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ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF OLEORESIN OF CAPSICUM SPECIES

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ABSTRACT

Oleoresin from two varieties (Round and long) of *Capsicum annum* have been extracted by using *n*-hexane and ethyl acetate through Soxhlet apparatus. Antioxidant activity was estimated by using DPPH through UV-Vis spectroscopy and was found to be comparable to reported activity. Antimicrobial activity has been evaluated by using Agar well diffusion method. Antioxidant activity was determined by using DPPH in UV-Visible spectrophotometer and then its %inhibition at different concentrations was measured. At maximum concentration 50µL showed maximum %inhibition about 66.79% for round shaped pepper and 55.67% for long shaped *capsicum annum*. The antibacterial activity of peppers was also tested by disc diffusion method against bacteria; strain such as gram-negative bacteria and it was found that peppers had the ability to inhibit the selected microbes It also presented significant antioxidant and antimicrobial activity.

INTRODUCTION

Capsicum is a genus of family Solanaceae which contain pepper and chilies. In Pakistan, mostly chilies are cultivated in Sindh but also in other provinces including Punjab producing the second most content of total production. Varieties of *capsicum frutescens* and *Capsicum annum* are commonly spelled as chilli in Asia due to their pungent property. While there are some sweet bell peppers are called as capsicums. In American English, it is usually identified as the Chili Pepper or Bell Pepper. In British English, they are all recognized as Peppers (Kappal *et al.*, 2008). Peppers are preferred as a source for pungency, colorant and flavoring. Peppers are used as various ways such as fresh, fermented, dried and as extract of oleoresin.

Peppers can also be used as chopped, whole, finely ground and coarsely ground either in the absence or presence of seeds. There are various products of peppers are used industrially such as chili sauce, pickled fruits, crushed flakes of red pepper, chili powder, paprika, fermented squash and three kinds of extracted oleoresin (Hervert-Hernandez *et al.,* 2010). Antioxidants works as protection for the body cells from the harms of oxidative damage which are caused by species of reactive oxygen and free radicals by inhibiting the formation free radicals, they act this as their substrates and behave as scavengers. *Capsaicinoids* are a chemical group of alkaloid compounds that are formed by fruits and this group of chemicals is responsible for the particular characteristic 'pungency' of Peppers. Different foods contain different type of composition (some of which have quite complex composition) comprising both non-volatile and volatile contents. Some of the components of food are responsible and are the basic reasons for the flavor of foods. Volatile compounds in this regard have earned a special attention of researches because these compounds are responsible for the characteristic aroma and flavor of foods (Pino *et al.,* 2006). Phenolic compounds have many biological properties such as anticancer, antiallergenic, antiproliferative, antimicrobial and anti-inflammatory activities. These compounds are responsible for reduction of lipids, protein and enzymatic oxidation. Many phytochemicals such as eugenol, farnesol, nerolidol, vanillin, tannins, pinene, phenolic acids, flavonoids, isoeugenol, acetyl eugenol, α cububene and β caryophyllene are present in spice. These phytochemicals from spices are not only used in food but also used in food packaging. Lipid oxidation can occur in fat based food packaging and this packaging is important because this lipid oxidation spoil the food (van Ruth *et al.,* 2003). Keeping in view the information regarding phytochemicals and their potential antioxidant and antimicrobial activity, the present study was designed and antioxidant and antimicrobial potential of oleoresin of capsicm annum round and long varieties were evaluated.

Raw material

The dried two verities of *capsicum annum* were purchased from local market of Pattoki. Seeds of both verities were separated. The weight of round chilli seeds was 1040 g and the weight of long chilli seeds was 790g. *capsicum annum* seeds were grinded by using grinder for increasing surface area. These were taken as such used for further processing for the extraction of oleoresin.

Extraction of oleoresin

200g of powdered of *capsicum annum* were placed inside the thimble. The solvents used for extraction were *n*-hexane ad ethyl acetate. *n-hexane* for the removal of oil and Ethyl acetate was used for the removal of active content (colors). These solvents were taken into the round bottom flask. The solvent was heated to reflux for 5 hours. After the extraction product was collected and purified by removing the solvent. Solvent was removed by the process of distillation. The resulted sample was taken in the petri dishes and left under the air for one hour to remove all the solvent. The oleoresin was obtained as a dark red liquid and had strong aroma.

Determination of Antioxidant activity by using 2, 2-diphenyl-1-picryhydrazyl (DPPH)

DPPH solution with methanol is monitored on UV visible spectrophotometer is simple and much effective. The absorbance of DPPH occurred at 517 nm at maximum level. The color change occurred from purple to yellow due to due to absorption from oxidant. Antioxidant activity was determined by the hydrogen donating ability of stable free radical DPPH. Antioxidant activity of oil was determined by DPPH radical scavenging with UV-Visible spectrophotometer.

The DPPH solution was made with methanol as solvent and DPPH as solute. Sample was used to prepare different concentrations of 10 m, 20 m, 30 m, 40 m and 50 m respectively with methanol. After this different concentration of sample were mixed with 3 mL methanol of DPPH solution in different test tube after this, absorbance of blank and resultant solution was determined at 517 nm by using UV Visible Spectrometer at normal temperature under light protected area. After all percentage inhibition was determined by using following equation.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Balnk} - \text{Absorbance of Sample}}{\text{Absorbance of Blank}} \text{x}100$$

Antimicrobial activity of seed oil of *capsicum annum* against different bacteria

Antimicrobial activity was carried out against different confirmed bacterial strains. Gram +ve bacteria were *bacillus substilus*, *Staphylococcus aureus* and gram –ve bacterial strain was *Escherichia coli*. These strains were confirmed on their selective media and on the basis of microscopic analysis and biochemical test i.e. catalase and oxidase test. Antibiotic *Imipenem* was used as a standard against these bacterial strains.

Preparation of muller hinton agar

Weigh 9.5g of Muller Hinton agar and dissolved it in 250ml of distilled water. Auto claved it at 121°C for 15 minutes at 15 lb. After this allows it cool and 20-25ml prepared media was poured into Petri plates within aseptic conditions under laminar air flow. Allowed it to solidify and leave it for 24 hours in laminar air flow to cross check these plates.

Antibacterial activity

Antibacterial activity was performed using agar well diffusion method. In the Muller Hinton agar plate well was created. The cultured bacterium was spread uniformly with the help of swab on the agar by to and fro movement. The antibiotic Tetracycline was placed on the agar and 100 µL of *capsicum annum* was poured into the well. After that the plates were left for 48 hours incubation at 37°C in the incubator. After 48 hours the plates were checked and clear portion around the wells were formed which showed that sample poured into the well has the ability to inhibit the selected microbe. The zone of inhibition was measured for sample and standard in millimeters.

RESULTS AND DISCUSSIONS

Oleoresin was extracted from red chilli (*capsicum annum*) through Soxhlet apparatus by using polar and non-polar solvent combination. Solvent was removed and oleoresin was collected, have deep red color and strong pungency. It was stored in the amber colored bottle for further evaluation. The meal was also collected which was light brown in color and having very faint pungency.

The *capsicum annum* was used for the treatment of many diseases as medicines about thousand years ago. It acts as natural antioxidant for the treatment of oxidative species in our body that decrease the production of oxidative species. DPPH was used as stable free radical for the determination of antioxidant. Naturals and artificial antioxidants convert DPPH free radical into diphenylpicrylhydrazine which was of yellow in color.

In this work antioxidant activity was determined for extract obtained from seed oil of *capsicum annum* by using DPPH. The oil of *capsicum annum* showed strongest antioxidant activity of 87.04% at 50 µg/mL then concentration increases up to 50 µg/mL then % inhibition also increases.

Table 4.3: Antioxidant activity of Round and Long variety.

Sr. #	sample Concentration	Absorbance of round	Absorbance of long	% Inhibition round	% Inhibition long
1	10µL	0.4865	0.5481	41.70	26.03
2	20µL	0.4672	0.5360	48.50	37.22
3	30µL	0.4504	0.5223	53.55	44.32
4	40µL	0.4375	0.5192	59.76	48.80
5	50µL	0.4139	0.5094	66.79	55.67

The oil of *capsicum annum* seed can be used in the treatment of different disease due to its strong antioxidant activity. Oleoresin of *capsicum annum* was prepared by drying and grinding the seeds of *capsicum annum*. Oleoresin was extracted by using Soxhlet type apparatus. Solvents like *n*-hexane and ethyl acetate were used for extraction. Then extract was preserved in fridge for further evaluation.

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Capsicum annum seed extract was tested by diffusion method disc diffusion method against three bacterial strains such as gram positive *Staphylococcus aureus, Bacillus substilus* and gram negative *E. coli*. Oil of *capsicum annum* seed show strong antibacterial activity against gram positive bacteria *Staphylococcus aureus* and gram negative *E. coli* at 37°C.The zone of inhibition of *capsicum annum* seed oil against bacterial species was given below in table:

Table 4.5: Antibacterial activity of oleoresin of *capsicum annum*.

Test Organisms	Colony morphology	Incubation temperature	Culture media	Incubation zone (mm)	Efficacy
Staphylococcus aureus	+ve rods	37 °C	Mannitol salt agar	8	I
Bacillus subtilis	+ve rods	37 °C	Nutrients	-	I
E.coli	-ve coci	37 °C	Mac conkey agar	-	S

R=resistant, S= sensitive and I=intermediate+

Antibacterial activity shown by *capsicum annum* is due to the presence of different alkaloids like piperidine, piperine and volatile resins and oils. Present research has revealed that *capsicum annum* presented higher antibacterial activity against gram positive in compoarison with gram negative bacteria.

Chilli peppers are reported as beneficial antifungal and antibacterial agents. This was preferentially associated with presence of high capsaicin content present in oleoresins from chillies.

CONCLUSION

Two varieties of *capsicum annum* including round and long shaped were purchased from local market of Pattoki. Then these varieties were grounded with grinder and oleoresin was extracted from both these varieties through Soxhlet apparatus by using organic solvent. Antioxidant activity was determined by using DPPH in UV-Visible spectrophotometer and then its %inhibition at different concentrations was measured. At maximum concentration 50µL showed maximum %inhibition about 66.79% for round shaped pepper and 55.67% for long shaped *capsicum annum*. The antibacterial activity of peppers was also tested by disc diffusion method against bacteria; strain such as gram-negative bacteria and it was found that peppers had the ability to inhibit the selected microbes.

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