https://doi.org/10.30799/jnpr.119.25100203 J. Nat. Prod. Resour. - Volume 10 Issue 2 (2025) 322–324

Share Your Innovations through JACS Directory

Journal of Natural Products and Resources

Visit Journal at https://www.jacsdirectory.com/jnpr

ISSN: 2455-0299

Evaluation of Phytochemical Screening, *In vitro* Antioxidant Activity, Anti-Bacterial Activity and GCMS Studies on Aqueous Seed Extract of *Cucurbita pepo*

M. Mayil Arasi, S. Esakkiammal, M. Muthu Kani, D. Criskilda Vincy, V. Abi Karthika, S. Jamesammal*

Department of Microbiology, Kamaraj Women's College, Thoothukudi – 628 003, Tamil Nadu, India.

ARTICLE DETAILS

Article history:
Received 07 October 2025
Accepted 23 October 2025
Available online 22 December 2025

Keywords:
Cucurbita pepo
Phytochemical
Antioxidant
Antibacterial

ABSTRACT

The current study aimed to investigating the phytochemical constituents, *invitro* antioxidant activity, anti-bacterial activity and GCMS studies of aqueous extract of *Cucurbita pepo*. All the experiments were conducted using standard procedures. The result of the phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, phenolic content, tannins, steroids, and glycosides. The antioxidant activity of aqueous extract of *Cucurbita pepo* was evaluated by DPPH free radical scavenging assay. Rutin was used as a reference compound. This extract also possesses many phytochemicals and significant antioxidant activity. Therefore, *Cucurbita pepo* may be useful for therapeutic purposes. The gas chromatographic and mass spectra were observed there were three compounds obtained namely, 1) octanoic acid, silver (1+) salt, 2) trans-13-octadecenoic acid, and 3) octadecanoic acid, in spectra were also recorded in the seed extract of *Cucurbita pepo*.

1. Introduction

Pumpkin is derived from the family Cucurbitaceae and is considered as key crop in horticultural zone. Pumpkin is (Cucurbita) characterized among different species, but the *Cucurbita maxima, Cucurbita pepo, Cucurbita moschata, Cucurbita ficifolia, and Cucurbita mixta* pangalo are the most popular species [1]. Among these, only three species, *Cucurbita moschata, Cucurbita maxima* and *Cucurbita pepo*, present the importance with high economical cultivation and production across the world [2]. For edible and medicinal purpose, pumpkins are cultivated in subtropical zones and transit zones. The Seeds of *Cucurbita pepo* are usually left-over during processing. It has been reported, though, that these seeds are of considerable use. The pharmacological activities reported for the seeds include antibacterial, antidiabetic, antifungal, anti-inflammatory and antioxidant activity [3]. *Cucurbita pepo* seeds which were previously regarded as waste, but now can find an important role in the food industry [4]. This is due to their nutritious composition mainly potassium, phosphorous, magnesium, selenium and zinc [5]. The seeds are disbursed either in raw or in roasted form. The *Cucurbita pepo* seeds are an excellent nutrient source responsible for diseases such as arthritis and inflammation [6]. The seeds can be consumed regularly without any side effects on human health [7]. In developing countries, about 80 % of the population uses natural products for various diseases [8]. Herbal drugs are generally considered as safe and have no side effects. Phytochemicals are responsible for the medicinal activity of plants [9]. These are non-nutritive chemicals that have sheltered humans from various diseases. Phytochemical constituents are the essential source for the organization of numerous pharmaceutical industries. In modern years, there has been major importance in identifying plant products with antioxidant properties, as free radicals are considered to play a major role in human diseases such as Cancer, inflammation and diabetes [10]. Therefore, the aim of the present study is to investigate the phytochemical screening, in vitro antioxidant activity, anti-bacterial and anti-diabetic activity, Phyto analytical study such as GCMS spectra were recorded to characterization of the compounds.

2. Experimental Methods

The pumpkin fruit, *Cucurbita pepo,* was bought from VOC market, Thoothukudi, Tamilnadu in December 2024. It was packaged in a polythene bag and kept in the dept of Microbiology, Kamaraj College, Thoothukudi. The pumpkin was properly washed and sliced. The seeds were air dried by spreading on sacks in an aerated room for 6 weeks and take out adhering foreign matter and were powdered with the help of pestle and mortar.

2.1 Chemicals

The reagents and chemicals used in this study are ethanol, chloroform, isopropyl alcohol, sulphuric acid, hydrochloric acid, sodium phosphate, ammonium molybdate, peptone water, Muller Hinton agar.

2.2 Bacterial Culture

The pathogenic bacteria namely gram-negative bacteria *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and gram-positive bacteria like *Staphylococcus aureus* were obtained from the Department of Microbiology at Kamaraj Women's College, Thoothukudi.

2.3 Extraction

Fifty grams (50 g) of the powdered seeds was soaked with 500 mL of ethanol, chloroform and isopropyl alcohol in separate conical flasks and kept for 2 weeks in a shaker incubator then collect the sample and filtered then kept in the air tight bottle until use.

2.4 Qualitative Phytochemicals

The preliminary phytochemical screening tests were performed for the presence of following secondary metabolites such as alkaloids, phenols, saponins, tannins, steroids, glycosides and flavonoids [11]. Alkaloids, phenols and flavonoids estimation followed the procedure suggested by Harborene [12] and Thangaraj [13].

2.5 Anti-Bacterial Activity

2.5.1 Disc Diffusion Assay

Disc diffusion method for antimicrobial susceptibility testing was carried out according to the standard method [14] to access the presence of antibacterial activities of the plant extracts. The test bacteria were inoculated in peptone water and incubated for 3 to 4 hr at 35 °C. Muller Hinton agar plates were prepared and poured in sterile Petri plates. 0.1

*Corresponding Author:jamesstephen2305@gmail.com(S. Jamesammal)

https://doi.org/10.30799/jnpr.119.25100203

mL of bacterial culture was inoculated on the surface of Muller Hinton agar plates and spread by using L-rod. The inoculated plates were allowed to dry for 5 min. The disk loaded with samples conc (1000 µg/mL) was placed on the surface of inoculated Petri plates using sterile technique. The plate was then incubated at 37 °C for 18 to 24 hours depending on the species of bacteria used in the test. After the incubation, the plates were examined for inhibition zone. The inhibition zones were then measured using calipers and recorded.

2.5.2 Alpha Amylase Inhibition Assay

The assay mixture containing 200 µL of 0.02 M sodium phosphate buffer, 20 µL of enzyme and the plant extracts in concentration range 20-100 µg/mL were incubated for 10 min at room temperature followed by addition of 200 µL of starch in all test tubes. The reaction was terminated with the addition of 400 µL DNSA reagent and placed in boiling water bath for 5 min, cooled and diluted with 15 mL of distilled water and absorbance was measured at 540 nm. The control samples were prepared without any plant extracts.

2.6 Total Antioxidant Capacity Assay

Total antioxidant capacity is a spectroscopic method for the quantitative determination of antioxidant capacity, through the formation of phosphomolybdenum complex. The assay is based on the reduction of Mo(V) complex at acidic pH. Total antioxidant capacity can be calculated by the method described by Prieto et al. [15]. 0.1 mL of sample (100 µg) solution is combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube is capped and incubated in a boiling water bath at 95 °C for 90 min. After cooling the sample to room temperature, the absorbance of the aqueous solution is measured at 695 nm against blank in UV spec. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it is incubated under same conditions as rest of the sample. Ascorbic acid was used as standard.

2.7 Gas Chromatography Mass Spectrometry (GC-MS)

The sample analyzed was labelled CU PE01 15325. It was prepared and placed in ALS vial 6, with a sample multiplier of 1. The sample was processed using an automated injector. Detection Mode: Total Ion Chromatogram (TIC) Data processing – The mass spectra of the unknown compounds were compared against the NIST 11. L spectral Database, with a minimum quality threshold set for identification. The software Chem Station Integrator was used for peak integration and identification.

3. Results and Discussion

3.1 Qualitative Phytochemical Screening

The *Cucurbita pepo* seed extract has an excellent source of nutritional diversity and health-protective qualities of the seeds, which are abundant in polysaccharides, carotene, mineral salts, vitamins and other useful substances. In the current study, the presence of alkaloids, phenolic compounds, flavonoids, saponins, steroids, tannins, and terpenoids in the *Cucurbita pepo* seed extract was confirmed and depicted in Table 1.

Table 1 Preliminary phytochemical screening of *Cucurbito pepo*

S. No.	Phytochemical constitution	Ethanol	Chloroform	Isopropyl alcohol
1	Alkaloids	+	+	+
2	Phenolic compound	+	+	+
3	Flavonoids	+	+	+
4	Terpenoids	+	+	+
5	Saponins	+	+	+
6	Steroids	+	+	+
7	Tannins	+	-	-
8	Glycosides	+	+	+
9	Proteins	-	-	-
10	Amino acids	-	-	-

(+): Presence of chemicals, (-): absence of chemicals or not detectable concentration

The strong phytochemicals have health benefits and provide protection from the risk of cholesterol, diabetes, cardiovascular disease and hypertension [16]. Alkaloids are naturally occurring chemical compounds in plant parts that normally have pharmacological effects. Phenolic compounds have potent antioxidant properties as oxygen scavengers, peroxide decomposers, free radical inhibitors and metal chelating agents. It also possesses anti-bacterial, anti-viral properties [17]. Flavonoids capable of scavenging oxygen-derived free radical and also possess anti-viral and anti- inflammatory properties [18]. Terpenoids are plant-based compounds and is widely used in the food industry and also in pharmaceutical and chemical products [19]. Saponins are bioactive compounds over biological and pharmacological properties that naturally arise in plants as triterpenes [20]. Steroids have antibacterial properties, and medicinal and pharmaceutical activities and boost the immune response [21]. The phytochemicals in the seed extract of Cucurbita pepo identified in this study may support the contribution and prevention of various diseases.

3.2 In Vitro Antioxidant Assay

Antioxidants are the organic constituents are highly used over natural sources, which are also a mixture of phytocompounds. The intense generation of oxidative stress by prooxidants damages the cellular molecules such as proteins, nucleic acids, and lipids which may cause tissue interruption. In such conditions, antioxidants perform a vital role in eliminating oxidative stress by scavenging the radicals causing oxidative damage. Therefore, the antioxidants found in the seed extract of *Cucurbita pepo* can play a vital role in scavenging the free radicals. This study aimed to evaluate the antioxidant capacity of *Cucurbita pepo* to identify its ability to scavenge the free radicals. The results are reported in Table 2. The DPPH scavenging assay was used to study the free radical scavenging activity of the seed extract by calculating the IC_{50} values of the extract. The lower IC_{50} value of *Cucurbita pepo* reflects higher DPPH radical scavenging activity. It can be concluded that the Cucurbita pepo extract showed more potent in vitro antioxidant activity in a dose-dependent manner with a higher percentage of inhibition at 50 mcg/ml. The IC_{50} value of *Cucurbita pepo* extract was found to be % antioxidant activity of this *Cucurbita pepo* might be due to the presence of higher amounts of phenolic compounds. Generally, the phenolic compounds are considered primary antioxidants.

Table 2 Determination of DPPH scavenging activity

S. No.	Extract	IC50 value (µg/mL)
1	Ethanolic extract	1.54
2	Chloroform extract	1.54
3	Isopropyl alcohol extract	1.05
4	Ascorbic acid (Std)	1.83

3.3 Antibacterial Activity

The maximum zone of inhibition against *Pseudomonas aeruginosa* exhibited the highest sensitivity to the chloroform extract with a maximum zone of inhibition of 9 mm. The inhibition zones for other bacteria were as follows: *Staphylococcus aureus* (5.5 mm), *Klebsiella pneumoniae* (5 mm), and *E. coli* (4 mm). In positive control sample ciprofloxacin exhibited inhibition zones ranging from 19 mm to 21 mm, it indicates a significant increase in antibacterial activity than the chloroform extract (Table 3 and Figs. 1-3)

Table 3 Determination of antibacterial activity of *Cucurbita pepo*

S.No.	Species name	Ethanolic extract		Chloroform extract		Isopropyl alcohol	
		Std	Sample	Std	Sample	Std	Sample
1	*E.coli*	21	7	19	4	16	8
2	*S. aureus*	20	5	19	5.5	20	5
3	*P. aeruginosa*	17	-	20	20	24	7
4	*K. pneumoniae*	21	4	21	5	20	5.5

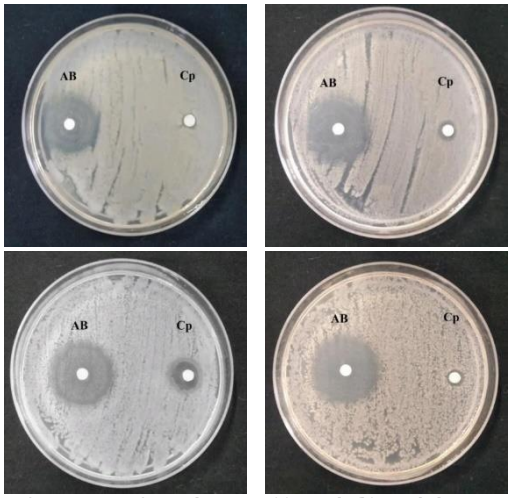

Fig. 1 Chloroform extract of *Cucurbita pepo* (a) *E. Coli*, (b) *Staphylococcus aureus*, (c) *Pseudomonas aeruginosa* and (d) *Kleibsiella pneumoniae*

https://doi.org/10.30799/jnpr.119.25100203

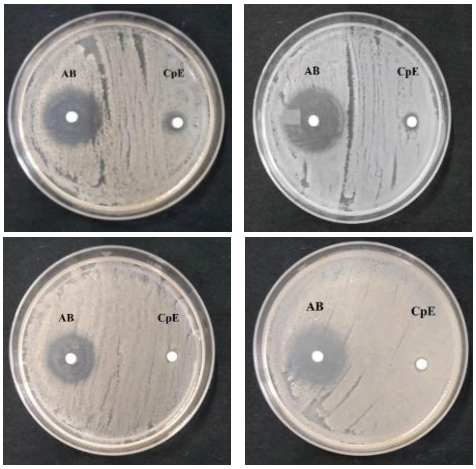

Fig. 2 Ethanolic extract of *Cucurbita pepo* (a) *E. Coli*, (b) *Staphylococcus aureus*, (c) *Pseudomonas aeruginosa* and (d) *Kleibsiella pneumoniae*

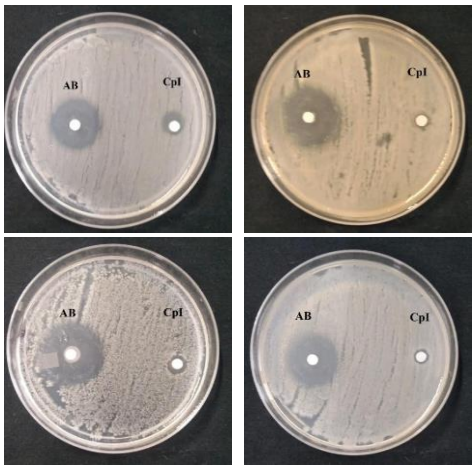

Fig. 3 Isopropyl alcohol extract of *Cucurbita pepo* (a) *E. Coli*, (b) *Staphylococcus aureus*, (c) *Pseudomonas aeruginosa* and (d) *Kleibsiella pneumoniae*

3.4 Antidiabetic Activity

The alpha amylase inhibition was observed in the ethanol extract of *Cucurbita pepo*. At a concentration of 1000 µg/mL, it shows the highest inhibition of 72.80%. The other extract isopropanol extract 40.80% of inhibition at 1000 µg/mL, and chloroform extract has 36.80% of inhibition at 1000 µg/mL. This indicates that the ethanol extract is the most effective inhibiting the alpha-amylase enzyme, which is relevant for controlling postprandial blood sugar levels.

3.5 Gas Chromatography and Mass Spectrometry (GC-MS)

The GCMS spectrum of *Cucurbita pepo* seed extract showed three major peaks at retention times (RT) 10.842, 12.261, and 12.412 min in GC. The GC-MS spectrum and peak fragmentation are shown in Fig. 4.

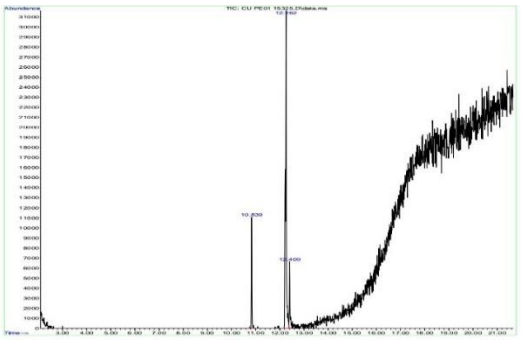

Fig. 4 GC-MS chromatogram of *Cucurbita pepo* seed extract

GC-MS plays a key role in the analysis of known and unknown components of the plant origin. GC-MS ionizes compound and measures their mass numbers. The use of mass spectrometry (MS) is most cases coupled to an appropriate separation technique as gas chromatography. GC-MS analysis of phyto constituents in plants gives a clear picture of the pharmaceutical value of the plant. There are very less reports on the analysis of phytochemicals in plants. The result of the GC-MS analysis led to the identification of number of compounds peak from GC fractions of the seed extract of *Cucurbita pepo* (Table 4).

Table 4 Phytocomponents identified in *Cucurbita pepo* seed extract by GC-Ms

S.No.	RT	Area %	Library/ID	Mol. F	Mol. W
1	10.842	14.15	Octanoic acid, silver (1+) salt	$C_8AgF_{15}O_2$	520.93
2	12.261	75.03	Trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.5
3	12.412	10.82	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5

4. Conclusion

Cucurbita pepo is a valuable medicinal plant with diverse pharmacological applications. Its antioxidant, antibacterial, and antidiabetic properties make it a promising candidate for functional foods and pharmaceuticals. Phytochemical analysis shows that the ethanol extract contained the highest levels of flavonoids, saponins, tannins, terpenoids, and steroids. The health benefits of pumpkins are mainly attributed to their Phenolic, flavonoid, terpenoids. The disc diffusion method was used to evaluate the antibacterial activity against selected bacterial strains. GC-MS results identified that the bioactive compounds include n-hexadecanoic acid (antimicrobial, anti-inflammatory), cis-13-octadecenoic acid (heart health, cholesterol balance). The results of this study support the idea that the pumpkin seed collected are promising sources of natural antioxidants. The consumption of pumpkin is linked, through mechanisms including antioxidant and anti-inflammatory capabilities, with the onset of many diseases or their symptoms. Pumpkin seeds are valued for their oil, rich in essential fatty acids, and as a strong source of proteins with essential amino acids. especially antioxidant properties, antibacterial and antidiabetic properties.

References

[1] A.W. Oyeleke, D.T. Oluwajuyitan, O.M. Oluwamukomi, N.V. Enujiugha, Amino acid profile, functional properties and in Vitro antioxidant capacity of *Cucurbita maxima* and *Cucurbita mixta* fruit pulps and seeds, European J. Nutr. Food Saf. 10(4) (2019) 224-241.

[2] B.A. Kulczyński, A. Sidor, A. Gramza-Michałowska, Antioxidant potential of phytochemicals in pumpkin varieties belonging to *Cucurbita moschata* and *Cucurbita Pepo* Species, CyTA- J. Food 18 (1) (2020) 472-484.

[3] C.Z. Nkosi, A.R. Opoku, S.E. Terblanche, Antioxidative effects of *Cucurbita pepo* seed (*Cucurbita pepo*) protein isolate in CCl_4-induced liver injury in low-protein fed rats, Phytother Res. 20(11) (2006) 935-940.

[4] J.K. Karanja, B.J. Mugendi, F.M. Khamis, A.N. Muchugi, Nutritional evaluation of some kenyan *Cucurbita pepos* (*Cucurbita spp.*), Int. J. Agricul. Forest. 2(9) (2008) 296-304.

[5] M. Kaur, S. Sharma, Development and nutritional evaluation of *Cucurbita pepo* seed (*Cucurbita moschata*) supplemented products, Food Sci. Res. J. 8(2) (2017) 310-318.

[6] M. Batool, M.M.A.N. Ranjha, U. Roobab, M.F. Manzoor, U. Farooq, H.R. Nadeem, et al., Nutritional value, phytochemical potential, and therapeutic benefits of pumpkin (*Cucurbita* sp.), Plant. 11(11) (2022) 1394.

[7] Q.A. Syed, Nutritional and therapeutic importance of the *E. Middleton*, Effect of plant flavonoids on immune and inflammatory cell function. In: Flavonoids in the Living System, Springer Boston MA, New York, 1998, pp.175–182.

[8] A. Salatino, M.L.F. Salatino, G. Negri, Traditional uses, chemistry and pharmacology of croton species, J. Braz. Chem. 18 (2007) 11-33.

[9] N. Savithramma, M. Linga Rao, D. Suhrulatha, Screening of medicinal plants for secondary metabolites, Middle-East J. Sci. Res. 8(3) (2011) 579-584.

[10] B. Halliwell, Reactive oxygen species in living systems: sources, biochemistry and role in human diseases, Am. J. Med. 91 (1991) 14s-22s.

[11] W.C. Evans, Trease and Evans pharmacognosy, 13th Ed., Cambridge University Press, Elsevier, London, 1989.

[12] A.J. Harborne, Phytochemical methods a guide to modern techniques of plant analysis, 3rd Ed., Chapman & Hall, London,1998.

[13] P. Thangaraj, Pharmacological assays of plant-based natural products, Springer International Publishing, India, 2016.

[14] A.W. Bauer, W.M. Kirby, J.C. Sherris, M. Turck, Antibiotic susceptibility testing by a standardized single disk method, Am. J. Clinic. Pathol. 45 (1966) 493-496.

[15] P. Prieto, M. Pineda, M.Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vit. E, Anal. Biochem. 269 (1999) 337-341.

[16] W.J. Craig, Phytochemicals: guardians of our health, J. Am. Diet Assoc. 97(10) (1997) 199-204.

[17] S.A. Van Acker, G.P. van Balen, D.J. van den Berg, A. Bast, W.J. van der Vijgh, Influence of iron chelation on the antioxidant activity of flavonoids, Biochem. Pharmacol. 56(8) (1998) 935-943.

[18] E. Middleton, Effect of plant flavonoids on immune and inflammatory cell function, In: Flavonoids in the Living System, Springer Boston MA, New York, 1998, pp.175-182.

[19] D. Tholl, Biosynthesis and biological functions of terpenoids in plants, Adv. Biochem. Eng. Biotechnol. 148 (2015) 63-106.

[20] M.A. Lacaille-Dubois, H. Wagner, Bioactive saponins from plants: An update, Stud. Nat Prod. Chem. 21 (2000) 633-687.

[21] R.F. Epand, P.B. Savage, R.M. Epand, Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins), Biochim. Biophys. Acta Biomembr. 1768(10) (2007) 2500-2509.

https://doi.org/10.30799/jnpr.119.25100203