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, 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.

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

| Plant constituent/test | *C. pepo* 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

### 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<br>0.275<br>0.276 | 0.274 | 20.17±0.27 | 44.43±0.26 |
| 200 | 0.205<br>0.210<br>0.215 | 0.210 | 40.05±0.11 | 55.03±0.46 |
| 300 | 0.102<br>0.110<br>0.106 | 0.106 | 70.17±0.14 | 72.17±0.32 |

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

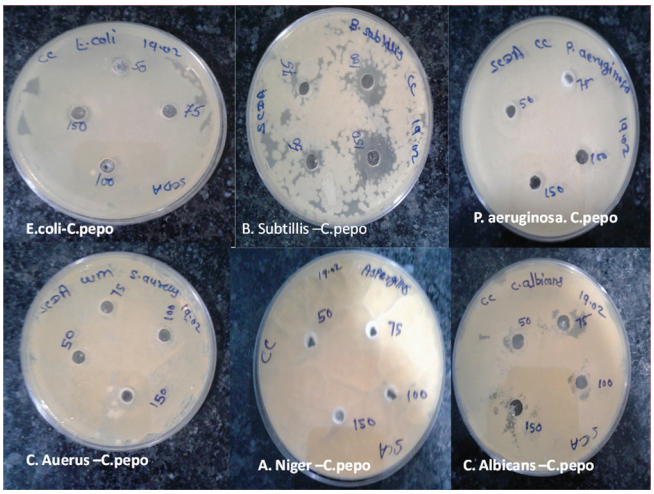


**Fig. 1: Inhibition of bacterial growth of the methanolic extract of *Cucurbita pepo*. seeds by disc diffusion method**

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

| S. No | Component | Concentration (µg/ml) | Zone of inhibition for bacteria | | | |
|---|---|---|---|---|---|---|
| | | | *B. subtilis* | *E. coli* | *S. aureus* | *P. aeruginosa* |
| 1 | *C. pepo* var. fastigata | 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 (µg/ml) | Zone of inhibition for fungus | |
|---|---|---|---|---|
| | | | *A. niger* | *C. albicans* |
| 1 | *C. pepo* var. fastigata | 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, 75µl, 100µl, 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 µg/ml is 63±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 µg/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 µg/ml and toward *E. coli* and *P. aeruginosa* at a concentration of 75 µg/ml, 100 µg/ml, and 150 µg/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 µl/ml (14 mm), 100 µl/ml (16 mm), and 150 µg/ml (19 mm) and *S. aureus* 75 µg/ml (13 mm), 100 µg/ml(14 mm), and 150 µg/ml (15 mm). The extract showed high activity against *B.subtillus* and *S. auerus* 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 $H_2O_2$ 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.auerus* & *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. auerus* 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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