.31% | Yellowish Brown | Mild | Liquid |
| 2. | Chloroform | 3.48% | Light yellow | Mild | Semisolid but non-sticky |
| 3. | Methanol | 19.90% | Brownish Black | Odourless | Semisolid and sticky |
| 4. | Aqueous | 20.19% | Brownish Black | Characteristic | Semisolid and sticky |

**Table 4: Results of phytochemical screening on different fruit extracts of *C. colocynthis***

| Sr. No | Test | | Pet ether | Chloroform | Methanol | Aqueous |
|---|---|---|---|---|---|---|
| 1. | Alkaloids | Mayer's | - | + | + | + |
| | | Dragendroff's | - | + | + | + |
| | | Wagner | - | + | + | + |
| | | Hager's | - | + | + | + |
| 2. | Glycosides | Keller Killiani | - | - | - | - |
| | | Modified Borntager's | - | - | + | + |
| 3. | Proteins/AA | Millon's Reagent | - | - | + | - |
| | | Xanthoprotein | - | - | + | - |
| 4. | Saponins | Froth/Foam Test | - | - | - | + |
| 5. | Carbohydrates | Benedict's | - | + | + | + |
| | | Fehling solution | - | + | + | + |
| | | Molisch's Reagent | - | - | + | + |
| 6. | Sterols | Salkowski Test | + | + | + | - |
| 7. | Terpenoids | Antimony trichloride | + | + | + | - |
| 8. | Flavonoids | Shinoda Test | - | + | + | + |
| | | Alkaline reagent | - | + | + | + |
| | | Lead acetate test | - | + | + | + |
| | | Sulphuric Acid Test | - | + | + | + |

| | | | | | | |
|---|---|---|---|---|---|---|
| 9. | Tannins | Gelatin Test | - | + | + | - |
| 10. | Phenols | $FeCl_3$ Test | - | - | + | + |

**Present=+, Absent=--**

**Table 5: Results of TPC and TFC of different fruit extracts of *C. colocynthis***

| Sr. No. | Extract | Total Phenolic Content (mg GAE/g extract) Mean$^n$±S.D | Total Flavonoid Content (mg QE/g extract) Mean$^n$±S.D |
|---|---|---|---|
| 1 | Petroleum-ether | --- | --- |
| 2 | Chloroform | 2.38±0.37 | 0.38±1.13 |
| 3 | Methanol | 9.44±0.43 | 2.88±0.15 |
| 4 | Aqueous | 18.10±0.27 | 1.13±0.24 |

***n=3; NA=Not applicable**

**Table 6: Results of DPPH and ellman's assay of different fruit extracts of *C. colocynthis***

| Sr. No. | Extract | DPPH assay $IC_{50}$ (µg/ml) Mean$^n$±S.D | Ellman's assay $IC_{50}$ (mg/ml) Mean$^n$±S.D |
|---|---|---|---|
| 1 | Petroleum-ether | --- | ---- |
| 2 | Chloroform | 1197.96±3.19 | 36.48±4.90 |
| 3 | Methanol | 313.11±15.24 | 10.46±2.10 |
| 4 | Aqueous | 186.85±2.19 | 15.92±3.72 |
| 5 | Ascorbic acid (std) | 4.25±0.1 | NA |
| 6 | Tacrine (std) | NA | 7.42±1.3 |

***n=3; NA=Not applicable**

**Table 7: Pearson correlation coefficient (r) of total phenolic and flavonoid content with their antioxidant and anticholinesterase activities**

| Sr. No. | Parameter | Antioxidant Activity (DPPH Assay) | Anticholinesterase Activity (Ellman's Assay) |
|---|---|---|---|
| 1 | Total Phenolic Content (TPC) | -0.89 | -0.70 |
| 2 | Total Flavonoid Content (TFC) | -0.65 | -0.85 |

## CONCLUSION

Biological activities on various medicinal plants sometimes assessed by using uncharacterized crude extracts prepared from plants that are not authenticated and thus, it is very difficult to reproduce the results of such studies without the identification of the crude drug as well as phytochemicals responsible for the activity. Hence, there is a need of phyto-pharmacognostic standardization and bioactivity-guided identification of active phytochemicals. The standards establish in present investigation on pharmacogonostic and phytochemical studies may be used as diagnostic tool for authentication and minimizing the adulteration of raw material as well as help in determining the quality of *C. colocynthis* fruit.

The phytochemical and *in-vitro* biological evaluation results revealed that methanol extract of *C. colocynthis* is rich in polyphenols and flavonoids which probably confer the remarkable antioxidant and anticholinesterase activity to the plant. Further research is needed to isolate and identify the polyphenols/ flavonoids from the bioactive extract as well as to *in-vivo* appraisal of isolated fraction/ compounds in detail.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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