

Phytoconstituents, bioactivity and antioxidant potential of some commercial brinjal (*Solanum melongena* L.) cultivars of Bangladesh

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Abstract

Comparative phytochemical composition, antibacterial activity, cytotoxicity and antioxidant potentiality of seven brinjal cultivars *Solanum melongena* L. were determined. Strong presence of carbohydrates, phenolic glycosides and weak presence of alkaloids, flavonoids, tannins, terpenoids, steroids and saponins were recorded in all the brinjal varieties. Antibacterial assay of brinjal extracts showed varying degree of sensitivity against both gram-positive and gram-negative bacteria. In cytotoxicity assay, the LC₅₀ values ranged between 59.91 to 216.45 mg/ml after 6 hours was an indication of poor cytotoxic potential of brinjal cultivars. The DPPH free radical scavenging activity ranged between 80.79% to 87.64 although the phenolic content was found between 6.082 to 9.292 mg GAE/g among the brinjal cultivars.

Key words: Antioxidants, Phytoconstituents, *Solanum melongena*.

INTRODUCTION

Brinjal (*Solanum melongena* L.) is an important solanaceous crop of tropics and sub-tropics. Brinjal fruit (unripe) is primarily consumed as cooked vegetable. In addition, it has some medicinal uses like antihemorrhoidal and hypotensive effect and used as an antidote to poisonous mushrooms (Sudheesh *et al.*, 1999). It is a good source of minerals and vitamins and is rich in total water soluble sugars, free reducing sugars, and proteins (Noda *et al.*, 1998). Some phytochemicals of vegetables are strong antioxidants and are thought to reduce the risk of chronic disease by protecting against free-radical damage, by modifying metabolic activation and detoxification of carcinogens, or even influencing processes that alter the course of tumor cells (Herrera *et al.*, 2009). Steroidal glycoalkaloids found in potatoes, tomatoes, and brinjals may lower blood cholesterol and offer protection against infection by *Salmonella typhimurium* (Friedman *et al.*, 2000 and Gubarev *et al.*, 1998). Mahmood *et al.* (2012) determined the cytotoxic effect of methanolic extract of *Datura innoxia* and *Solanum surrattense* on brine shrimps. But still there is no record on assessing cytotoxicity of brinjal cultivars on brine shrimps. Furthermore, in recent years, much emphasis has been placed on antioxidant properties of plant parts. Anthocyanins, an important group of naturally occurring phenolic compounds found in brinjal peel (Mazza *et al.*, 2004).

Brinjal fruit with different size, shape and color can be seen in vegetable-markets in all over Bangladesh. Diversity in fruit morphology of brinjal gives rises to the question whether they contain similar type of phytochemicals or have identical bioactivity. From these point of view the present investigation was carried out to make a comparative

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profile on phytoconstituents, antibacterial activity, cytotoxicity and antioxidant potential of seven brinjal cultivars collected from vegetable markets Dhaka, Mymensingh and Jessore districts of Bangladesh.

MATERIALS AND METHODS

Seven commercial brinjal cultivars were collected from different vegetable markets of Dhaka, Mymensingh and Jessore and named as collection no. JUBS1 to JUBS7 (Plate 1). Here, JUBS1 and JUBS7 were local cultivars known as 'Kanta begun' and 'Kamranga begun' collected from Jessore and Mymensingh districts respectively. Rests of the five cultivars were known as 'hybrid begun' were collected from different vegetables markets of Dhaka and Savar.

The collected fruit samples were chopped into pieces and sun-dried separately under open sunlight followed by drying in a hot air oven (Gallenkamp) at reduced temperature (not more than 50°C) for 48 hours to make suitable for grinding. After that fruits chips were ground into coarse powders using high capacity grinding mill and were stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation. About 500 g powder was digested with 1500 ml of 100 % of methanol for three days accompanying with occasional shaking and stirring. The whole mixtures then underwent a coarse filtration by a piece of clean, white cotton material followed by filtration using Whatman no.1 filter paper. This digestion procedure was repeated twice for each of the seven sample to obtain maximum amount of extract. The extract was concentrated at 45°C under reduced pressure using a rotary evaporator and resultant residue was stored under refrigerated conditions until further studies.

The crude extracts of fruits were subjected to different chemical tests to find out the qualitative presence of phytochemicals which were identified by characteristic color changes using standard procedure as described for alkaloids (Harborne, 1973), steroids (Trease & Evans, 1989), phenolics and flavonoids (Awe & Sodipo, 2001), saponins and cardiac glycosides (Sofowora, 1993), tannins (Odebiyi & Sofowora, 1978) terpenoids and saponins (Obadoni & Ochuko, 2001).

Antibacterial sensitivity test was estimated by the disc diffusion method following Bauer *et al.* (1966). The bacterial strains used for the experiment were collected as pure cultures from Microbiology laboratory, Department of Botany, Jahangirnagar University, Savar, Dhaka. Gram positive bacteria (*Bacillus subtilis*, *B. cereus*) and Gram-negative bacteria (*Escherichia coli* ATCC25922, *Vibrio cholera*, *Pseudomonas sp.*, *Salmonella typhi*, *Salmonella sp.*, *Serratia sp.*, *Erwinia sp.*, *Proteus mirabilis*, *Shigella flexneri*, *Staphylococcus aureus* ATCC25923) were considered for the test. 100µl of bacterial cell suspension was spread throughout the plates by spread plate method. The sample discs, the standard antibiotic disc and the control disc were placed gently on the previously marked zones in the nutrient agar plates. The plates were then inverted and kept in an incubator at 37° C for 24 hours. After incubation, the antimicrobial activities of the test

materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale.

The cytotoxicity assay was carried out following Brine shrimps (*Artemia salina*) lethality bioassay as described by Meyer *et al.*, 1982. The antioxidant activities of the extracts were measured on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical following the method described by Blois (1958) and Aoshima *et al.* (2004). The absorbance of each of the samples were taken at 517 nm after 30 min of incubation of DPPH solution and the samples.

The total concentration of phenolic compound (TPH) in methanol extracts were determined by Folin-Ciocalteu method (Vinson *et al.*, 1995) using the Folin-Ciocalteu reagent (FCR) with Gallic acid (GA) as the standard and expressed as milligram (mg) Gallic acid equivalents (GAE)/g of extract. Results are expressed as mean \pm SE for a given number of observations (n = 3). The level of significance was set at *p* value of 0.05.

RESULTS AND DISCUSSION

The qualitative chemical examination of crude methanolic (MeOH) extract of different brinjal plant samples have been presented in Table 1. Phytochemical analysis of fruit extracts of the seven brinjal cultivars (sample no. JUBS1 to JUBS7) showed strong presence of carbohydrate and phenolic glycosides including cardiac and anthraquinone glycosides and weak presence of alkaloids, flavonoids, tannins, terpenoids, steroids and saponins.

According to Ghani (2003) the presence of phytochemicals may account for their various pharmacological activities. Chinedu *et al.* (2011) reported on the presence of phytosterols, saponins and vitamin C, cardiac glycosides, steroids and tannins, and trace amount of terpenoids from *S. aethiopicum*. However, Nicoletti *et al.* (2008) mentioned that the *Solanum* sp. contains bioactive compounds like alkaloids, flavonoids, steroids and saponins. Agoreyo *et al.* (2012) showed that phytate, oxalate and tannin were higher in the round variety of *S. melongena* than that the oval variety. In contrast, the present findings indicated that amount of various phytochemicals did not alter with types of samples.

The antibacterial property of methanolic extracts of brinjal fruits have been tested against twelve bacterial strains to evaluate antimicrobial potential. The findings suggest that fruit extracts of brinjal cultivars were moderately active against the gram-positive and gram-negative bacteria. The antibacterial activity of JUBS2, JUBS3, JUBS4 and JUBS5 showed similar propensity of sensitivity against *B. subtilis*, *B. cereus*, *E. coli* ATCC25922 and *Pseudomonas* sp whereas JUBS1 and JUBS7 showed greater sensitivity against *S. aureus* ATCC25923 and *V. cholerae* along with *B. subtilis*, *B. cereus*, *E. coli* ATCC25922 and *Pseudomonas* sp.

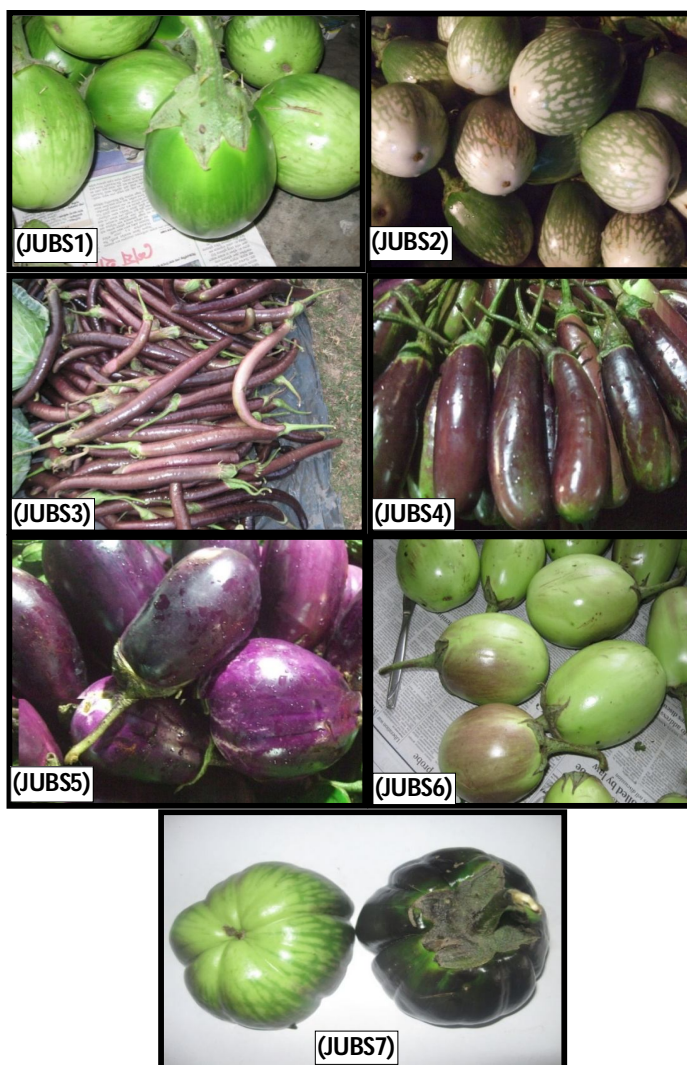


Plate 1. Commercial brinjal cultivars collected from different vegetable markets of Bangladesh

Largest inhibition zone 18.5 mm was observe *B. cereus* at 30 mg/disc concentration by JUBS1 followed by 16.4 mm at 30 mg/disc concentration by JUBS7 (Fig. 1). Cowan (1999) mentioned that plants which have been found *in vitro* to have antimicrobial property contains wide variety of secondary metabolites, such as tannins, terpinoids, alkaloids and flavonoids. Anushia *et al.* (2009) reported on the antibacterial activities of the *S. melogena* investigated against some common human pathogenic bacteria which was in favor of present findings. Sinden *et al.* (1986) reported that glycoalkaloides found in root of brinjal have antibacterial and diuretic properties.

Table 1. Qualitative chemical examination of fruit extracts of different brinjal cultivars

Sl no.	Name of the phytochemicals	Name of the reagents/test	Brinjal cultivars						
			JUBS1	JUBS2	JUBS3	JUBS4	JUBS5	JUBS6	JUBS7
01	Carbohydrates	Molish's	(++)	(++)	(++)	(++)	(++)	(++)	(++)
		Fehling	(++)	(++)	(++)	(+++)	(+++)	(++)	(+++)
02	Alkaloids	Hagger's	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		Wagner's t	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		Mayer's	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		Draggendorf's	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		Tannic acid solution (10%)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
03	Glycosides a. Cardiac glycosides b. Anthraquinone glycosides	FeCl ₃	(+++)	(++)	(++)	(++)	(++)	(++)	(+++)
		Keller	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		Killiani's	(+)	(+)	(+)	(+)	(+)	(+)	(+)
		Modified Borntrager's	(+)	(+)	(-)	(+)	(+)	(+)	(-)
04	Flavonoids	Lead acetate	(+)	(+)	(+)	(+)	(+)	(+)	(-)
		Alkali	(+)	(+)	(+)	(+)	(+)	(+)	(-)
		Conc.H ₂ SO ₄	(-)	(-)	(-)	(-)	(-)	(-)	(-)
05	Terpenoids a. Terpene b. Triterpene	CHCl ₃ +Conc. H ₂ SO ₄	(++)	(+)	(+)	(+)	(+)	(-)	(++)
		CHCl ₃ + Acetic anhydride	(+)	(+)	(+)	(+)	(+)	(+)	(+)
06	Steroids	Salkowski	(+)	(+)	(+)	(+)	(+)	(+)	(+)
07	Phenols	FeCl ₃	(+)	(+)	(+)	(+)	(+)	(+)	(-)
		Ammonia	(-)	(-)	(-)	(-)	(-)	(-)	(-)
		Lead acetate	(+)	(+)	(+)	(+)	(+)	(+)	(-)
08	Saponins	Foam	(+)	(+)	(+)	(+)	(+)	(+)	(+)

NB: +++ =Strong, ++ = moderate, + = weak, - = Negative

Our results indicated that the inhibition zone production by the extracts were proportional to the concentration of extracts and local cultivars (JUBS1 and JUBS2) showed rather greater antibacterial activity than of the rest. Al-Janabi & Al-Rubeey (2010) given an acceptable explanation for the antimicrobial activities of *S. melogena* based on the presence of phenolic and alkaloid compounds, especially chlorogenic acid that considered the predominant phenolic compound found in all varieties of brinjals.

Brine Shrimp Cytotoxicity Test (BSCT) is used as a useful tool to screen a wide range of chemical compounds for their cytotoxicities. It has been well utilized to screen out physiologically active compound as well. In cytotoxicity assay, the LC₅₀ values that ranged between 59.91 to 216.45 mg/ml after 6 hours was an indication of poor cytotoxic potential

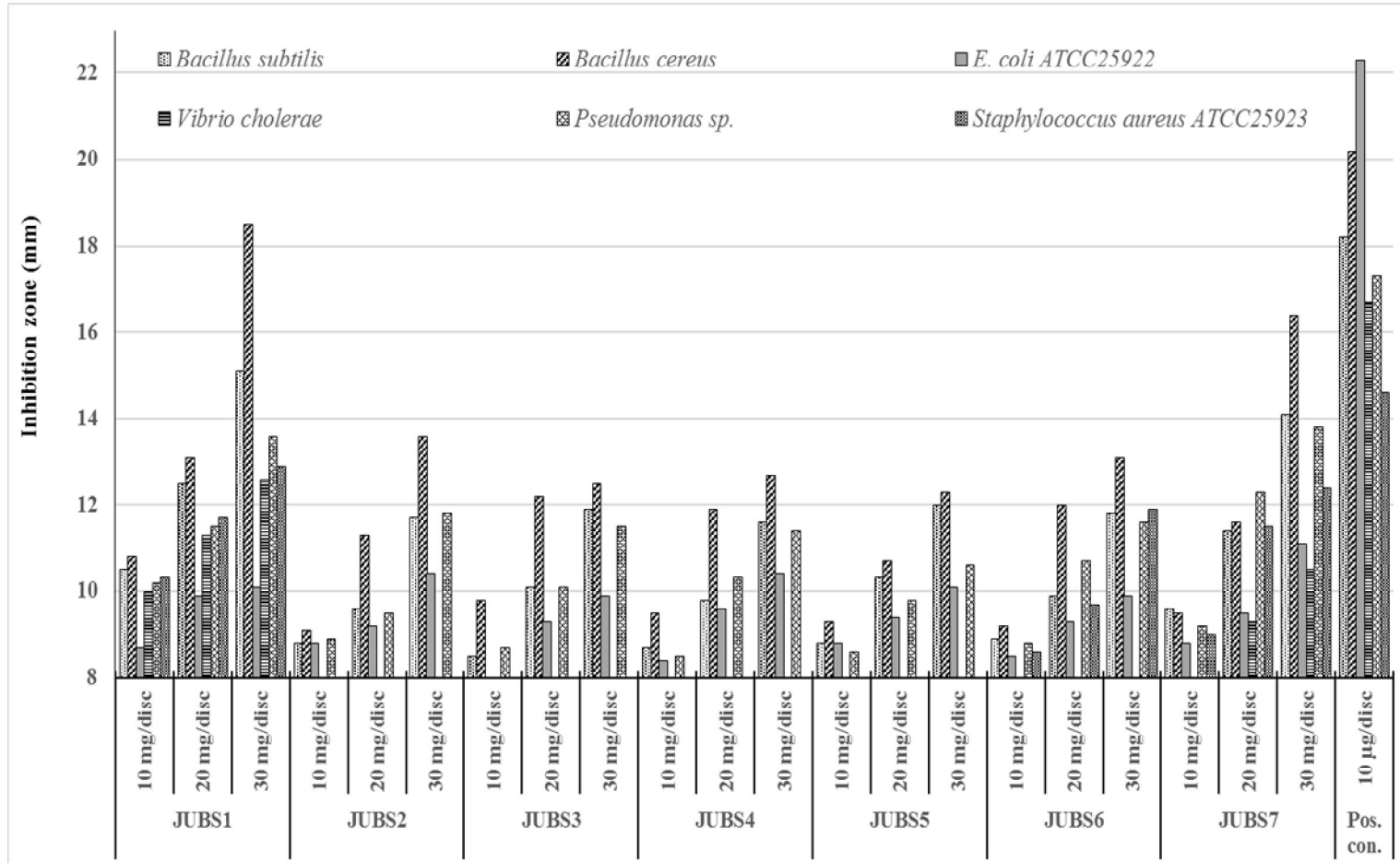


Fig. 1. Antibacterial activities of crude methanolic extracts of seven brinjal (*S. melongena*) samples using disc diffusion method. Positive control= Streptomycin 10 µg/ disc

of brinjal cultivars in comparison to Vincristine Sulphate ($LC_{50} = 0.069$ mg/ml). Concentration dependent increment in percent mortality of Brine Shrimp nauplii produced by the methanolic extract of JUBS7 collected from Mymensingh with $LC_{50} = 59.91$ mg/ml after 6 hours indicates the presence of cytotoxic principles in the extract followed by JUBS5 with an $LC_{50} = 79.16$ mg/ml. However, after 6 hours the LC_{50} values of rest of the samples namely JUBS1, JUBS2, JUBS3, JUBS4 and JUBS6 ranged between 95 mg/ml to 216 mg/ml.

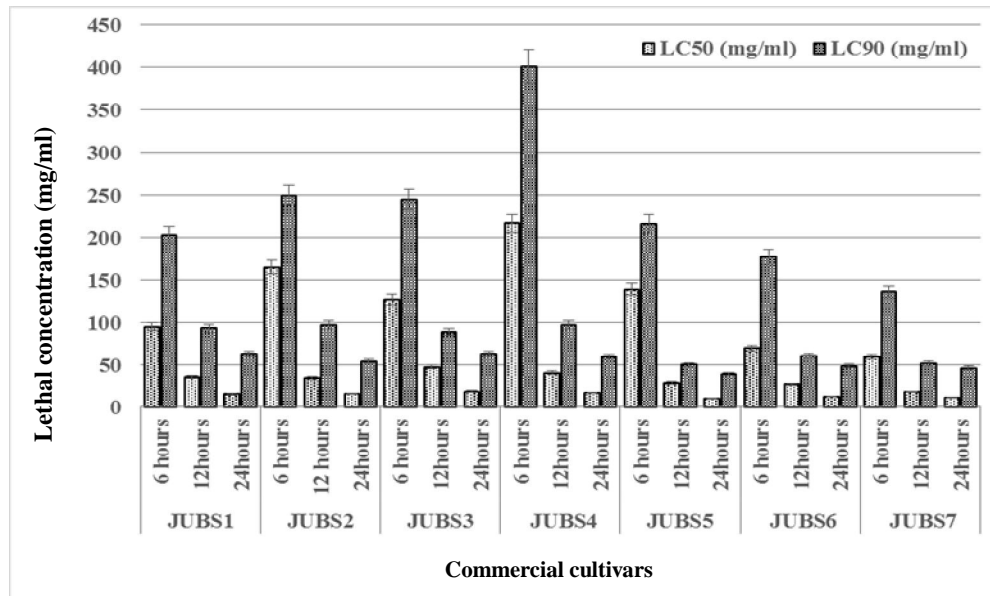


Fig. 2. LC_{50} and LC_{90} value of seven brinjal cultivars after 6,12 and 24 hours

After 12 hours the LC_{50} values of all the samples ranged between 17.88 to 47.38 mg/ml and 11.19 to 18.38 mg/ml after 24 hours respectively (Fig. 2). Dried fruit extract of *Solanum nigrum* have been reported to show cytotoxicity with LC_{50} value of 63.10 μ g/ml following brine shrimp lethality test (Karmakar *et al.*, 2010). According to Bello *et al.* (2005) saponins and tannins have been reported to exhibit cytotoxic effects and growth inhibition making them suitable as tumor inhibiting agents. However, Amash *et al.* (2007) showed that pipit brinjal had cytotoxic effects against MDA-MB-231, CaOV3 and HepG2 with IC_{50} value (concentration causing 50% inhibition of the tumor cell line) of 93.5, 6.15 and 35.4 μ g/ml, respectively.

Antioxidant potential was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. In the present experiment, DPPH free radical scavenging activity was found maximum sample JUBS7 (87.64%) and minimum in sample JUBS5 (80.79%). Nonetheless, rest of the samples namely JUBS1, JUBS2, JUBS3, JUB4 and JUBS6 shown above 80% DPPH free radical scavenging activity that ranged between 81.80 to 87.07% (Fig. 3).

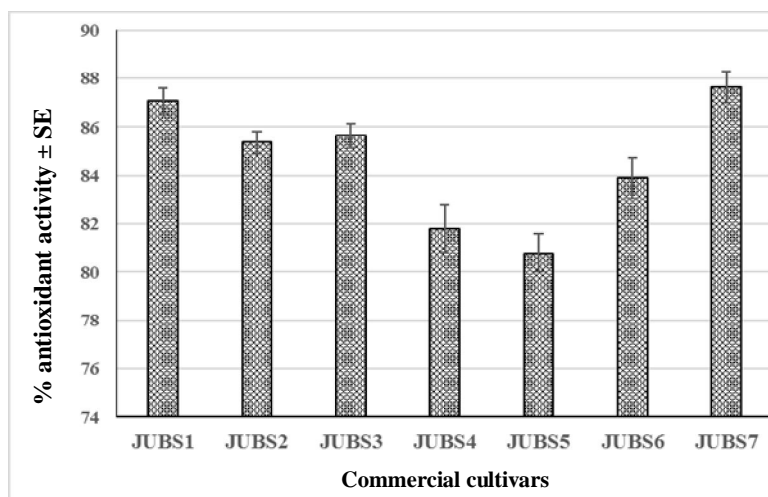


Fig. 3. DPPH free radical scavenging activity of crude extract of seven brinjal cultivars

The present results on DPPH free radical scavenging activity of different cultivars of brinjal corroborates with the findings of Nisha *et al.* (2009). Noda *et al.* (1998) found that extracts from brinjal fruit skin possess high capacity in scavenging of superoxide free radicals and inhibition of hydroxyl radical generation by chelating ferrous iron.

Polyphenols are the large group of phytochemicals that are gaining acceptance as being responsible for the health benefits associated with fruits and vegetables. Because of their chemical structure, plant polyphenols can scavenge free radicals and inactive other pro-oxidants, and also interact with a number of biological relevance. Total phenolic content of the crude extract of brinjal cultivars were determined by Folin-Ciocalteu reagent. Maximum phenolic content observed in this experiment was 9.292 mg GAE/g in sample JUBS7 followed by JUBS1 expressed as 8.312 mg GAE/g (Fig. 4). While minimum amount of phenolic content was recorded in sample JUBS2 (6.821 mg/ GAE/g). The phenolic content varied irrespective to the color and size of fruit. In contrasts to the present findings, Nisha *et al.* (2009) reported that extracts from purple colour small size brinjal fruit demonstrated better antioxidant activities than the other samples which may be attributed to the higher phenolic and anthocyanin content. Shaheen *et al.* (2013) reported that total phenol content (TPC) in different varieties of *Solanum melongena* L. ranged between 3.16 ± 0.04 - 7.86 ± 0.33 mg GAE/g of fresh weight.

The present study suggests that fruits extract of *S. melongena* could be potential source of antimicrobial agent and natural antioxidant. Thus further extensive work on isolation of novel phytochemicals from brinjal extract may lead to find interesting therapeutic compound and future work in this regard is needed.

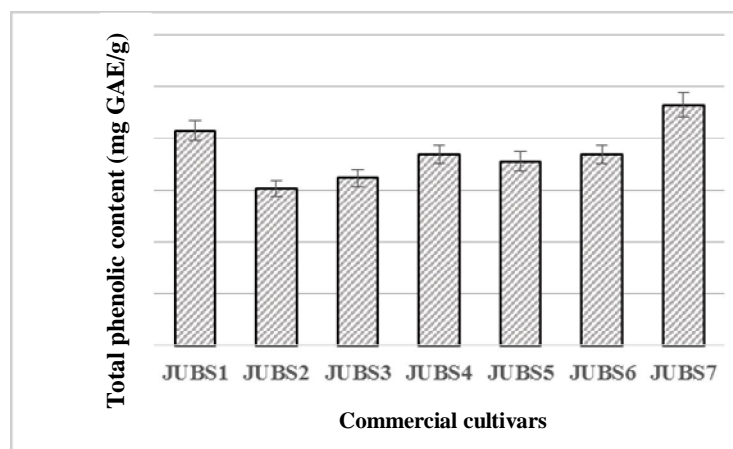


Fig. 4. Total phenolic content of methanolic crude extract of seven brinjal cultivars

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