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RESEARCH ARTICLE

ASSESSMENT OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF CAPSAICIN EXTRACTED FROM CHILI SAMPLES OF NEPAL

Sanjogta Thapa Magar and Rajani Shrestha
Department of Microbiology, St.Xavier's College, Maitighar, Kathmandu, Nepal.

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Abstract

Chilies are widely used as vegetables, spices, and external medicines throughout the world. Capsaicin, the potent alkaloid responsible for the pungent flavor of chilies, has been shown to have antioxidant and antimicrobial properties. In this study, the capsaicin content and antioxidant and antimicrobial activity of five different types of chilies were evaluated, namely Green chilies, Habanero chilies, Jalapeno chilies, Chile chilies, and Green capsicum. Fresh chilies were obtained from the market and divided into two halves. The dried chilies were prepared by sun drying the fresh chilies. Capsaicin was extracted from the samples using methanol and a Soxhlet extraction apparatus, and the presence of capsaicin was confirmed using TLC and chemical tests. The amount of capsaicin present in the samples was quantified using a UV spectrophotometer. The antioxidant activity of the capsaicin extracts was determined using the DPPH scavenging assay, and the IC_{50} values were calculated using Graph Pad Prism software. Capsaicin antibacterial effectiveness was tested against bacterial strains: *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 43888), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella* Typhi (ATCC 14028), and *Listeria monocytogenes* (ATCC 19115). The results showed that fresh chilies had lower capsaicin content than dried chilies, with the capsaicin content ranging from 94.41% to 98.03% for fresh chilies and from 99.59% to 111.12% for dried chilies. Fresh chilies also exhibited higher antioxidant activity than dried chilies, with the IC_{50} values ranging from 18.02 to 94.47 mg/ml for fresh chilies and from 20.86 to 121.40 mg/ml for dried chilies. Capsaicin also showed some level of inhibitory effect on bacterial strains, with the ZOI ranging from 10 to 28 mm depending on chili extracts. Compared to commercial antibiotics, capsaicin was more effective against the tested microorganisms. The rise of antibiotic resistance and the use of artificial food preservatives are both major concerns. Therefore, capsaicin is very useful in the food industry as a preservative but also in the pharmaceutical sector as a replacement for many synthetic antibiotics.

Corresponding Author:- Sanjogta Thapa Magar
Address:- Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu, Nepal.

Introduction:-

Human use of chili dates back to prehistoric times. Chilies are grown almost all over the world, being fairly easy to cultivate in a wide range of climatic and environmental conditions. Since the ancient period time, chili has been known all over the world as a delicious spice with a characteristic smell and taste. It is an integral component of every Nepalese kitchen. Both green and dried chilies are commonly used for various purposes. Chilies are a good source of compounds with biological activities, such as capsaicinoids, phenolics, and antioxidants [2]. Chili is used for flavoring in food manufacturing and colorants, sauces, and pickles. Chili varieties are also used as active ingredients in cosmetics industries, pharmaceuticals industries, and pest management [3]. Chili exhibit capsaicinoids, carotenoids, and phenolic compounds[4]. Capsaicin, a chemical component of chili is one the pungent capsaicinoids found in chili, has already demonstrated a high degree of biological activity affecting the nervous, cardiovascular, and digestive systems. Chemical analysis has demonstrated that chili contains relatively high concentrations of several essential nutrients, including vitamin C[5]. It has been found that capsaicin extracts possess both antioxidant and antibacterial properties, which is of great interest as natural additives both in academia as well as the food industry since there is a tendency to replace synthetic antioxidants with natural ones [6]. Today's consumer demands for more natural and fresh-like food without the addition of chemical preservatives are increasing. Hence capsaicin extract might be useful in food industries as a natural preservative as well as an antioxidant for eliminating free radicals and in pharmacy industries to use as an antibiotic compound against pathogenic production [7–9].

Method and Materials:-

Study design

The evaluation of capsaicin showing antioxidant activity and antibacterial extracted from chilies was conducted using a random sampling method.

Study period and site

This study was carried out in Pharmaco Industries Pvt. Ltd., Ramkot, Sitapaila, Kathmandu, and Mithula Analytics Pvt. Ltd., Baluwatar, Kathmandu from the period of April 2019 to February 2022 to extract the capsaicin content of the chilies and determine the antioxidant and antibacterial property of capsaicin.

Sample size

Based on ICMSF (International Commission on Microbiological Specifications for Foods), five fresh chilies and five dried chilies were taken. Each sample was processed thrice for triplicate data[10].

Sample used

The five different chilies samples used in this study are Green chilies, Habanero chilies, Jalapeno chilies, Chile chilies, and Green capsicum.

Sample collection

The fresh chilies were collected from the Kalimati vegetable market, Kalimati, and Balkhu vegetable market, Balkhu. A good shape, texture, color, and fresh smell of chilies were chosen and the bruised or damaged were eliminated. After purchasing, the fresh chilies were then divided into halves. To prepare dried chili, fresh chilies were washed and deseeded. They were distributed in stainless steel trays and dried under direct sunlight at temperatures in the range of 25–38°C for 2 weeks [11].

Capsaicin extraction

Capsaicin in chili was extracted using methanol as solvent. The fresh chili was ground to a paste and the dried chili was ground to powder form [12]. 20 g powder of the samples was mixed with organic solvents (methanol) for capsaicin extraction using the Soxhlet apparatus. The apparatus will be set up and the extraction was carried out extraction until the chili color appeared in the percolated methanol at 65ºC. After extraction, the solvent was evaporated from the extracts. The remaining traces of extracts were further dried in an oven at 40℃[13–15].

Confirmation test of capsaicin

The Mayer's Test was performed to determine the presence of alkaloids i.e. capsaicin. To a few drops of Mayer's reagent, 2 mg of prepared extract was added. A yellow precipitate confirmed the positive result of Mayer's test. It was expected to be a complex of potassium-alkaloid. In the formation of Mayer's reagent, the mercury (II) chloride solution was added to potassium iodide and produced a red precipitate of Mercury (II) iodide. The excess of potassium iodide leads to the formation of potassium tetraiodomercurate (II). Alkaloids consist of nitrogen atoms that have lone-pair electrons. The lone pair electrons are examined to form covalent coordinate bonding with metal ions. In alkaloid identification, the nitrogen in alkaloids would react with the metal ion of potassium (K+) from potassium tetraiodomercurate (II) to produce a precipitating complex of potassium-alkaloid. The formation of white or pale yellow precipitate indicated the presence of alkaloids [16].

TLC analysis

The standard capsaicin and chili extracts were spotted on a silica gel aluminum sheet using petroleum ether: chloroform: acetonitrile as the mobile phase and viewed under the UV light at 302 nm [16,17].

Quantification of Capsaicin extracted from different chilies

1 mg of the standard capsaicin was weighed accurately and transferred to a 1000 ml volumetric flask. It was dissolved in methanol to obtain a final concentration of 1 mg/ml as a stock solution and different concentrations were prepared from 50 mg to 400 mg. The simple linear regression graph was constructed using standard capsaicin. To determine the amount of capsaicin extracted from chilies, a UV-visible spectrophotometer was used. The optical density was determined at 280 nm. Based on linear regression equations, the contents of samples were calculated as micrograms per millimeter of capsaicin [13,18,19].

Antioxidant assay

The free radical scavenging capacity of the standards and samples was determined by using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. Sample solutions of 100–400 mg /ml were prepared in methanol and a 0.5 ml aliquot was added to a tube containing 1.5 ml of DPPH solution (The reaction mixtures were kept in the dark for 60 min. The absorbance was measured using a spectrophotometer at 517 nm. A DPPH solution (1.5 ml) and methanol (0.5 ml) were used as the negative control. Ascorbic acid and capsaicin were used in five concentrations (100 to 400 mg/ml) in methanol to obtain the calibration curves.

Based on the ability of capsaicin extract to scavenge DPPH free radicals, the inhibitory percent of DPPH was determined using the formula:

$$\%\mathbf{AA} = [1 - (A_1 - A_2)/A_0)] \times 100$$

Where,

- % AA is the antioxidant activity,
- A_0 the absorbance of the negative control (original DPPH solution without sample),

•A_1 the absorbance of the test sample (DPPH solution in the presence of sample), and
•A_2 is the absorbance of the sample without DPPH.

The relationship between DPPH inhibition percentage and equivalent sample concentration was used to determine the half-inhibitory concentration (IC_{50}) value of each sample in Prism Graph Pad [20,21].

Methanol residue test

All 10 chili samples were tested for methanol residue since methanol could lead to false positive results during antibacterial tests. To perform this test, 0.5 ml of standard (126 µl methanol and 10 ml distilled water) and dilution samples (100 µl and 10 ml distilled water) were added to a test tube. 50 µl of 0.5 M sulphuric acid and 0.1 M Potassium permanganate solutions were added to the testing solutions. After 5 minutes, an examination was continued by adding 50 µl of 0.15 M sodium hydrogen sulfite solution, and the samples were hardly shaken for a full discoloring mixture. Then, 50 µl of 0.025 M chromotropic acid solution and 1 ml of concentrated sulfuric acid were added to all testing samples respectively, and shaken well. The result of the reaction was observed after five minutes. The negative results were concluded if the mixture remained colorless, while positive results were concluded if purple appeared at every intensity. Afterward, the obtained positive color was compared with standard test tubes for estimating the methanol content of each sample [22].

Antibacterial activity test of extracted crude capsaicin

Chili extracts were suspended in dimethyl sulfoxide (DMSO) to obtain stock concentrations of 100 mg/ml (Loizzo et al 2017). The antibacterial activity was checked by a well diffusion assay. 20 ml of sterilized MHA was poured into sterile Petri plates, and after solidification, a fresh culture of ATCC culture i.e. *Staphylococcus aureus* (ATCC 6538), *Escherichia coli*(ATCC 43888), *Pseudomonas aeruginosa*(ATCC 9027), *Salmonella*Typhi (ATCC 14028), and *Listeria monocytogenes*(ATCC 19115) were swabbed on the respective plates. The wells were then punched over the agar plates using a cork of each capsaicin extract that will be added to the wells. All the plates were incubated for 24 hours at 37°C. After incubation, the diameter of inhibitory zones formed around each disc was measured (mm) and recorded [23,24].

Antibacterial activity test of antibiotics

The sterile swab was dipped into the inoculum tube. The swab was rotated against the side of the tube to remove excess inoculums. Streaking on the MHA plate was done by rotating the plate approximately 60º each time to ensure an even distribution of the inoculums. At last, swabbing was done around the edge of the agar to remove any excess moisture. The plates were allowed to set for 3-15 minutes before applying disc namely Cefixime (5mcg), Erythromycin (15 mcg), Azithromycin (15mcg), Chloramphenicol (30 mcg), Ceftazidine (30mcg), Gentamicin (10mcg), Cefotaxime (30mcg), Nalidixic acid (30mcg), Ciprofloxacin (5mcg), Vancomycin (10 mcg), Clindamycin (2 mcg), Ceftriaxone (30 mcg), and Ampicillin (10 mcg). The antibiotic disc was applied to plates using sterile forceps and the disc was gently pressed to ensure complete contact of the disc in agar. Then the plates were inverted and incubated at 37°C within 15 minutes of disc application. The zone was measured using a ruler and zones were interpreted using the guidelines provided by the CLSI. The result was interpreted as resistant (R), intermediate (I), or susceptible(S) [25].

Data analysis

All analyses were performed in triplicate, and the results were expressed as means±standard deviation. The results were analyzed using statistical software, and the means were accepted as significantly different at a 95% confidence interval ($P<0.05$). Pearson's correlation and ANOVA test were done to find out the correlation between the two variables using SPSS software. IC_{50} values were calculated by plotting the percentage of inhibition versus concentrations using Prism Graph Pad. Further, data were analyzed using Microsoft Excel.

Results:-

Linearity curve of standard Capsaicin

A linear graph of absorbance and concentration of standard capsaicin in mg was plotted and the R^2 value was found to be 0.9999. The regression equation of the calibration curve was $y=0.0032x+0.003$.

Quantification of extracted capsaicin from fresh and dried chilies

The capsaicin content of five varieties of fresh and dried chilies was determined. Among five chilies, Habanero chili showed high content of capsaicin in both fresh and dried form as compared to the other four chilies i.e. 98.03±2.33 mg/ml and 111.12±1.40 mg/ml respectively whereas Chile chili showed the lowest capsaicin content as compared to another sample i.e. 94.15±0.82 mg/ml and 99.59±1.08 mg/ml respectively.

DPPH radical scavenging activity

The antioxidant activity of capsaicin from fresh and dried chilies was determined by DPPH radical scavenging activity using ascorbic acid as a standard as shown in Table 2. The maximum inhibition of DPPH free radicals was shown by fresh Habanero chilies and the lowest inhibition of DPPH free radicals was shown by Chile chili.

IC_{50} (inhibitory concentration) value

IC_{50} value of the extracted capsaicin samples was calculated by using Graph pad Prism software. All samples including standard capsaicin IC_{50} value were found to be higher than the IC_{50} value of ascorbic acid.

The antibacterial property of extracted crude capsaicin

The inhibitory effect of all extracted capsaicin from various fresh and dried chilies concerning a variety of ATCC organisms such as *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 43888), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella* Typhi (ATCC 14028), and *Listeria monocytogenes* (ATCC 19115) was determined by the technique of agar well diffusion. The results show the presence of antibacterial activity in all chili samples.

Antibacterial activity test of antibiotics

The antibacterial activity of various antibiotics disc namely, Cefixime (5mcg), Erythromycin (15 mcg), Azithromycin (15mcg), Chloramphenicol (30 mcg), Ceftazidine (30mcg), Gentamicin (10mcg), Cefotaxime (30mcg), Nalidixic acid (30mcg), Ciprofloxacin (5mcg), Vancomycin (10 mcg), Clindamycin (2 mcg), Ceftriaxone (30 mcg), and Ampicillin (10 mcg) was conducted against a variety of ATCC organisms such as *Staphylococcus aureus* (ATCC 6538*), Escherichia coli* (ATCC 43888), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella* Typhi (ATCC 14028), and *Listeria monocytogenes* (ATCC 19115). The zone was measured using a ruler and zones were interpreted using the guidelines provided by the CLSI. The result was compared with extracted capsaicin.

Discussion:-

This study was conducted at Pharmaco Industries Pvt. Ltd. and Mithula Analytics Pvt. Ltd. in Kathmandu from April 2019 to February 2022. Fresh chilies, including Green chilies, Habanero chilies, Jalapeno chilies, Chile chilies, and Green capsicums, were collected from Kalimati and Balkhu vegetable markets. The chilies were dried under direct sunlight for 2 weeks to obtain dried chilies. The capsaicin content of the chilies was extracted to determine their antioxidant and antibacterial properties. The Soxhlet extraction method with methanol as a solvent was used to extract capsaicin from 20 g of coarsely ground chilies. Extraction continued until no more color was visible in the methanol. The extracted capsaicin showed a creamy pale yellow precipitate, indicating its presence according to Mayer's test. The extracts were then qualitatively analyzed using thin-layer chromatography and were resolved on a TLC plate viewed under UV light at 308 nm, similar to previous studies.

Capsaicin concentrations in methanol extracts were measured using UV spectrophotometry. Habanero chili exhibited higher capsaicin content (98.03±2.33 mg/ml) than Chile chili (94.15±0.82 mg/ml), consistent with prior research by [26]. Pearson's correlation coefficient between absorbance and capsaicin standard was found to be 1, indicating a total positive correlation. The capsaicin content of dried chilies was significantly higher than that of fresh chilies ($P<0.05$), with dried chili content being 4 to 7 times higher than fresh chili, consistent with prior research by [27]. This is attributed to the dehydration of the chili matrix and improved extractability of capsaicin by cell disruption during the thermal process, as reported by [1].

A DPPH method was used to determine the antioxidant activity of capsaicin. The method involved using the free radical DPPH and observing its reduction on a UV spectrophotometer at 517 nm. Ascorbic acid (vitamin C) was used as a positive control in the study. The percentage of all chili extract DPPH inhibitors was compared with ascorbic acid, and it was found that the free radical inhibitor activity of all chilies extract was lower than that of ascorbic acid. This result is consistent with a previous study by [20].

The antioxidant assay's results are normally expressed as IC_{50} values (Half maximal Inhibitory Concentration) which represents the concentration of the sample that is capable of scavenging 50% of the DPPH. It is said that the free radical scavenging (antioxidant activity) of the sample is inversely proportional to its IC_{50} value. That means the sample will require less amount in scavenging the free radical if the IC_{50} value is less or vice versa. The lower the IC_{50} value, the higher the antioxidant activity of the samples. All assays were analyzed in triplicate and the results are expressed as means ± standard deviation of various extracts for different antioxidant assays. IC_{50} values were calculated by plotting the percentage of inhibition versus concentrations using Graph Pad Prism 7.05.

Fresh and dried chilies showed significant antioxidant activity. The Habanero chili exhibited the highest antioxidant activity among fresh chilies, and the dried Jalapeno chili showed the highest among dried chilies. All chili extracts had IC_{50} values within the range of intermediate to strong antioxidant activity. The capsaicin extracts are good antioxidant agents and can delay or prevent free radicals and inhibit oxidation. However, the length of the sample extraction process and storage of the samples can affect the outcome

Natural compounds are being investigated as alternative antibacterial agents to combat multidrug-resistant bacteria. The inhibitory effect of the samples was tested using the agar well diffusion technique against various bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* Typhi, and *Listeria monocytogenes*. All 10 extracted crude capsaicin samples showed activity against all isolates. Controls were run in parallel to ensure the inhibition was due to the extract and not the solvent. This study contradicts the findings of [28]. The results of the study showed that all the tested chilies exhibited significant inhibitory effects against pathogenic bacteria. Specifically, the 100 μg/ml concentration of crude extract from all the chilies samples inhibited the growth of all the tested bacteria. These findings were consistent with several previous studies, including [29–33]. These results suggest that chilies may be a promising natural source for developing antibacterial agents to combat multi-drug-resistant bacterial infections

In addition to the effectiveness of the extracted crude capsaicin, a comparison was made among different types of chilies. It was found that dried Habanero chilies showed higher antibacterial activity against all pathogenic bacteria than other types of chilies. This suggests that the drying process may enhance the antibacterial properties of chilies.

Moreover, a significant difference was observed between the antibacterial activity of capsaicin extracted from fresh and dried chilies. The ANOVA test showed that the p-value was less than the α-value of 0.05, indicating that there were significant differences among them. This finding highlights the importance of considering the processing methods when studying the antibacterial properties of chilies.

The study also analyzed the antibacterial activity of the crude capsaicin extracts by comparing the diameter of the growth inhibition zone with that of commonly used antibiotics. The antibiotics tested included Cefixime, Erythromycin, Azithromycin, Chloramphenicol, Ceftazidine, Gentamicin, Cefotaxime, Nalidixic acid, Ciprofloxacin, Vancomycin, Clindamycin, Ceftriaxone, and Ampicillin. The zone of inhibition (mm) was compared with the Antibacterial Disk Diffusion Zone Interpretation Guide chart.

Interestingly, the results showed that *Staphylococcus aureus* (ATCC 6538) was the most susceptible bacteria to antibiotics, while *Pseudomonas aeruginosa* (ATCC 9027) was the most resistant bacteria to antibiotics. These findings were contrary to a previous study conducted by [30].

The study found that there was no significant difference in the zone of inhibition of capsaicin extracted from fresh and dried chilies, as well as different antibiotics, against *Staphylococcus aureus, Escherichia coli, Salmonella* Typhi, and *Listeria monocytogenes*. However, there was a significant difference in the zone of inhibition against *Pseudomonas aeruginosa*. These results suggest that the crude capsaicin extracts have a comparable or greater antibacterial effect than the tested antibiotic.

Conclusion:-

The Habanero chili pepper had the highest capsaicin content, with values of 98.03±2.33 mg/ml for fresh and 111.12±1.40 mg/ml for dried. The Jalapeno chili and Green chili had similar capsaicin content when fresh, but the Jalapeno chili pepper has a slightly higher capsaicin content when dried. The Chile chili pepper and Green capsicum had lower capsaicin content compared to the Habanero and Jalapeno chili, and their capsaicin content was higher when dried. The drying process tends to concentrate the capsaicin in chili. The Habanero chili had the highest DPPH scavenging activity (above 90% at all concentrations tested) among the chili pepper samples tested. The Green chili, Jalapeno chili, and Chile chili had intermediate activity (75-90% at the highest concentration tested), while the Green capsicum had the lowest activity (below 75% at the highest concentration tested).

The Habanero chili had the highest antioxidant activity (lowest IC_{50} value of 18.02 and 20.86 mg/ml for fresh and dried, respectively) among the chili pepper samples tested. The Green chili, Chile chili, and Green capsicum have lower activity, while the Jalapeno chili pepper had intermediate activity. Antioxidant compounds have advantages that include preventing cell damage, lowering inflammation, enhancing the immune system, and enhancing cognitive function.

However, it was discovered that when compared to different commercial antibiotics, the capsaicin concentration of diverse chili samples has the strongest antibacterial action against four bacterial strains*: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*, and *Salmonella* Typhi. The zone of inhibition, measured in millimeters (mm), ranged from 10 mm to 28 mm depending on the sample and bacterial strain. Since antibiotic resistance and the use of chemical preservatives in food are huge problems. Capsaicin can be very useful in food industries as a preservative and also can replace many artificial synthetic antibiotics in pharmaceutical industries.

Recommendation:-

There are various solvents available for the extraction and isolation of capsaicin from chilies.

There are various other methods of extraction to extract capsaicin from chilies.

Various capsaicinoid components, as well as the capsaicin of chilies, can be identified and further tests can be carried out.
Purification methods like HPLC-MS can be used for crude extracts.

Comparing the efficacy of extraction in terms of bioactive components and antimicrobial components with different organic solvents in different proportions can be evaluated.

Research on the bioactive compounds present in chilies that act as antioxidants.

It is recommended that the capsaicin extract from the chilies can be further tested for its antimicrobial property against various microorganisms.

Tables and Figures

Figure 1:- Calibration curve of capsaicin standard in methanol.

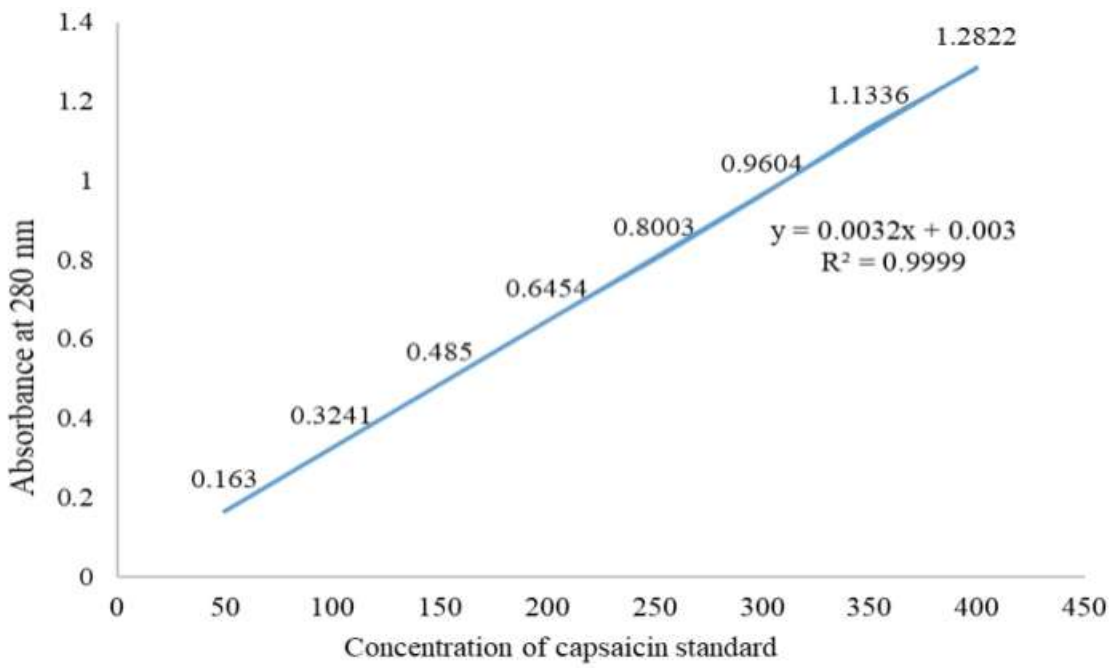


Table 1:- Quantification of Capsaicin extracted by UV spectrophotometric analysis using standard linearity curve graph.

Sample	**Capsaicin content (mg/ml)**	
	Fresh	**Dried**
Green chili	94.41±1.86	102.07±0.58
Habanero chili	98.03±2.33	111.12±1.40
Jalapeno chili	94.24±1.51	99.94±0.33
Chile chili	94.15±0.82	99.59±1.08
Green capsicum	94.47±0.56	100.31±0.46

Figure 2:- Comparison of crude capsaicin extract of fresh and dried chilies content obtained from linearity curve method.

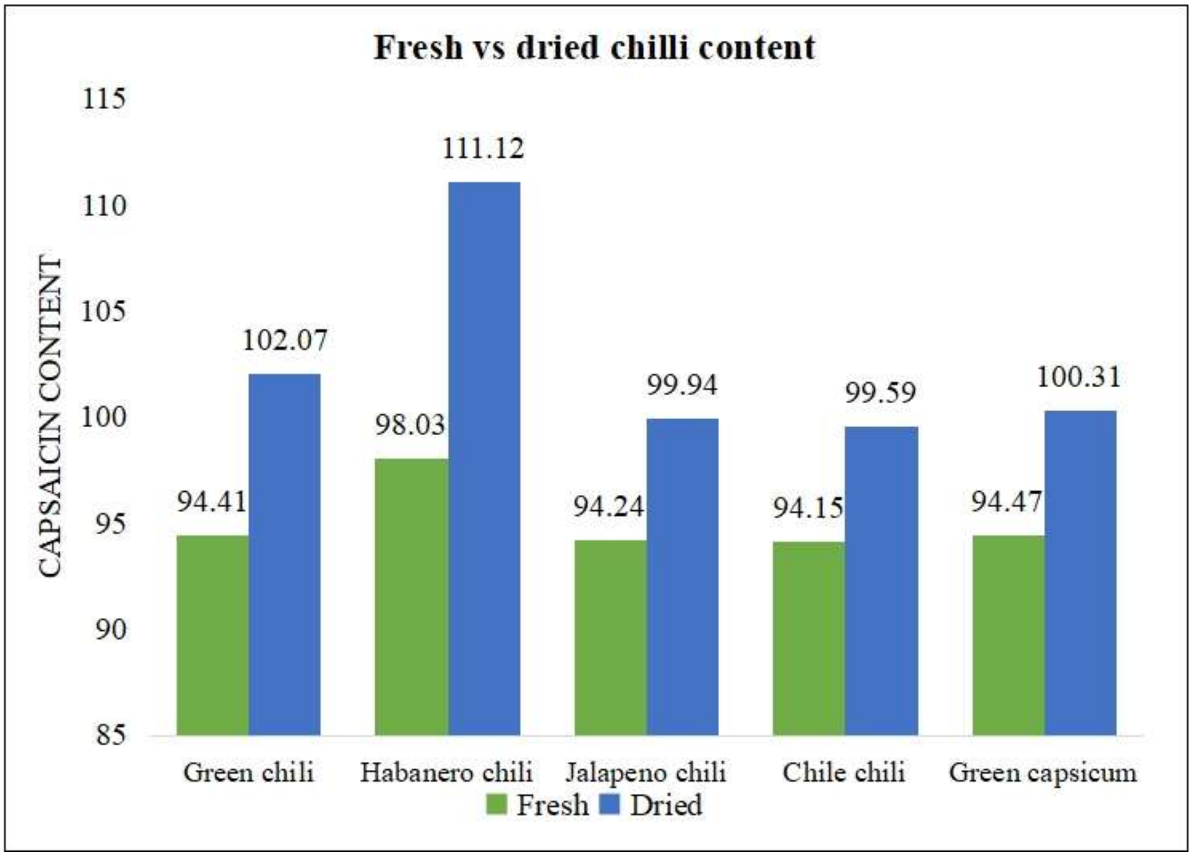


Table 2:- Antioxidant effect of capsaicin on DPPH radicals.

Sample	DPPH scavenging % at different concentrations (mg /ml)			
	400	300	200	100
Ascorbic acid	97.49±0.01	97.42±0.05	97.15±0.10	96.33±0.01
Capsaicin standard	99.01±0.07	92.49±6.14	88.62±5.75	80.46±0.01
Green chili (Fresh)	89.24±0.03	79.57±0.07	74.84±0.04	72.30±0.06
Green chili (Dried)	77.64±0.02	69.69±0.40	62.65±0.01	56.29±0.07
Habanero chili (Fresh)	97.57±0.03	89.02±0.06	83.40±0.22	77.42±0.04
Habanero chili (Dried)	89.80±0.03	81.35±0.56	79.42±0.02	71.87±0.02
Jalapeno chili (Fresh)	96.40±0.08	88.05±0.05	82.93±0.04	77.12±0.05
Jalapeno chili (Dried)	94.50±0.12	85.62±0.03	76.98±0.01	70.30±0.04
Chile chili (Fresh)	92.63±4.16	86.22±0.01	79.35±0.03	74.55±0.01
Chile chili(Dried)	84.75±0.11	84.60±0.05	74.88±0.04	71.28±0.01
Green capsicum (Fresh)	75.83±0.62	63.75±0.02	57.85±0.04	52.35±0.01
Green capsicum (Dried)	72.50±0.04	63.15±0.02	55.94±0.04	48.14±0.01

Figure 2:- Comparison of DPPH inhibitor percentage of various fresh and dried chili extracts with Ascorbic acid.

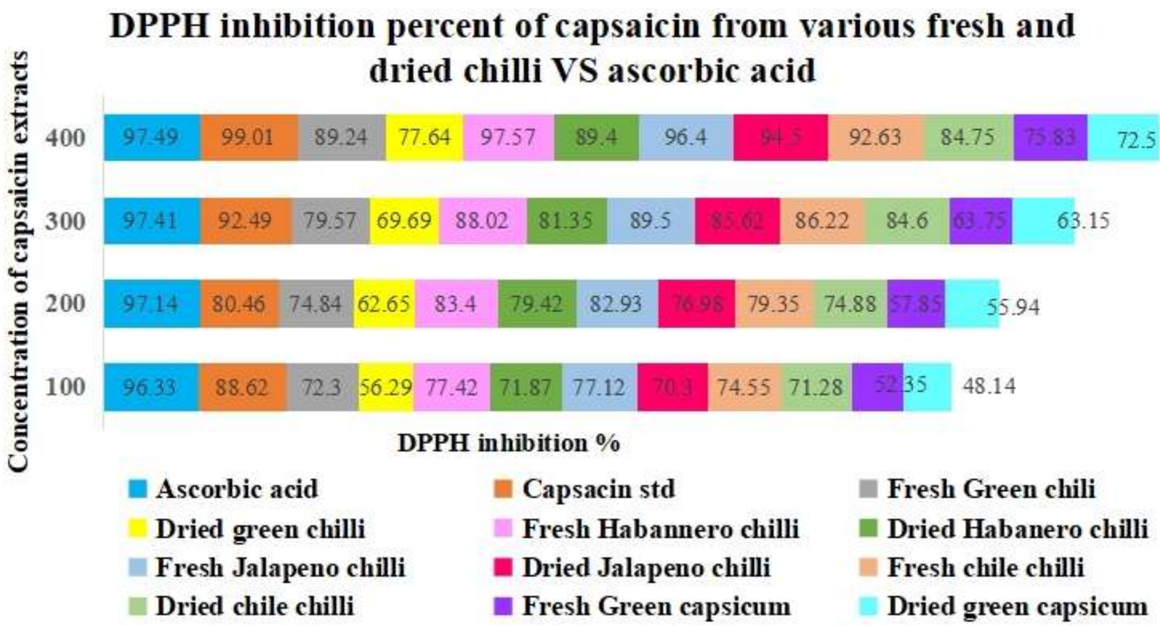


Table 3:- IC_{50} (inhibitory concentration) value of DPPH radical scavenging activity.

Sample	**IC_{50} value of DPPH radical scavenging activity (mg/ml)**
Ascorbic acid	12.14
Capsaicin standard	24.59
Green chili (Fresh)	19.43
Green chili (Dried)	72.74
Habanero chili (Fresh)	18.02
Habanero chili (Dried)	20.86
Jalapeno chili (Fresh)	28.79
Jalapeno chili (Dried)	44.09
Chile chili (Fresh)	32.54
Chile chili (Dried)	33.02
Green capsicum (Fresh)	94.47
Green capsicum (Dried)	121.40

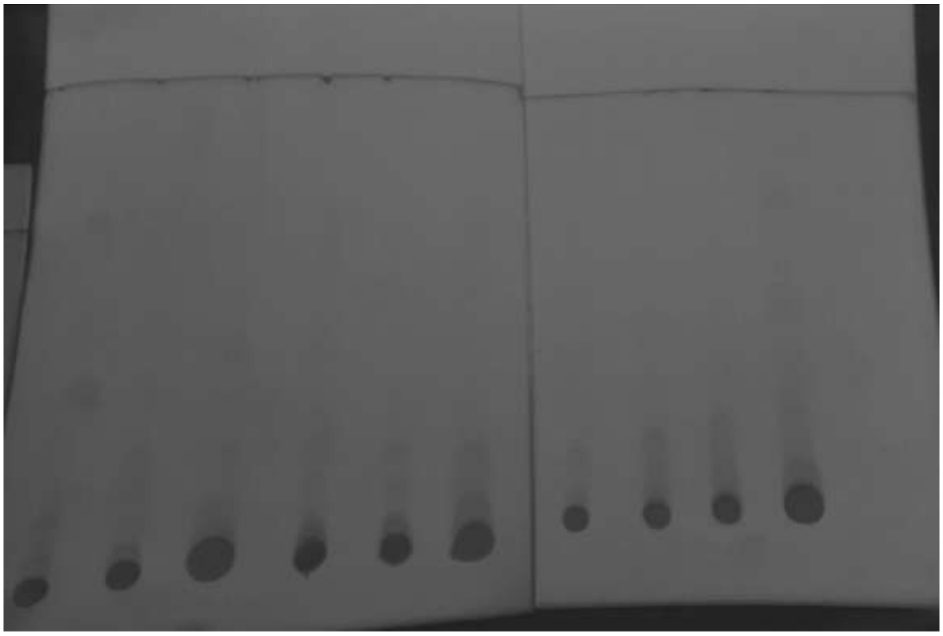
Table 4:- Antibacterial properties of extracted capsaicin against various bacteria.

Sample	**Zone of inhibition mm per 100 µl**			
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas Aeruginosa***	***Salmonella Typhi***
Capsaicin	17	16.7	15.3	15.3
Green chili chili(Fresh)	10.3	13.0	13.7	13.7
Green chili chili(Dried)	13.3	18.3	15.3	17.3
Habanero chili(Fresh)	11.3	12.3	12.0	14.3
Habanero chili(Dried)	16.0	22.0	15.0	28.0
Jalapeno chili(Fresh)	10.0	10.3	11.0	11.7
Jalapeno chili(Dried)	15.0	14.0	13.7	23.7
Chile chilis (Fresh)	11.7	14.0	10.3	17.7
Chile chilis (Dried)	14.7	20.7	13.7	22.3
Bell chili(Fresh)	10.3	12.3	13.0	17.7
Bell chili(Dried)	14.0	21.0	16.7	20.3

Table 5:- Antibacterial properties of different antibiotics against various bacteria.

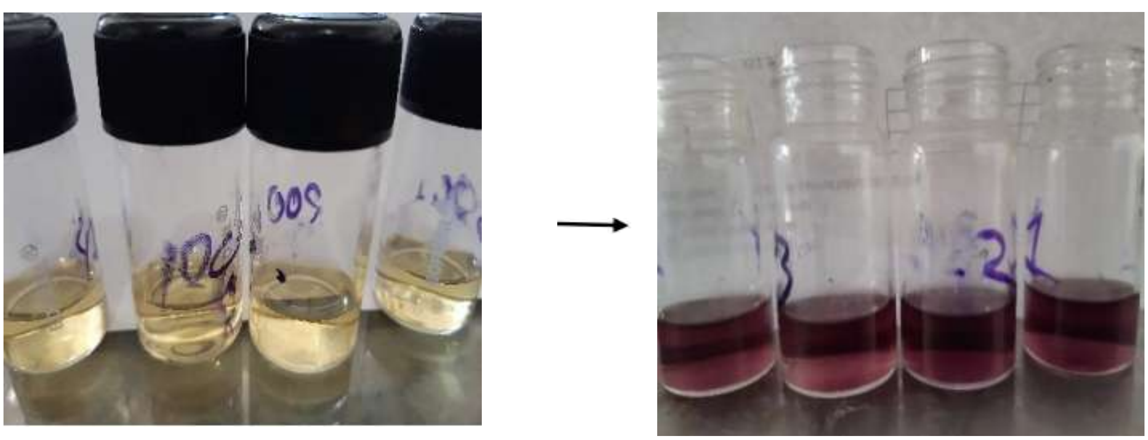
Antibiotic disc	Concentration	Zone of inhibition (mm)				
		***Staphylococcus aureus* ATCC**	***E.coli* ATCC**	***Pseudomonas aeruginosa* ATCC**	***Salomonella* Typhi ATCC**	***Listeria monocytogenes* ATCC**
Cefixime	5	15	-	-	15	-
Erythromycin	15	-	30	-	30	17
Azithromycin	15	27	28	-	28	16
Chloramphenicol	30	24	18	3	26	18
Cefoxitin	30	11	-	-	5	-
Gentamicin	10	24	15	29	12	19
Cefotaxime	30	13	15	-	14	-
Nalidixic acid	30	15	16	-	26	21
Ciprofloxacin	5	28	21	34	22	25
Vancomycin	10	18	8	-	-	15
Clindamycin	2	28	-	-	-	17
Ceftriaxone	30	29	20	20	19	-
Ampicillin	10	10	-	-	-	-

Photograph 1:- All five fresh and dried chili samples.

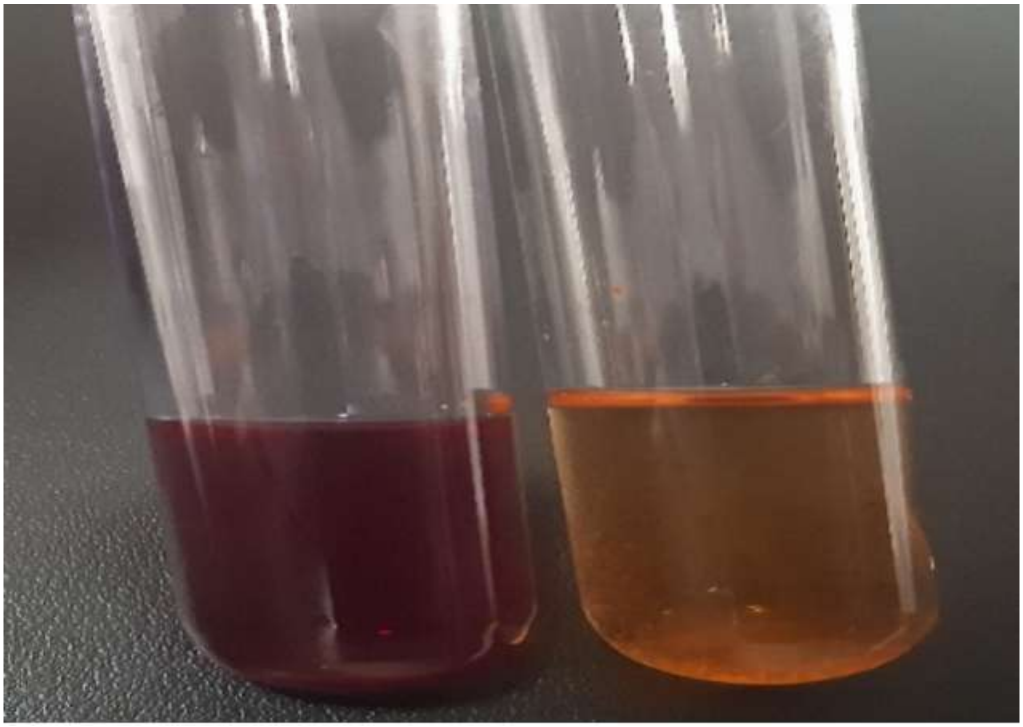


Green chili (Fresh) | Green chili (Dried) | Habanero chili (Fresh) | Habanero chili (Dried) | Jalapeno chili (Fresh) | Jalapeno chili (Dried) | Chile chili (Fresh) | Chile chili (Dried) | Green capsicum (Fresh) | Green capsicum (Dried)

Photograph 2: Thin layer Chromatogram of capsaicin extracted from chili samples

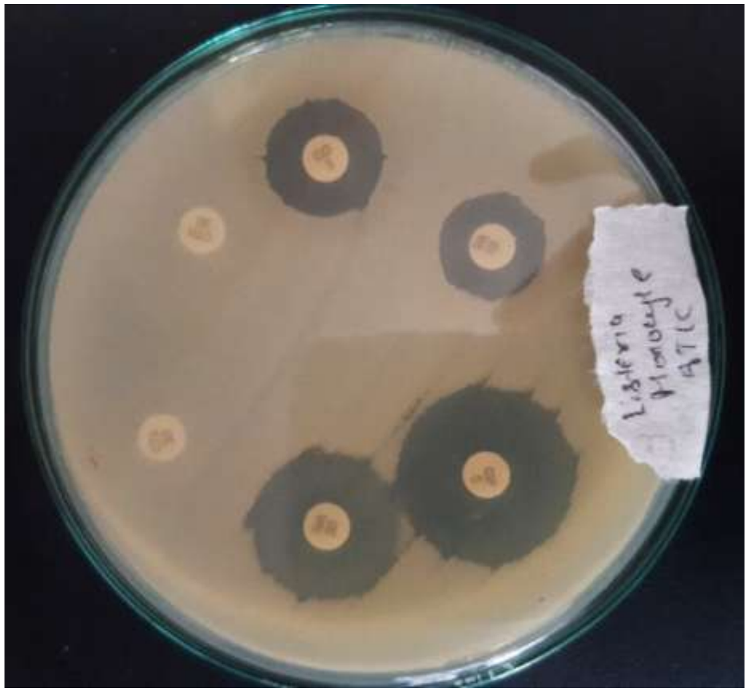


Photograph 3:- Antioxidant test by DPPH scavenging activity



Photograph 4:- Methanol residue test of crude capsaicin along with methanol as positive content.

Photograph 5:- Antibacterial activity of extracted capsaicin *Listeria monocytogenes* (ATCC 19115).



Photograph 6:- Antibacterial activity of commercial antibiotics against *Listeria monocytogenes* (ATCC 19115).

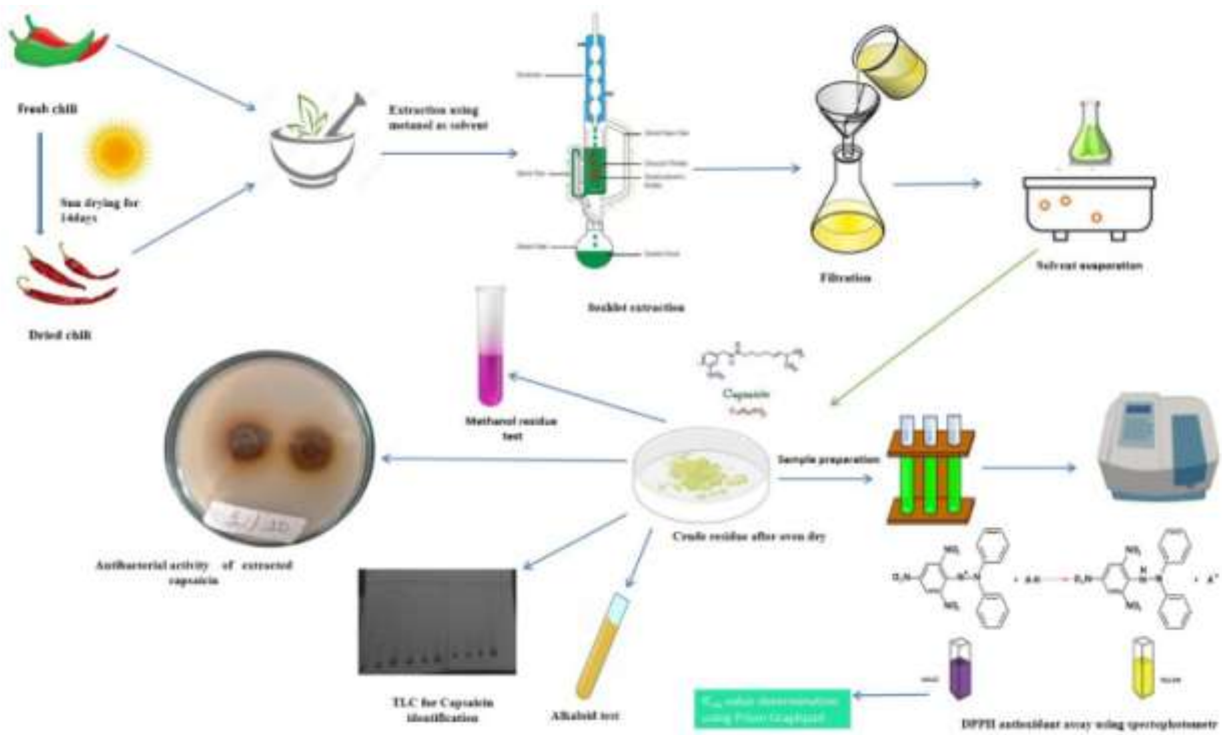


Figure 3: Graphical abstract

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