Characterization, Antioxidant Assessment and Antifungal Activity of Cyamopsis tetragonoloba

Braivy Anto[1], Suriyavathana. M.[2], Thamaraiselvi. G.[3], Anandhi. E[4], Manikandan Mathaiyan[5], Indumathi. K.P.[6]

[1,2,3,4,5,6] *Plant Therapeutics Laboratory (PTL), Department of Biochemistry, School of Biosciences, Periyar University, Salem-11, Tamil Nadu, India.*

[2]*Corresponding Author: Dr.M.Suriyavathana, Professor and Research supervisor, Plant Therapeutics Laboratory (PTL), Department of Biochemistry, Periyar University, Salem-11.*

***Abstract*—The key industrial commodity guar gum is derived from the underutilized semi-arid legume *Cyamopsis tetragonoloba*, often known as guar, which has been used as a traditional meal in India. Like antioxidants, there isn't much research on its biological function, though. The characterization technique GC-MS was used to identify the various components found in the ethanolic extract of *Cyamopsis tetragonoloba*. The DPPH and FRAP Assays were used to analyze the in vitro antioxidant evaluation of sample. These extracts' antifungal ability against *Aspergillus nidulans* and *Pencillium notatum* was later examined after their GC-MS analysis and in vitro antioxidant assessment determined their free radical scavenging activity.**

I. INTRODUCTION

Common names for *Cyamopsis tetragonoloba* l. include guar and cluster bean. Native to Africa and mostly grown in northwest India, the drought-resistant guar or cluster bean belongs to the Fabaceae family. A guar seed is composed of 27–30% (w/w) endosperm, 30–33% hull, and 43–47 percent germ (Tahmouzi, S, et al., 2023). Guar gum, a naturally occurring polymer called galactomannan, is made from the seeds of the *C. tetragonoloba* bean. Its pods are used in herbal medicine due to their strong anti-diabetic effects. *C. tetragonoloba* is a well-known folk remedy. Anorexia, dyspepsia, obesity, arterial hardening, laxatives, cooling agents, digestive aids, and—most importantly—diabetes are all helped by it (Jamshed, M, et al., 2018). Only esters, fatty acids, alcohol, aldehydes, terpenes, etc. can be analyzed using GC-MS. The GC-MS is a special and potent technique that offers a unique chance to analyze novel compounds for the purpose of characterizing and identifying compounds that have been produced or derivatized (Abeer fauzi AL Rubaye et al, 2017). Antioxidants are essential for accelerating the healing of wounds and protecting tissues from oxidative damage. Numerous substances have been found to have potent antioxidant qualities, including flavonoids, anthraquinones, and naphthoquinones. For example, ellagic acid, lawsone, emodin, shikonin, alkanin, and some herbal extracts have shown strong antioxidant activity by efficiently scavenging reactive oxygen species (ROS), preventing lipid peroxidation, and increasing the intracellular concentrations of antioxidant enzymes such as glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) **(**Yazarlu, O., et.al.,2021**).** One popular technique that offers a preliminary way to evaluate antioxidant activity is the DPPH radical scavenging assay. Electron transfer (ET) is the main basis of this technique, with the hydrogen atom transfer (HAT) process contributing only slightly to the assay (Prior et al., 2005). The FRAP test is a traditional ET-based method used to measure the reduction in ferric ion (Fe3+) ligand complex, which is then converted by antioxidants into the bright blue ferrous (Fe2+) complex in an acidic environment. The increase in absorbance at 593 nm is used to measure antioxidant activity, and the results are either reported as micromolar Fe2+ equivalents or in proportion to an antioxidant standard (Antolovich et al., 2002). An agent's antifungal efficacy is mostly ascribed to two methods: chemically disrupting the production or function of essential fungal components and/or evading the traditional mechanisms of antifungal resistance (Laxminarayan, R., et al., 2016).

II. MATERIALS AND METHODS

Collection of the Sample

Pods of *C. tetragonoloba* were collected from Trivandrum District, Kerala and authenticated by TamilNadu Agricultural University, Coimbatore.

Fig 1: *Cyamopsis tetragonoloba*

Soxhlet extraction

Soxhlet extraction was used to get an ethanolic extract from the plant sample. 200 milliliters of ethanol were mixed with 10 grams of powdered pod sample, and the mixture was extracted using soxhlet for a full day. Solvents were separated when extraction was finished, and the volume was lowered under low pressure. After being given a solid mass, it is then moved into sterile bottles and kept in storage.

GCMS

A GC-MS system (Clarus The phytochemical USA) with Elite-5MS (5% diphenylV95% dimethyl Perkin Elmer, CT, 500) was used to identify the chemical contents. Duff: omx0.25 mm x 0.25 um. The carrier, helium gas, had a flow rate of 30 mL/minute (olysiloxane). After two minutes at 110°C, the oven temperature was raised to 280°C in nine minutes. The entire analysis took 36 minutes, and the injector temperature was 250°C. Once a clear baseline was obtained, two-mL aliquots of ethanol extract were fed into the chromatographic column. With the use of the mass spectrum library and a computer-driven algorithm, the major elements were determined. The analyzer made use of the Turbo mass 5.2 software.

Invitro Antioxidant Assessment

DPPH Assay

Samples of *C. tetragonoloba* pods in varying volumes (2–20 µl) were prepared up to 40 µl using DMSO, and 2.96 ml of DPPH (0.1 mM) solution was added. For twenty minutes, the reaction mixture was incubated at room temperature in a dark environment. The mixture's absorbance was measured at 517 nm after 20 minutes. The control was 3 milliliters of DPPH. The following formula was used to determine the *C. tetragonoloba* pod sample's 1% radical scavenging activity:

$$\%\ \text{RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times$$

Where, RSA is the Radical Scavenging Activity, Abs control is the absorb radical + ethanol, Abs sample is the absorbance of DPPH radical + ethanolic extract of *C.tetragonoloba*.

Ferric Reducing Antioxidant Power

A 5 ml phosphate buffer was added to each tube containing the plant sample, which was collected at different concentrations ranging from 1 ml to 2.5 ml of 1% ferrocyanide. After that, it was wrapped in aluminum foil and incubated for 20 minutes at 37ºC. Following incubation, 2.5 ml of 10% TCA is added to each tube, and the tubes are centrifuged for 5–6 minutes at 3000 rpm. To get the 2.5 ml of distilled water, 2.5 ml of the supernatant was collected. Water was added. Ferric chloride (0.5 ml) was then added to each tube. The blue colour developed was read at 700nm. The blank consists of about 1ml of water.

Antifungal Study

Using an ethanolic extract of *C. tetragonoloba* pods, the antifungal activity was evaluated using the Rose Bengal agar cup diffusion method. This method involved subculturing a pure isolate of each fungal strain on agar media plates for 24 hours at 37°C.

III. RESULTS

GC-MS Analysis

One of the best ways to identify the bioactive compounds of non-polar components and volatile essential oil, fatty acids, and lipids is to use GC-MS. Using a Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m × 0.25 mm × 0.25 µm film thickness), GC/MS analysis qualitatively screened the compounds present in CTJLE and found bioactive phytochemical constituents, including sugars, amides, alcohols, aldehydes, ethers, ketones, carboxylic acids, amino acids, fatty acids, alkaloids,

phenolic compounds, flavonoids, tannins, and terpenoids.

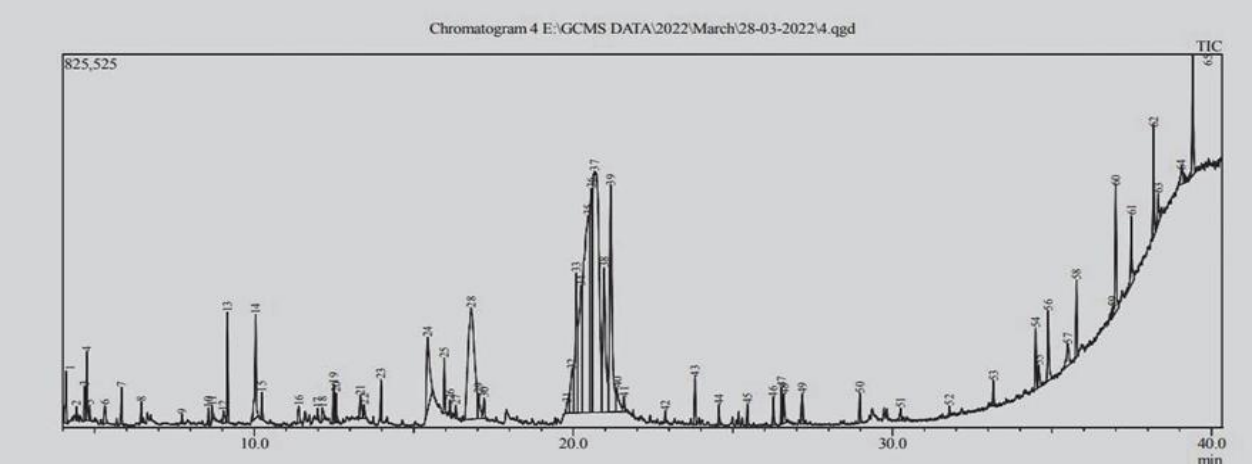


Fig 2: GC-MS analysis of *Cyamopsis tetragonoloba* pod ethanolic extract

The result analysis for the ethanol extract of *Cyamopsis tetragonoloba* by GCMS reveals the presence of (65) compounds which has been screened and analyzed which provides the complete spectrum of phytochemical constituents present in the ethanol extract of *Cyamopsis tetragonoloba* (Figure 2).

Antioxidant Assessment

DPPH Activity

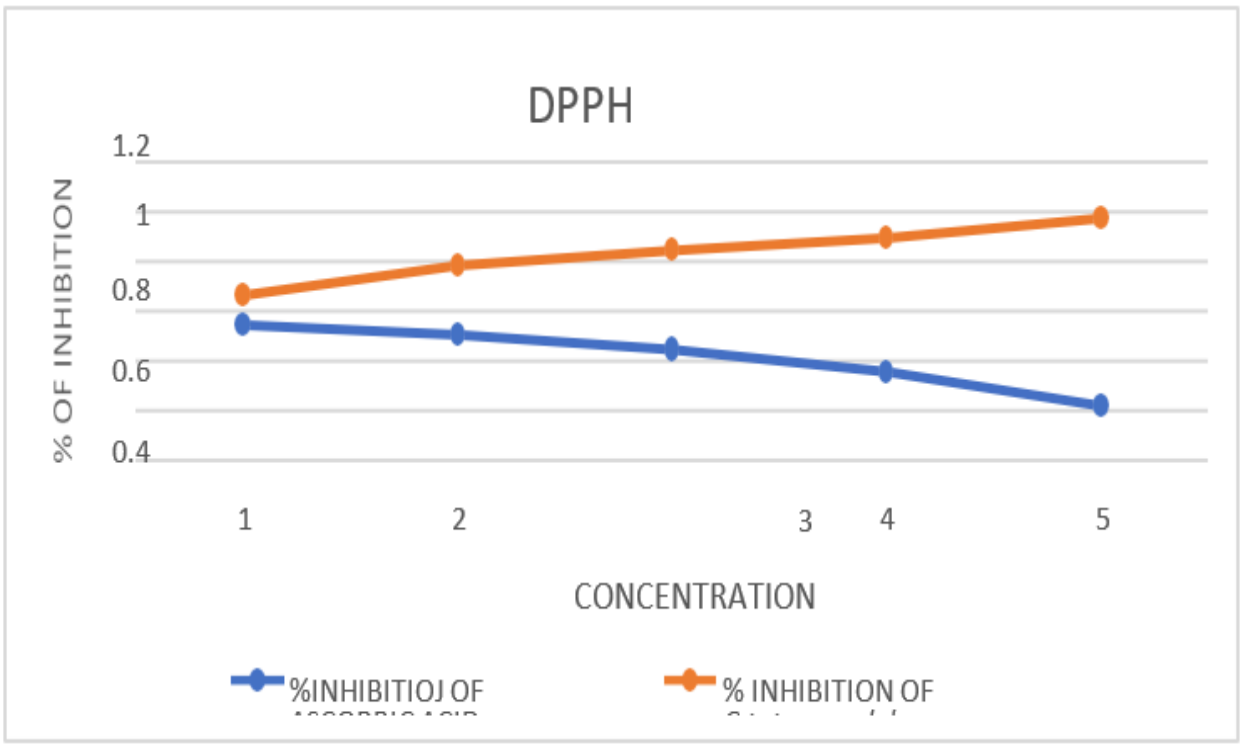


Fig 3: - Graphical representation of DPPH assay of *C.tetragonoloba*

C. tetragonoloba pods extract showed the varied potential of antioxidant capacities in terms lower the IC50 (µg/ml) ranges (0.24-0.03) is shown in the figure (3). The stable DPPH radical activity is determined by the radical decolorization in the presence of antioxidant generated by the extract.

Ferric Reducing Antioxidant Assay

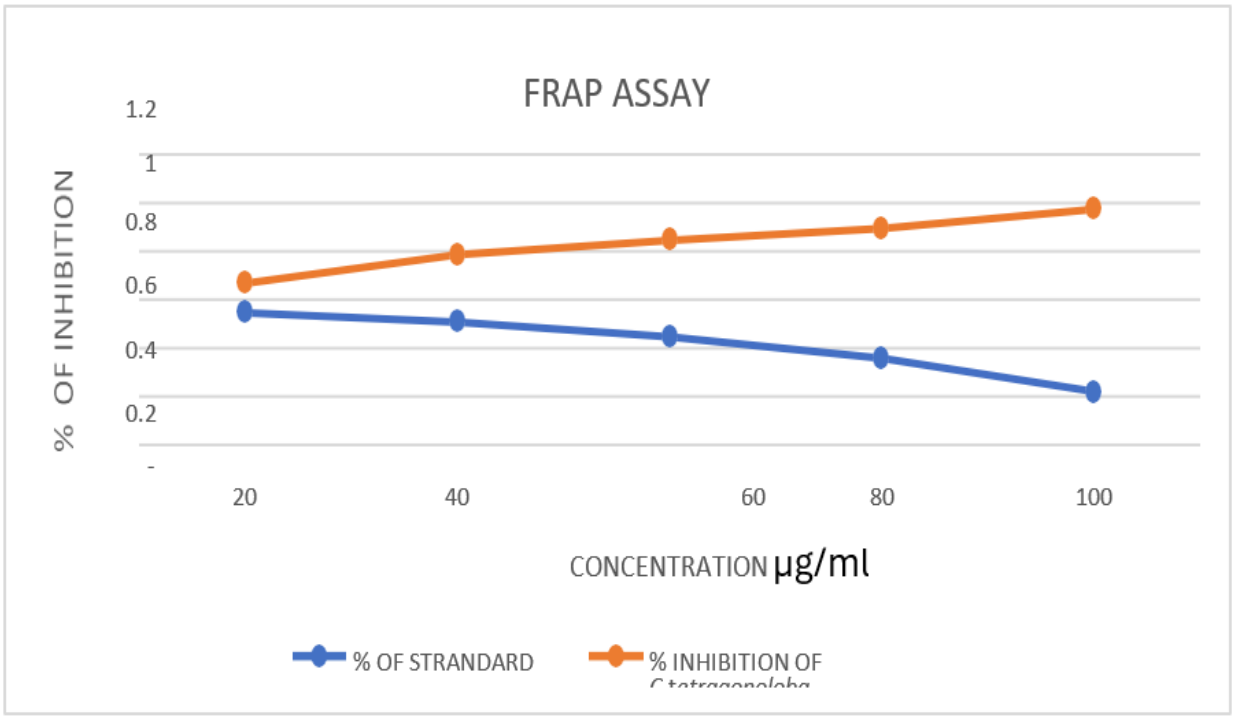


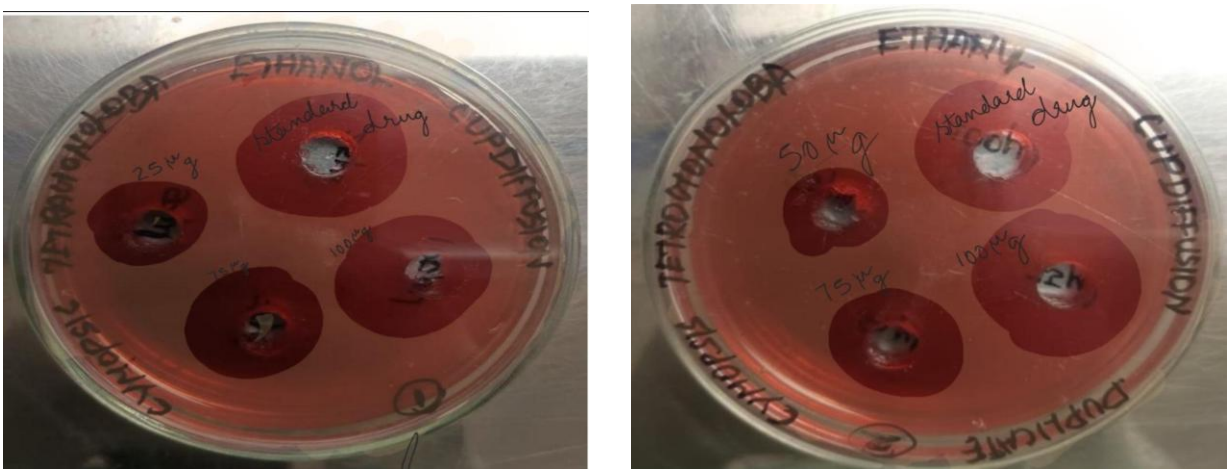
Fig 4:- Graphical representation of FRAP Assay of *C.tetragonoloba*

The analysis evaluates the FRAP levels (20-100µg/ml) concentration highlighting ascorbic acid as most effective with 20% and 80% (Figure 4). The stable hydroxyl activity was widely used for the determination of oxidation activity whereby hydroxyl stable radical decolorized the free radicals by the antioxidant present in the *C.tetragonoloba* pod extract.

Antifungal Activity

The antifungal assay was done by using a cup diffusion method with standard antifungal drugs. The solvent of Ethanol was used against *Aspergillus nidulans* and *Penicillium notatum* fungal strains.

The result of antifungal screening by cup diffusion method using different fungal strains (*Aspergillus nidulans* and *Penicillium notatum*) (Figure 5) shows in plate.



a) Aspergillus nidulans *b) Penicillium notatum*

Fig 5: - Antifungal effect of ethanolic extract of C.tetragonoloba against a) Aspergillus nidulans and b) Pencillium notatum.

The results clearly show distil notable inhibitory zone against *Cyamopsis tetragonoloba*. The presence of inhibitory zone specifies *Cyamopsis tetragonoloba* ethanolic extract antifungal effect against the selected organisms such as *Aspergillus nidulans* (3.52±1.45) and *Penicillium notatum* (3.61±0.58).

IV. CONCLUSION

A traditional system of medicine continues to be widely practiced on many accounts population rise, inadequate supply of drugs, Prohibitive cost of treatment side, effects of several allopathic drugs and development of resistance to currently used drugs for infectious disease have led to increased emphasis on the use of Herbal materials as a source of medicine for a while of various human ailments. The analysis for the ethanolic extract of *Cyamopsis tetragonoloba* by GCMS reveals the presence of (65) compounds which has been screened and analyzed which provides the complete spectrum of phytochemical constituents present in the ethanolic extract of *Cyamopsis tetragonoloba.* In vitro antioxidant status of *Cyamopsis tetragonoloba* pod extract exhibited notable antioxidant radical scavenging activity. FRAP showed effective antioxidant potential. The global prevalence of infectious disease caused by fungi is a major public health issue. Which causes human infectious diseases. Hence, there is an increased demand for accurate knowledge for MIC (Maximum Inhibitory Concentration). Plants derived compounds *Cyamopsis tetragonoloba* are enabled to balance between sensory accessibility and antifungal efficacy that can be determined by in vivo and in vitro studies. The tested microorganism was more sensitive to their ethanol pod extract compared with standard antifungal drug (Ceftriaxone). This novel study has explored the antioxidant status from *C. tetragonoloba* pods possess a good store of antioxidant and also it acts as a natural herbal remedy to combat infectious disease in near future.

REFERENCES

[1] Tahmouzi, S., Meftahizadeh, H., Eyshi, S., Mahmoudzadeh, A., Alizadeh, B., MollakhaliliMeybodi, N., & Hatami, M. (2023). Application of guar (Cyamopsis tetragonoloba L.) gum in food technologies: A review of properties and mechanisms of action. Food Science & Nutrition.

[2] Jamshed, M., Ali, S. T., Rizwani, G. H., Zahid, H., & Asif, S. T. (2018). Pharmacognostic evaluation of pods of Cyamopsis tetragonoloba L. Int J Herb Med, 6(1), 51-53.

[3] Abeer Fauzi Al-Rubaye, Imad Hadi Hameed, Mohanad Jawad Kadhim. Uses of Gas Chromatography- Mass Spectrometry (GC-MS) Technique for analysis of bioactive natural compounds of some plants. International Journal of Toxicological and Pharmacological Research 2017; 9(1); 81-85.

[4] Yazarlu, O., Iranshahi, M., Kashani, H. R. K., Reshadat, S., Habtemariam, S., Iranshahy, M., & Hasanpour, M. (2021). Perspective on the application of medicinal plants and natural products in wound healing: A mechanistic review. Pharmacological research, 174, 105841.

[5] Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of agricultural and food chemistry, 53(10), 4290-4302.

[6] Antolovich, M., Prenzler, P. D., Patsalides, E., McDonald, S., & Robards, K. (2002). Methods for testing antioxidant activity. Analyst, 127(1), 183-198.

[7] Laxminarayan, R., Matsoso, P., Pant, S., Brower, C., Røttingen, J. A., Klugman, K., & Davies, S. (2016). Access to effective antimicrobials: a worldwide challenge. The Lancet, 387(10014), 168-175.