

EVALUATION OF ANTIOXIDANT, ANTIMICROBIAL, AND ANTIFUNGAL POTENTIAL OF
CUCURBITA PEPO VAR. FASTIGATA SEED EXTRACTSROSHNI RS SONI^{1*}, MANOJ BALI²¹Department of Applied Sciences, Quest Group of Institutions, Jhanjeri, IKG-Punjab Technical University, SAS Nagar, Punjab, India.²Baba Hira Singh Bhattal Institute of Engineering and Technology, Lehragaga, IKG-Punjab Technical University, Sangrur, Punjab, India
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ABSTRACT

Objective: The current study aims to study the antioxidant and antimicrobial and antifungal potential of the methanolic extract of *Cucurbita pepo* var. fastigata seeds (MECS).

Methods: Extraction of the seeds has been carried out with solvents of increasing polarity (chloroform, acetone, and methanol) and the phytochemical study of the methanolic extract have been carried out using standard methods. The free radical scavenging activity of all the extracts was evaluated by DPPH and H₂O₂ methods. Standard disk diffusion method was used to evaluate antibacterial and antifungal activities.

Results: Phytochemical evaluation showed the maximum presence of triterpenoids, phenolic compounds, tannins and small amount of Coumarins. Methanolic extract revealed momentous antioxidant activity as compared to chloroform and ethyl acetate extract. Hence, methanolic extract of *C. pepo* seeds (MECS) at a dose level of 100, 200 and 300 µg/ml was evaluated for antioxidant potential. Maximum free radical scavenging activity of methanolic seed extract of *cucurbita pepo* var. fastigata has been found at a dose of 300 µg/ml to be 63±0.16 % by 1,1-diphenyl-2-picryl hydrazyl model and at a value of 78% at 300 µg/ml with H₂O₂ model. Methanolic extract also showed the presence of antibacterial activity.

Conclusion: Presence of phytochemicals in the methanolic extract is responsible for the antioxidant potential. Extracts were investigated for antibacterial activity using the standard disc diffusion assay method against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and for the antifungal activities against *Aspergillus niger* and *Candida albicans*. The seed extract showed the presence of antibacterial activity, but the antifungal activity was found to be absent in the extract.

Keywords: Antimicrobial, Antioxidant, *Cucurbita pepo* var. fastigata, Free radical scavenging.

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INTRODUCTION

According to the WHO, medicinal plants as therapeutic aids have momentous role in curing and maintaining appropriate health [1-3]. Nature has been practicing combinational chemistry for eons [4] and numerous natural products and synthetically modified natural product have been developed for treatment [5]. The structural analysis and the ability to amalgamate them permitted chemists to amend them to repress or enhance their solubility and efficiency [6]. The ethanolic and aqueous extract of *Cassia fistula* Linn. shows the presence of various antioxidants such as kaempferol, gallic acid, ellagic acid, coumaric acid, rutin, myricetin, and quercetin [7].

The *Cucurbita pepo* var. fastigata belongs to the Cucurbitaceae family which consists of 130 genera and 800 species. Some of the important plants are *Momordica charantia*, *C. pepo*, *Cucurbita andreana*, *Cucurbita ficifolia*, *Cucumis sativus*, *Cucumis melo*, *Citrullus colocynthis*, *Luffa echinata*, *Trichosanthes kirilowii*, *Lagenaria siceraria*, and *Benincasa hispida* [8]. Literature reveals the traditional use of the various parts of the plant by Ayurvedic & Chinese systems as anti-inflammatory, analgesic, antidiabetic, antiulcer, and antioxidant. The fruit is used to cure fatigue & thirst, acts as a blood purifier, treats cold. The seeds are diuretic, helpful in headaches neuralgia, bronchitis, fever, Gastritis, burns, febrile diseases, irritable bladder & prostatic complains. The seeds are also beneficial to spleen, lungs and act as taenicide. The leaves are used for the treatment of nausea & helps to boost haemoglobin [9]. Oil obtained from *C. pepo* seeds is recommended in nutritional and medicinal purpose as it acts as a potential drug able to heal wounds in animal [10]. *C. pepo* is chiefly known for its enhancement in prostatic

hyperplasia (benign prostatic hyperplasia) [11], urinary dysfunction, and cytotoxic properties and also has also been used widely as a hypoglycemic agent. Many pharmacological studies have established hepatoprotection, restrain benign prostatic hyperplasia, antioxidant, anticancer, anti-inflammatory, antidiabetic, and antiulcer activities supporting its customary uses [9,12]. The seeds, in addition to their roles as food additives and supplements, may also be used as an efficient and economical source of antibacterial agents for the treatment of bacterial infections [13]. The methanolic, chloroform, and ethyl acetate extracts of *C. pepo* fruits significantly reduced the paw swelling in mice dose dependently suggesting the immunomodulatory effects of extracts and can, therefore, act as immunonutrient [14,15]. The natural plant components found in pumpkin could recover the liver against alcohol-induced liver toxicity and oxidative stress in rats [16]. The cell growth inhibition of prostate, breast, and colon cancer cells by ethanolic seed extract authenticates the ethnomedical use of pumpkin seeds for a treatment of benign prostate hyperplasia [17].

The adequate ROS & RNS is vital for the efficient immune response as the imbalance between the production of oxidants/RONS and their removal by antioxidants lead to increased accumulation and, hence, the condition of oxidative stress [18]. Oxidative stress has been recognized as the major reason of the development and progression of numerous ailments. Supplements rich in exogenous antioxidants or boosting endogenous antioxidant defenses of the body are a capable way of skirmishing the unwanted effects of ROS-induced oxidative damage. Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS-induced oxidative damage [19]. Antioxidant defense system plays a key role to overcome

different diseases caused as a result of free radicals by neutralizing the excess of free radicals. An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property [20]. However antioxidant, antimicrobial and antifungal studies have not yet been reported for the seeds of *Cucurbita pepo* var. fastigata extract. So the present study has been carried out to evaluate the antifungal, antimicrobial and antioxidant potential of *Cucurbita pepo* var. fastigata seeds.

METHODS

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide were procured from Hi-Media. Carrageenan and ascorbic were obtained from Jackson Laboratories, Amritsar, Punjab. The solvents such as hexane, chloroform, ethyl acetate, and methanol were of analytical grade and procured from SD Fine Chemical.

Plant material

The seeds of *C. pepo* var. fastigata were bought from Local Market of Kharar (PB)/Roopnagar (PB)/Chandigarh (UT) and Delhi, 2013. The seeds were authenticated by Prof. Satwinderjeet Kaur and the letter vide ref no: 0176 has been deposited in the Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar. The seeds were cleaned, washed, dried for 2 days, and crudely powdered in a grinder at room temperature. The sample was kept in light-protected air-tightened containers.

Extraction

The powdered seed material was subjected to defatting i.e., removal of fats using hexane, and then, extraction was carried out using solvents of increasing polarity such as chloroform, acetone, and methanol by cold maceration process for 24 h. The solvents were completely removed by rotary evaporator and crude extracts were obtained and stored in the refrigerator. These extracts were further used for evaluation of their antioxidant, antibacterial, and antifungal activities.

Phytochemical screening

Standard procedures for preliminary phytochemical screenings of the extracts were carried out to analyze the presence of various constituents. The extracts obtained were analyzed for flavonoids, tannins, alkaloids,

Table 1: Phytochemical screening of *C. pepo* var. fastigata seeds extracts/isolated comp

Plant constituent/test	<i>C. pepo</i> var. fastigata
Alkaloids	-
Carbohydrates	-
Phytosterols	-
Phenolic compounds and tannins	++
Triterpenoids	+++
Flavonoids	-
Coumarins	+

C. pepo: *Cucurbita pepo*

Table 2: DPPH scavenging activity by metabolic seed extract of *Cucurbita pepo* var. fastigata.

Concentration (µg/ml)	% age scavenging	
	Methanol extract	Ascorbic acid
100	45.73±0.27	50.23±0.49
200	58.72±0.45	64.74±0.58
300	76.35±0.52	82.88±0.87

Values are the average of triplicate experiments and represented as mean±standard error of the mean. DPPH: 1,1-diphenyl-2-picrylhydrazyl

triterpenes, sterols, protein, carbohydrates, and amino acids. The identification was done on the basis of color change for the respective components [21,22]. Further, seclusion and characterization of pure compounds from the extract are in progress.

Antioxidant activity

Qualitative scavenging activity on DPPH radical

The qualitative assays were accomplished according to simple screening method for antioxidants [23]. Dilution of 2 mg of each extract was done with 1 ml of the suitable solvent, following which a small quantity of each dilution was cautiously laden onto the baseline of the 20 cm by 10 cm TLC plates, and the sample was allowed to dry for some time. Hexane-ethyl acetate in ratio of 7:3 was used as the mobile phase. The dried plates were sprayed with a 0.2% solution of DPPH in ethanol. The extracts having antioxidant constituent displayed a yellow on purple spot due to the discoloration of DPPH [24].

Quantitative scavenging activity on DPPH radical

Antioxidant potential of methanolic seed extract of *C. pepo* var. fastigata was evaluated by 1, 1-diphenyl-2-picryl hydrazyl radical scavenging activity. The reduction capability of 1, 1-diphenyl-2-picryl hydrazyl radical was determined by the decrease in its absorbance at 517 nm. DPPH radical is scavenged by antioxidants through the donation of a proton, which forms the reduced DPPH and lead to decrease in absorbance at a wavelength of 517 nm [25]. Ascorbic acid was used as standard and blank was used to remove the influence of the color of the samples. A methanolic solution of DPPH was used as negative control.

Table 3: Antioxidant activity of metabolic seed extract of *Cucurbita pepo* var. fastigata by hydrogen peroxide method

Concentration (µg/ml)	Absorbance (nm)	Mean	% age scavenging	
			Methanol extract	Ascorbic acid
100	0.273	0.274	20.17±0.27	44.43±0.26
	0.275			
	0.276			
200	0.205	0.210	40.05±0.11	55.03±0.46
	0.210			
	0.215			
300	0.102	0.106	70.17±0.14	72.17±0.32
	0.110			
	0.106			

Values are the average of triplicate experiments and represented as mean±standard error of the mean

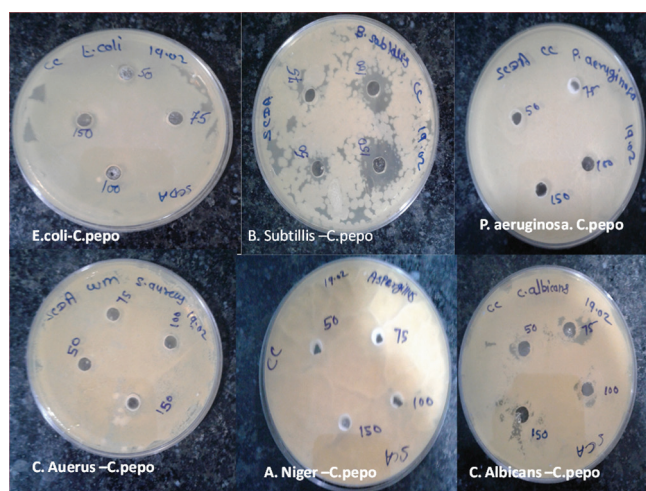


Fig. 1: Inhibition of bacterial growth of the methanolic extract of *Cucurbita pepo* seeds by disc diffusion method

Table 4: Antibacterial activity of methanolic seed extract of *Cucurbita pepo* var. *fastigata* by disk diffusion method

S. No	Component	Concentration ($\mu\text{g/ml}$)	Zone of inhibition for bacteria			
			<i>B. subtilis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
1	<i>C. pepo</i> var. <i>fastigata</i>	50	Resistance	Resistance	Resistance	Resistance
		75	14 mm	Resistance	13 mm	Resistance
		100	16 mm	Resistance	14 mm	Resistance
		150	19 mm	Resistance	15 mm	Resistance

B. subtilis: *Bacillus subtilis*, *E. coli*: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 5: Antifungal activity of methanolic seed extract of *Cucurbita pepo* var. *fastigata* by disk diffusion method

S. No	Component	Concentration ($\mu\text{g/ml}$)	Zone of inhibition for fungus	
			<i>A. niger</i>	<i>C. albicans</i>
1	<i>C. pepo</i> var. <i>fastigata</i>	50	Resistance	Resistance
		75	Resistance	Resistance
		100	Resistance	Resistance
		150	Resistance	Resistance

A. niger: *Aspergillus niger*, *C. albicans*: *Candida albicans*

Tests were carried out in triplicate. Percentage inhibition was evaluated by using the equation 1.

$$I (\%) = (A_0 - A_s) / A_0 \times 100 \quad (1)$$

Where, A_0 = values for the absorbance of the negative control.

A_s = The absorbance of the sample [26].

Free radical scavenging activity of metabolic seed extract by hydrogen peroxide method

Hydroxyl radical formation can occur in several ways by far the most important mechanism *in vitro* is the Fenton reaction where a transition metal involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [27]. Hydroxyl radicals are the most reactive and predominant radical generated endogenously during aerobic metabolism among the ROS which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and causing some diseases and ageing as well [28,29]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [30]. In the present study, the seed extract was evaluated for their hydroxyl radical scavenging activity.

Antibacterial activity

Extracted dilution was prepared in 50 μL , 75 μL , 100 μL , and 150 μL [31]. Required glassware was washed and dried in a hot air oven. The sterilized agar medium was transferred into the Petri dishes and was allowed to solidify at room temperature. The selected test organism (bacterial) was spread over the solidified agar with the help of a swab stick. Sterile borer was used to make wells of 8 mm diameter. The dilutions of Extracted sample (50 μL , 75 μL , 100 μL , 150 μL) were done in the wells with the help of a sterile syringe needle. The Petri plates were placed in a refrigerator for 5 min to allow diffusion. Later, the Petri plates were incubated in inverted position at 37°C for 24 h in the incubator. After 24 h, the zone of inhibition was observed and diameter in mm was measured and recorded.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical evaluation revealed that the methanolic extract of *Cucurbita pepo*. var. *fastigata* seeds showed maximum presence of triterpenoids, phenolic compounds, tannins and small amount of

Coumarins (Table 1), due to which it was further subjected to *in vitro* antioxidant studies. Polyphenolic compounds, triterpenoid and steroid found in plants, have been reported to have multiple biological effects including antioxidant activity [32-35].

Qualitative DPPH radical scavenging activity

DPPH method was used to estimate the antioxidant activity of the *C. pepo*. seed's extracts. The qualitative DPPH radical scavenging activity was demonstrated due to change in the coloration from purple to yellow on the TLC plate by the extract.

Quantitative DPPH radical scavenging activity

In quantitative estimation, DPPH radical was used as a substrate to evaluate the free radical scavenging activity of the methanolic extract of *C. pepo* var. *fastigata* was shown at a dose of 300 $\mu\text{g/ml}$ is $63 \pm 0.16\%$ by 1,1-diphenyl-2-picryl hydrazyl model as shown in Table 2.

Free radical scavenging activity of metabolic seed extract by hydrogen peroxide method

Hydroxyl radical formation can occur in several ways by far the most important mechanism *in vitro* is the Fenton reaction where a transition metal involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [27]. Hydroxyl radicals are the most reactive and predominant radical generated endogenously during aerobic metabolism among the ROS which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and cause aging of human and some diseases [28,36]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [30]. In the present study, the seed extract was evaluated for their hydroxyl radical scavenging activity. Maximum free radical scavenging activity of methanolic seed extract of *C. pepo* var. *fastigata* was shown at a dose of 400 $\mu\text{g/ml}$ is 78% by H_2O_2 model as shown in Table 3.

Antibacterial activity

It is evident from the data presented in Table 4 that the sample possesses antibacterial activity. Disk diffusion method showed the resistance of the seed extract to *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* at a concentration of 50 $\mu\text{g/ml}$ and toward *E. coli* and *P. aeruginosa* at a concentration of 75 $\mu\text{g/ml}$, 100 $\mu\text{g/ml}$, and 150 $\mu\text{g/ml}$. It implies that the isolates with a minimum inhibitory concentration at or above or zone diameters at or below the resistant breakpoint are not inhibited by the usually achievable concentration of the agent with normal dosage schedules [36]. As shown in Fig. 1, the largest zones of inhibition were observed in *C. pepo* seed extract against *B. subtilis* 75 $\mu\text{g/ml}$ (14 mm), 100 $\mu\text{g/ml}$ (16 mm), and 150 $\mu\text{g/ml}$ (19 mm) and *S. aureus* 75 $\mu\text{g/ml}$ (13 mm), 100 $\mu\text{g/ml}$ (14 mm), and 150 $\mu\text{g/ml}$ (15 mm). The extract showed high activity against *B. subtilis* and *S. aureus* species.

Then, it is evident from the data presented in Table 5 that the sample does not possess antifungal activity. The disc diffusion method result showed the resistance of the *C. pepo*. seed extract toward both *Aspergillus niger* and *Candida albicans* at all the four concentrations.

DISCUSSION

Oxidative stress and microbial infections pose a serious health problem around the world & plants have been an inestimable source of valuable natural products since these are potential source of antioxidants and antimicrobial agents [37]. World Health Organization (WHO) advocates the medicinal plants as the best source of diverse range of drugs and active compounds. Therefore investigations are required in order to explore their properties and understand their safety and efficiency [38]. The present study reports the antioxidant, antibacterial and antifungal activities of methanolic extract of cucurbita pepo seeds. The evaluation of the antioxidant potential was done using H₂O₂ and DPPH (qualitative and quantitative methods). Hydroxyl radical formation can occur in several ways by far the most important mechanism in vitro is the fenton reaction where a transition metal involved as a prooxidant in the catalyzed decomposition of superoxide and hydrogen peroxide [27]. Hydroxyl radicals are the most reactive and predominant radical generated endogenously during aerobic metabolism among the ROS which could be formed from superoxide anion and hydrogen peroxide, in the metal ions, such as copper or iron and cause ageing of human and some diseases [28,39]. The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells [30]. Both the results thus established the therapeutic potential against oxidative stress. The reason for the antioxidant activity is the presence of phenolic compounds, tannins and triterpenoids. The antimicrobial screening was performed for *B.Subtillis*, *E.Coli*, *S.aureus* & *P. aeruginosa* at a concentration of 50 µg/ml and towards *E.Coli* & *P. aeruginosa* at a concentration of 75 µg/ml, 100 µg/ml & 150 µg/ml. Disk diffusion method showed the resistance of the seed extract in all the samples. The cucurbita pepo var. fastigata seed extract showed high activity against *B.subtillus* and *S. aureus* species. The sample do not possesses antifungal activity. However no zone of inhibition against antifungal organism was found hence the disc diffusion method results showed the resistance of the *Cucurbita pepo*. seed extract towards both *A.niger* and *C.albicans* at all the four concentrations. The insight into the inactivity of the extra against *A.niger* and *C.albicans* will require further investigation. The growing bacterial resistant towards antibiotics has become a matter of great concern for researchers' worldwide [40]. The antibiotic resistant bacteria have been considered as a major problem by intensive care physicians in the treatment of patients [41]. The increase in bacterial resistant has prompted researchers to explore the antimicrobial role of natural herbs against resistant strains [42, 43]. Many infectious diseases caused by resistant microbes can be treated by seed extracts having potential antimicrobial compounds. The results from our study have shown extremely strong activity in the seed extracts of *Cucurbita pepo*. Traditionally, herbal medicine is used by folklore for the treatment of various infectious diseases. Although most of the cases are not evaluated scientifically but the chemical constituents of even the simplest medicinal preparations are beneficial [44]. Hence the seed extracts offer an ample potential for the development of novel agents effectual against infections that are presently difficult to treat.

CONCLUSION

On the basis of the results of the above study, it can be concluded that the methanolic extract of *Cucurbita pepo*. var. fastigata seeds possess notable antioxidant and antibacterial activity. The *Cucurbita pepo* var. fastigata seed extract showed high activity against *B. subtilis* and *S. aureus* species, but they have shown no zone of inhibition against antifungal organism which shows that the methanolic extract of seeds does not possess antifungal activity. However, further, investigations are required to comprehend the precise mechanisms of action and isolation of the compound(s) accountable for such activities.

AUTHORS' CONTRIBUTION

Roshni R.S. Soni - Conceived idea of the study, participated in its design, performed laboratory work and coordinated and helped

to draft the manuscript, and also performed statistical analysis. Manoj Bali - Participated in the sequence alignment and drafted the manuscript & Supervised the study from conceiving of idea to drafting of manuscript.

CONFLICTS OF INTEREST STATEMENT

We declare that we have no conflicts of interest.

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