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PHYTOCHEMICAL PROFILING AND ANTIMICROBIAL ACTIVITY OF BELL PEPPER (*CAPSICUM ANNUM* L.) FROM QUETTA CITY MARKETS

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Abstract

Herbal medicines are highly in demand for primary health care in developing countries, where they are widely used because of their historical use, cheap in use, low cost, and least side effects. This research was performed to evaluate the presence of phytochemicals and study the microbial activities of bell pepper (Capsicum annum L.) purchased from the local market of Quetta city. A qualitative analysis was carried out to determine the presence of metabolites. Results showed that Capsicum annum L. contained phytochemicals like carbohydrates, cardiac glycosides, phenols, tannins, steroids, saponins, terpenoids, and volatile oil whereas, anthraquinones were absent. For the determination of antioxidant activities TAC, DPPH, RP, and FRAP were tested. The antioxidant activity of plant extract was 32.64 gm ascorbic acid/gm of plant extract for TAC test and 5.355 gm of ascorbic acid/gm of plant extract for FRAP test, whereas it was also observed that as the concentration of the plant extract was increased, the RP was also increasing and DPPH test proved this plant extract to be a best free radical scavenger. The disc diffusion method was used to determine the antibacterial activity against two bacteria (Lactobacillus and Escherichia Coli) strains. The plant extract was found to be more effective against Escherichia Coli than Lactobacillus bacteria strains. With the help of proximate analysis, the percentages of moisture content (9.52%), volatile matter (83.52%), ash (4.52%), and fixed carbon (2.44%) were determined.

Keywords: *Antibacterial activities, Antioxidant activities, Phytochemicals, Proximate analysis*

INTRODUCTION

Many developing countries utilize herbal plants as their primary healthcare method because they possess important therapeutic properties. It is reported that in developed countries around 80% of people use herbal medicine for their treatment (1). In Pakistan, several medicinal plants, fruits, and vegetables are used as food and medicine. Fruits and vegetables are famous for their many healthy features. Evidence has proved that medicinal and culinary herbs have some endemic species, which are rich in phytochemicals that minimize cardiovascular diseases and cancer (2). In this research, the most popular worldwide crop bell peppers (*Capsicum annum* L.), which is particularly considered an alien fruit with great nutritional value, has been studied.

Capsicum annum L. is a popular and important spice and vegetable that is cultivated in the subtropical and tropical areas of the world (3). It is used in salads, Chinese dishes and as a vegetable in Pakistan. Its flavor and aroma attracted researchers to explore its nutritional values. It is an herbaceous annual or biennial herb. Its life cycle is divided into four stages: seedling, vegetative growth, fruiting, and flowering. The *Capsicum annum* L. fruit is fleshy large, quadrangular, 7-16 cm in size and 6-11 cm long in width, and weight 100 to 500g. They can also be processed into commercial products such as puree, sauces,

and powders. These are significant due to their high nutritional values, they contain bioactive phytochemicals e.g., carotenoids, flavonoids, phenolics, and other antioxidants (4). *Capsicum annum* L. is found in many colors (orange, yellow, red, and green) depending on its ripening phase and capacity to synthesize carotenoids or chlorophyll. Besides having an unusual flavor, they are an important source of vitamins (pro-vitamins A, E, and C) and they contain various bioactive compounds such as cartenoids and phenols, made them helpful for the health of consumers (European Commission 2015) (5, 6). The *Capsicum annum* L. belongs to the family Solanaceae, subfamily Solanoicleae, tribe Solaneae (4).

Out of more than 26 species of *Capsicum annum* L. only 5 species are cultivated by human. Nowadays, *Capsicum annum* L., grown in regions like China, India, Nigeria, Mexico, Spain, and Turkey, occupies high production of great economic importance (7). The names of 5 commercially cultivated species are *Capsicum annum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens, and Capsicum pubescens* (4). *Capsicum annum* L. has many common English names, including chili, sweet pepper, and hot pepper (8).

"Phyto" meaning "plant" is a Greek word and phytochemicals are natural plant components that are obtained from different parts of plants like roots, barks, seeds, flowers, leaves and fruits (9, 10). Vegetables and fruits are particularly high in phytochemicals and possess health benefits (11). In fruits, vegetables, and grains, nearly 5000 phytochemicals are identified (12). Phytochemicals are naturally occurring bio-active compounds that are found in plants that vary with the geographical origin, growing season, cultivar, maturity, and processing techniques (11). Phytochemicals work with fibers and nutrients to ward off disease (9). Studies showed that medicinal plants are valuable in curing human diseases due to the presence of phytochemicals (13). Many phytochemicals from several chemical classes have been found to have inhibitory effects on almost all types of microorganisms in vitro (10). Extensive research has proved the role of phytochemicals in the prevention of certain degenerative diseases such as cancer, arthritis, and cardiovascular disease (11). Phytochemicals are divided into primary and secondary compounds (14). The primary components that are important for cellular processes are proteins, amino acids, lipids, nucleic acids, and chlorophyll (9). The secondary metabolites or constituents are synthetically or systematically, different compounds with obscure functions (10) as they are produced due to the secondary reactions of primary carbohydrates, amino acids, and (15). In plants, secondary metabolites possess important biological and pharmacological activities, e.g. anti-oxidative anti-toxin, anti-inflammatory, hypoglycemia, and anti-carcinogenic (14). The secondary constituents comprise alkaloids, terpenoids, tannins, phenolic compounds, flavonoids, and many more (13). Plant phenols are a diverse group of compounds, ranging from simple benzoic acids to complex polymeric lagnins via flavonoids. Many studies have shown that phenol has many biological effects that help to prevent coronary heart disease and cancer (16). Tannins have anti-cancer, cardioprotective, stomach-protective, antibiotic, and cholesterol-lowering effects. In addition to this, they also promote urinary tract health (17). Terpenoids have various pharmacological properties like antimalarial, antitumor, antibacterial, and antiviral. They play an important role in attracting valuable ticks and consuming herbivorous insects. Alkaloids present in medicinal plants are used as anesthetics (13). Flavonoids present in *Capsicum annum* L. has strong antifungal, antibacterial, antiviral, anti-inflammatory, antioxidant, and antiallergic activities (18).

An antimicrobial is a substance that inhibits the growth of microorganisms such as protozoa, bacteria, and fungi. Antibiotics are classified into two types: microbicidal, which kills microbes and microbistatic which inhibits their growth (19). Screening of several medicinal plants for potential antibacterial effects is under progress due to the growing frustration towards chemotherapy and antibiotic resistance caused by pathogenic bacterial infections (20) as many synthetic antibiotics have increased the risk of bacterial infections in humans and animals (21). Literature shows that changes in chromosomes made these bacteria resistant to synthetic antibiotics (22), therefore, to find the solution to this problem, search for effective herbal antibacterial compounds got the importance as they possess extraordinary potential sources for viral and microbial inhibitors (20, 23). Reported studies prefer the use of plant extracts not only because of their antiviral, antibacterial, and antifungal activities (23) but also their fewer side effects, less cost, and lower toxicity than drugs. (24). Similarly, natural antibacterial depends significantly on the availability and

abundance of the source of raw materials (21). From various studies, it has been discovered that the peels of fruits and vegetables also have great potential to act as a source of strength, safe, new, and better antibacterial agents (22).

Overall no comprehensive data regarding the phytochemical, antioxidant and antibacterial activities of *Capsicum annum* L. (grown in Pakistan) is available so far. Therefore, the aim of present study is to determine the phytochemical properties, antibacterial activities, and proximate analysis of *Capsicum annum* L. from Quetta city markets.

MATERIALS AND METHODS

This study focused on the phytochemical analysis, proximate analysis, and antibacterial and antioxidant activities of *Capsicum annuum* L. purchased from the local market of Quetta City, Balochistan.

SAMPLE COLLECTION AND PREPARATION OF METHANOLIC EXTRACT

Samples of *Capsicum annuum* L. were obtained from the local market of Quetta City, Balochistan. A total of 500 g of shade-dried plant powder was soaked in methanol for fifteen days at room temperature. The extract was separated from solid residues by filtration and concentrated using a rotary evaporator at 35°C.

PHYTOCHEMICAL ANALYSIS

Phytochemical tests were conducted to identify the presence of bioactive compounds using standard methodologies.

Alkaloids were tested using Mayer's and Wagner's reagents. Presence was indicated by turbidity. Phenols were confirmed by an intense violet color with ferric chloride solution and bulky precipitates with lead acetate solution. Saponins were identified by froth formation after shaking the cooled aqueous solution. Steroids were indicated by a brown ring formation with sulfuric acid in chloroform and acetic anhydride. Terpenoids were confirmed by reddish-brown coloration at the interface with chloroform and sulfuric acid, Tannins were confirmed by bluish-green color formation with ferric chloride solution while coumarins were confirmed by the appearance of yellow color with sodium hydroxide. Glycosides and Carbohydrates were confirmed by bluish-violet zone in Molisch's test and reddish-brown precipitates in Fehling's test. Anthraquinones showed red precipitates with hydrochloric acid and Volatile Oils were showed white precipitates with sodium hydroxide and hydrochloric acid.

PROXIMATE ANALYSIS

The proximate composition, including moisture content (MC), volatile matter (V/M), ash content (AC), and fixed carbon, was determined using ASTM standard procedures.

Moisture Content was determined by ASTM: D3173. Volatile Matter was determined by ASTM: D3175. Ash Content was determined by ASTM: D3174-12. Fixed Carbon was calculated as 100 - (%MC + %V/M + %AC).

ANTIOXIDANT ACTIVITY

Antioxidant activities were assessed using various methods:

- Total Antioxidant Capacity (TAC): Determined by the phosphomolybdenum method, measuring absorbance at 695 nm.
- Iron-III Reducing Antioxidant Power (FRAP): Measured using TPTZ reagent, with absorbance at 593 nm.
- DPPH Radical Scavenging: Evaluated by absorbance at 517 nm, calculating inhibition percentage.
- Reducing Power (RP): Assessed by absorbance at 700 nm after treatment with phosphate buffer, potassium ferricyanide, and ferric chloride.

ANTIBACTERIAL ACTIVITY

The antibacterial activity of methanolic extracts was tested against *Escherichia coli* (gram-negative) and *Lactobacillus* (gram-positive) using the disc diffusion method. Bacterial cultures were grown on Mueller

Hinton agar, and discs with different concentrations (0.5 g, 1 g, and 1.5 g) of plant extract were placed on the agar. After 24-hour incubation, zones of inhibition were measured. Pure DMSO and amoxicillin were used as negative and positive controls, respectively.

This comprehensive approach evaluates the phytochemical properties, nutritional content, antioxidant potential, and antibacterial effectiveness of *Capsicum annum L.*

RESULTS AND DISCUSSION

This study was focused on the investigation of phytochemicals, proximate analysis, and antioxidant and antibacterial activities of C*apsicum annum* L. that is available in the local market of Quetta city, Balochistan.

PHYTOCHEMICAL ANALYSIS

The methanolic extract and fine powder of C*apsicum annum* L. were used to perform phytochemical analysis. Results as shown in Table I, confirmed the presence of various phytochemicals such as Phenols, Carbohydrates, Saponins, Cardiac glycosides, Tannins, Steroids, Terpenoids, and Volatile oil but Anthraquinones were absent. Alkaloids, tannins, and flavonoids are usually responsible for the plant's antibacterial and antioxidant properties. Behlil and his fellows also studied the qualitative phytochemical screening of different methanolic extracts of plants from Balochistan. In that study, they observed the presence of phenols, alkaloids, flavonoids, steroids, saponins, glycosides, fats, terpenoids, volatile oil, carbohydrates, xanthoproteins and the absence of resins, quinones, emodins, anthocyanins, quinones and anthraquinones (25). While, Bashir and his fellows observed the presence of carbohydrates, steroids, glycosides, tannins, flavonoids, phenols, and terpenoids in the powder of banana peel whereas, xanthoprotein and alkaloids were absent in their study (26). Literature and present research showed that phytochemicals are an essential part of most plant extracts and contribute a vital role as an herbal plant.

Table I. Phytochemical analysis of the methanolic extract of *Capsicum annum* L.

Tests	Results
Carbohydrates (Molisch's test)	+
Cardiac Glycosides	+
Phenolic	+
Saponin	+
Steroids	+
Terpenoids	+
Tannins	+
Coumarins	+
Quinones	+
Anthraquinones	–
Volatile oil	–

Plants having phenols show numerous biological, medicinal, and pharmacological properties and could be used as an anti-mutagenic, anti-carcinogenic, anti-inflammation, and anti-allergy substitute. It is also reported that such plants can modify the expression of genes as well (32).

PROXIMATE ANALYSIS

Proximate analysis is an important characterization method, that provides valuable information about the nutritional composition of the raw biofuels. These characteristics are important because moisture, volatile matter, and fixed carbon all have an impact on both the plant design and the combustion property (27). The proximate analysis also aids in determining sample quality and determining the nutritional value of the *capsicum annum* L. (33).

Table II presents the values obtained for proximate analysis of *capsicum annum*. There is a significant difference among the values of moisture content, volatile matter, ash content and fixed carbon contents of the *capsicum annum* L. as shown in Fig. 1. The amount of moisture content for the dried sample varies from 6-11 percent for pyrolysis of biomass as higher moisture because higher moisture content requires excess heat to vaporize water during the thermochemical conversion, affecting the efficiency of the

pyrolysis process and negatively impacting the fuel energy value. Furthermore, high moisture content reduces combustion yield and quality, whereas samples with lower moisture content aid in combustion and are less susceptible to degradation by microorganisms and chemical changes. Determined 9.52%of the moisture content in *Capsicum annum* waste based on the moisture content in *Capsicum annum* L.

Table II. *Capsicum annum* L powder proximate analysis

Samples	Moisture	Volatile matter	Ash	Fixed Carbon
Capsicum annum L.	9.52%	83.53%	4.52%	2.43%

Conversely, the level of volatile matter ranges at 83.53%. Organic materials mainly carbohydrates, fats, and proteins can be a good source of nutrients. Due to the high volatile matter ratio, the *Capsicum annum* L. was much ignited.

Ash is a non-combustible solid material that provides various micronutrients and minerals to the soil. The ash content was recorded as 4.52%. Therefore, low ash and high volatility biomass are best for bioenergy conversions, such as pyrolysis and gasification methods. The fixed carbon content of the *Capsicum annum* L. was 2.43%.

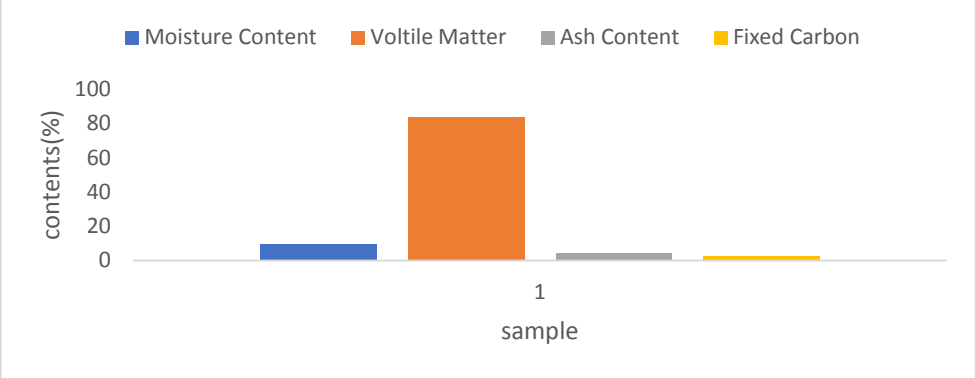

Fig. 1. Proximate analysis of *Capsicum annum* L.

ANTIOXIDANT ACTIVITY

Several factors affect the antioxidant activity, solvent types, nature of the antioxidant, temperature, methanolic extract, extract composition, and extraction method (34). Due to these factors, many methods were used to identify the antioxidant activity of plant extract. This helps in describing various mechanisms occurring in the action of antioxidants (35).

TOTAL ANTIOXIDANT CAPACITY (TAC)

To determine the TAC of methanolic extract of *capsicum annum,* the phospho molybdic method was used. This method involved Mo (VI) which is reduced into a green complex of Mo (V) by using the regression equation ($y = 0.0819x - 0.007$ with $R^2 = 0.9974$, whereas, x is the concentration of ascorbic acid in mg/mL and y is the absorbance). Fig. 2 (a) represents the direct relation between absorbance and concentration by using a regression equation. The measurement of the total antioxidant capacity of *capsicum annum* contained in mg ascorbic was 32.64 gm ascorbic acid /gm of plant extract.

FERRIC (FE-III) REDUCING ANTIOXIDANT POWER (FRAP)

The FRAP in the methanolic extract was calculated using the regression line equation. The regression line equation ($y = 0.2861x + 0.2257$ with $R^2 = 0.9983$, where x is the concentration and y is the absorbance of ascorbic acid in mg/mL of the calibration curve) is shown in Fig. 2 (b). The antioxidant activity of the *Capsicum annum* L. was obtained as 5.355 mg ascorbic acid/ gram of plant extract. The scavenging power of the selected plant sample was related to ascorbic acid used as standard. Through calculation, it is shown that the result of plant extract and ascorbic acid depends on their concentrations.

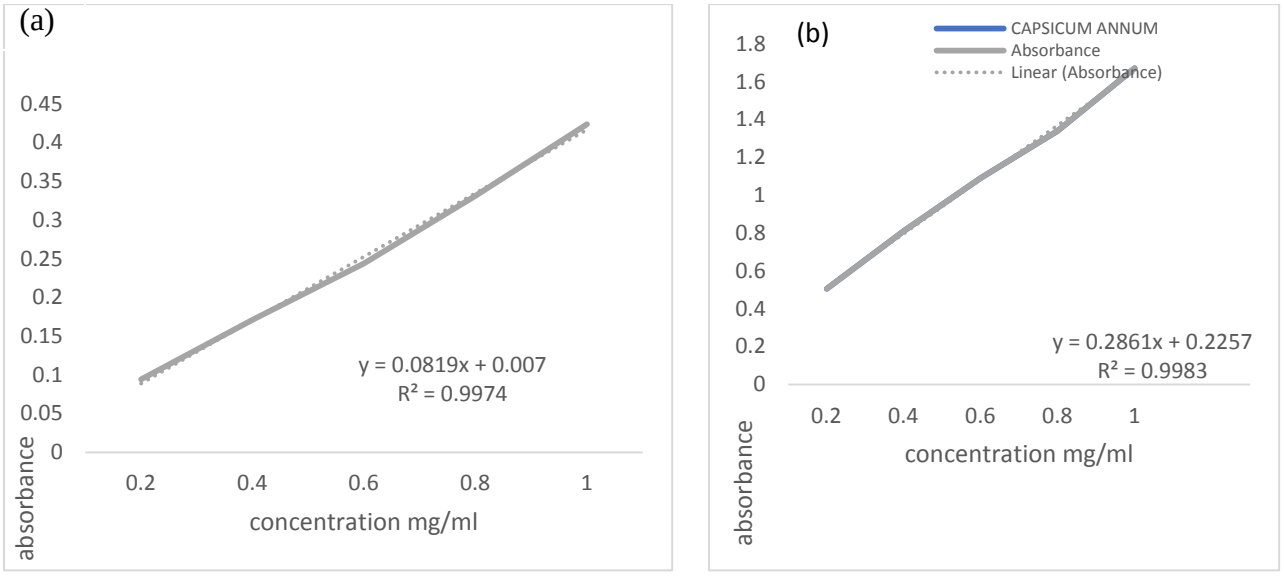

Fig. 2 (a). TAC standard graph **(b).** FRAP standard graph

REDUCING POWER (RP)

The RP of the selected plant sample was found to be associated with increasing the absorbance related to standard ascorbic acid. Fig. 3 shows the relative RP of the samples and standard ascorbic acid. Quantitively and accurately, different methods are used in the determination of the antioxidant activity of plant extract. Like, for the formation of phosphomolybdenum complex, TAC measurement is used. During this process, a green color complex is formed by the reduction of plant extract. It estimates both water and fat soluble antioxidants (36-38). The capacity of a substance to act as a good electron donor and contain antioxidant properties is commonly related to RP. Numerous research works show a correlation between the reducing ability and antioxidant potency of plant extract (39). At the time of determination of reducing potential, methanolic extract of the plant sample showed that increasing the concentration would thus increase the RP (35).

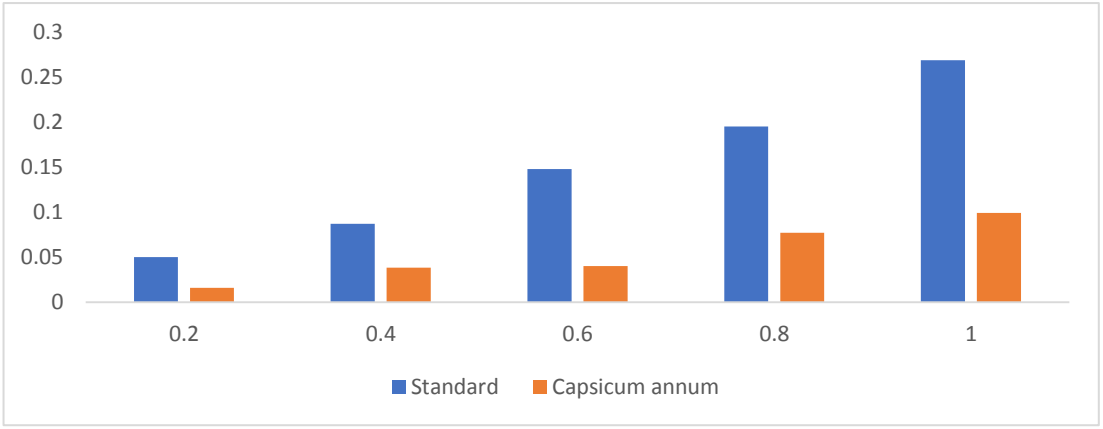

Fig. 3. RP standard graph

FREE RADICAL SCAVENGING POWER ASSAY (DPPH METHOD)

The DPPH method was used to check the antioxidant activity of the selected plant sample at a maximum wavelength of 517nm absorption. Further, the DPPH of the sample and standard ascorbic acid were also calculated (as Shown in Fig. 4). The change in color from purple to yellow was the result of accepting hydrogen atoms and a decrease in absorbance value. The quality of this method is that it can analyze numerous samples in a short period and can also detect active compounds at very low concentrations. The RP of the compound was also described by checking the absorbance value of the extract. The absorbance value and the RP are directly proportional to each other (36). Results showed that the methanolic extract of the selected plant sample is the best free radical scavenger which can prevent auto-oxidation and is also useful for the cure of different diseases.

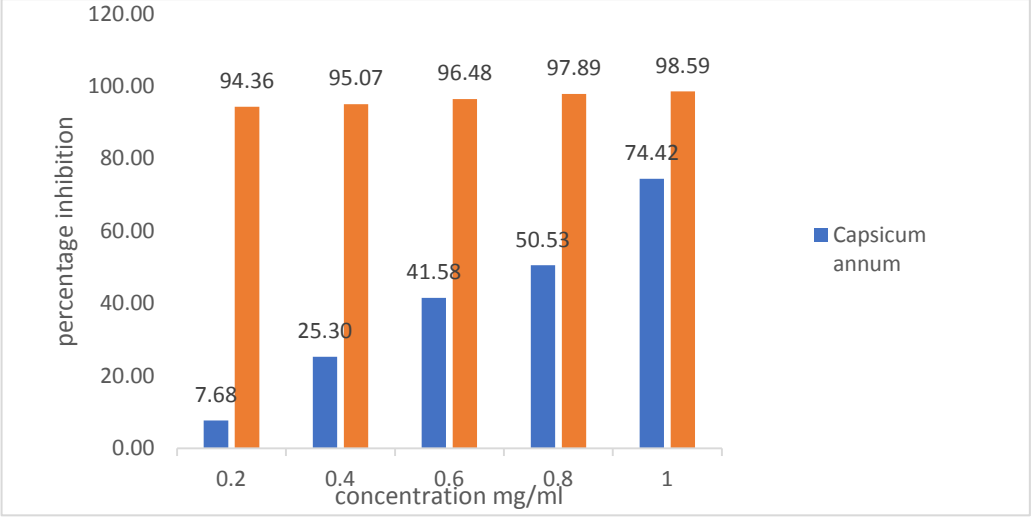

Fig. 4. standard graph of DPPH

ANTIBACTERIAL ACTIVITY

The extract of *capsicum annum* L. was used for antibacterial activity to investigate their bioactive chemical constituents. The activity was carried out by using an Agar disc diffusion method against chosen microorganisms. This method was already used by many researchers to check the antimicrobial of plant extract (23, 28). The activities were compared with the standard antibiotic drug amoxicillin. The methanolic extract of the plant showed positive antibacterial activity against two strains *Lactobacillus* and *Escherichia coli* bacteria. The inhibition zone was measured in millimeters. The crude extract of the plant sample showed remarkable activity against selected strains of bacteria. If both bacteria are compared, it is observed that the highest activity has been shown by *E. coli* as compared to *Lactobacillus* as shown in Fig. 5. Antibacterial activity is increased by the presence of antioxidant compounds due to the inhibition effect of antioxidants against the bacterial cells (23). The plants with high antioxidant activity show high antibacterial activity.

Table III. Antibacterial activity by disc diffusion method (Inhibition zone mm)

Concentration (mg/ml)	*Escherichia coli*	*Lactobacillus*
0.5	64	18
1	98	56
1.5	1.48	86

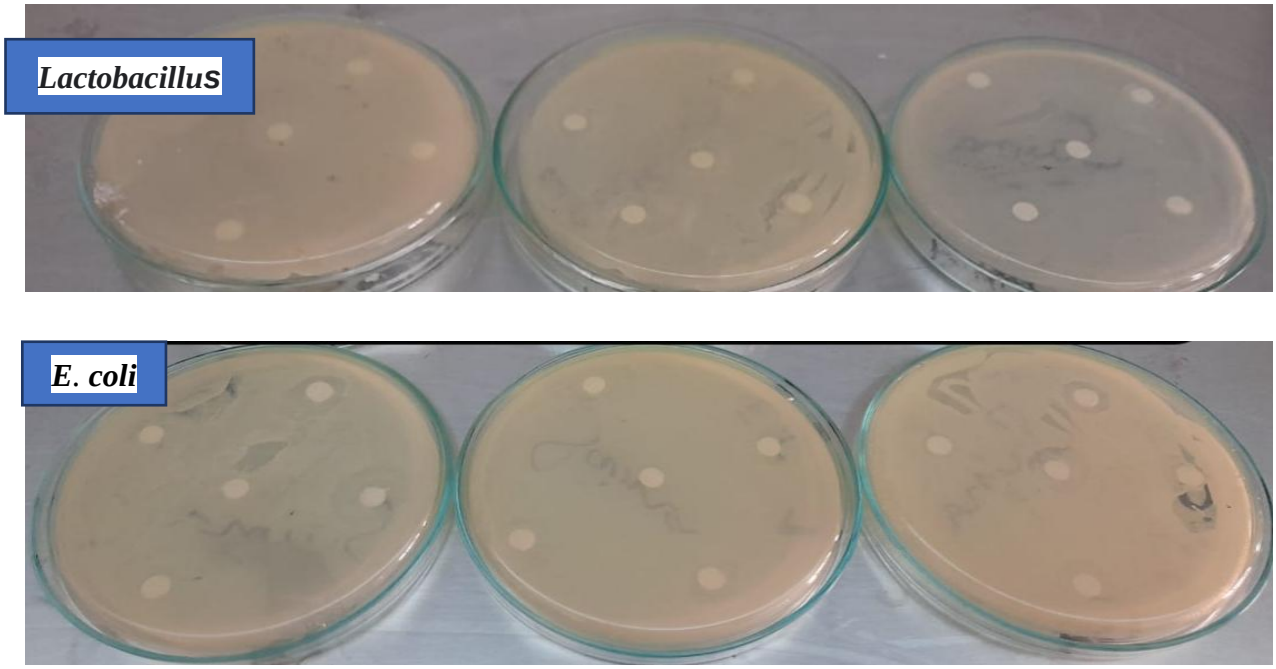

Fig. 5. Antibacterial activity of *capsicum annum* L. (well size 6mm)

CONCLUSION

The results indicated the that *capsicum annum* L. is abundant in phytochemical constituents which could be used in pharmaceutical industries and nutritionally *Capsicum annum* L. is phenol good source of a mixture of antioxidants. It exhibits antibacterial activities against some bacterial pathogens responsible for food borne disease. The presence of alkaloids, flavonoids and polyphenols, etc. make this plant useful in herbal treatment. Moreover, the proximate analysis showed that valuable bioproducts can be prepared from C*apsicum annum* L. due to high volatile matter, fixed carbon content, and low ash and moisture content.

Conflict of Interest:

Authors have no conflict of interest.

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