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Research Article

Phytochemical screening and antimicrobial activity of *Capsicum chinense* Jacq.

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Abstract

The present study investigated on the comparative evaluation of the extraction, quantification, phytochemical and antimicrobial activity of Capsaicin from acetone and acetonitrile extracts of *Capsicum chinense* Jacq. The polar aprotic solvent extracts showed high amount of Capsaicin with pungency level of 1,529,500 Scoville Heat Units. The Thin layer chromatography method is providing a fingerprint of plant extract. The Capsaicin extracted in the solvents on TLC chromatogram was viewed under UV 254 nm and UV 366 nm and documented. The extraction and estimation of chlorophyll and Carotenoids were also performed for the plant sample following standard procedure. Phytochemical analysis shows that acetone and acetonitrile extract of callus, leaf, shoot, fruit and seed which shows abundant presence of alkaloids, flavonoids, phenols, saphonins etc. The acetone and acetonitrile extract showed maximum zone of inhibition of *Klebsiella pneumonia* and *Staphylococcus aureus* against the gram positive and gram negative bacteria respectively through agar well diffusion method. The acetone and acetonitrile extract was found to be more effective at different concentration against all the tested bacteria and fungi. The results revealed that the Capsaicin and other secondary metabolites present in the acetone and acetonitrile extract of *Capsicum chinense* would contribute for the further extraction and purification of capsaicin as an antimicrobial agent.

1. Introduction

Nature is the paradise of medicinal principles offers to the society through plants which has richest source of phytochemical components since time immemorial. *Capsicum chinense* (Orange habenaro peppers) contains the highest concentrations of Capsaicinoid compared to other varieties and therefore may show more promise as a source of valuable Capsaicinoid [1]. The Capsicum genus contains numerous species of sweet and hot peppers [2]. It belongs to the family Solanaceae. Chilli is an important vegetable crop and used world-wide as for flavour, aroma, and add colour to foods. The genus *Capsicum* includes many species, in that *Capsicum chinense* is indigenous because it has a largest genetic diversity in the upper Amazon and it is well adapted to the diverse environmental conditions around the World [3].

Capsaicin is the compounds which are responsible for the pungency of pepper fruits and their products [4]. Capsaicin and several related compounds are called Capsaicinoid [5]. Pure capsaicin is a volatile, hydrophobic, colourless, odourless, and crystalline to waxy compound. The pungency and flavour are fruit attributes of *Capsicum chinense* [6] because Capsaicin and dihydrocapsaicin are an alkaloids which are responsible for 90 % of the intense organoleptic sensation of heat [7, 8]. The habenaro chilli as well as the Scotch Bonnet varieties are renowned to be very aromatic and the hottest pepper in the world [9].

Hot taste is due to the presence of Capsaicinoids, particularly Capsaicin (N-(4-hydroxy-3-methoxy-phenyl) methyl) 8-methyl-non-6-enamide) and dihydrocapsaicin (N-(4-hydroxy-3-methoxy-phenyl) methyl) 8-methyl-nonamide)

which are responsible for 80-90 % of the spiciness [10]. Several phytochemical studies have been carried out to find the techniques for purification of capsaicin to meet the higher demand for the compound. Phytochemical constituents are the chemical compounds formed during the plant normal metabolic growth and these are potential bioactive compound which are precursors for the synthesis of useful drugs [11].

Capsaicin have several medicinal properties and it is currently used in topical ointments, nasal sprays, as well as a high-dose dermal patch, to relieve the pain of peripheral neuropathy such as post-herpetic neuralgia caused by shingles [12] and also used as cream for the temporary relief of minor aches and pains of muscles and joint associated with arthritis, simple backache, strains and sprains [13]. Capsaicin is considered to be an active principle in arthritis pain reliever and anti-inflammation [14]. The plant flavonoids are potentially important dietary factory in cancer as chemo-protective agents and they show anti-allergic [15], anti-inflammatory [16], anti-microbial, anti-mutagenicity effect, anticancer and high antioxidant activities [17]. It is also used as traditional medicine for the treatment of ulcers, diabetes and Rheumatism [18]. Capsaicinoid content in peppers increases with maturing and climacteric ripening of the fruit [19].

In 2007, Guinness World Records ' Bhut jolokia' chilli pepper from the Assam region of India as being the World hottest chilli. The heat level of Bhut jolokia is 1,001,304 Scoville Heat Unit [20]. Accurate measurement of pungency is very important because of the increased demand by consumers for south-western foods. The amount of capsaicin is present in the given variety can be vary depending on the light intensity and temperature at which plant has been grown, the age of the fruit, and the colour and the position of the fruit on the plant [21]. The aromas of chilli pepper are the consequence of the characteristic components of their little stream essential oils [22, 23].

The bioactive compounds are the active principles found in plants and it has many pharmaceutical and therapeutic applications [24]. These compounds are vitamins and other secondary metabolites such as phenolic compounds, terpenoid, steroids and alkaloids [25].

2. Material and Methods

2.1 Sample Collection

The plant materials of *Capsicum chinense* such as callus, leaf, shoot, fruit and seeds were collected, shade dried, grounded, sieved through 20-30 mesh to obtain a coarse powder and stored in an air-tight container until further processes. The morphology of the fruit shape, colour, seed colour and size of the *Capsicum chinense* are important and examined following the methods of Moscone [26] and Dias [27]. The Standard capsaicin was purchased from Sigma Aldrich chemicals co. St Louis, MO, USA. All solvents used for Capsaicinoid analysis were of HPLC grade from Himedia.

2.2 Preparation of Extract

The extraction and quantification of Capsaicinoids in polar aprotic solvent was performed according to Collins *et al.*, with slight modifications [28]. The Chilli powder was mixed with acetone and acetonitrile solvents in the ratio of 1:10 (gram: millilitre). The mixture was placed in 120 ml glass bottle with Teflon lids. The bottles were capped and placed at 65°C in the Water bath for an hour and were swirled manually. The sample were removed from the water bath and cooled at room temperature. The supernatant was centrifuged at 10000 rpm and filtered through Whatman No.1 filter paper. The filtrates were evaporated to dryness and crude obtained was stored at 5°C in refrigerator until further analysis [29].

2.3 Phytochemical analysis

Freshly prepared extracts such as callus, leaf, shoot, fruit and seed were extracted from the solvent - acetone and acetonitrile were subjected to Standard phytochemcial analysis to find out the presence of the following phytoconstituents such as phenols, flavonoids, alkaloids, etc., Trease & Evans [30], method has been followed to find out the results.

2.4 Thin Layer chromatography Methodology

Qualitative analysis of the extract was done by Thin Layer chromatography. This was performed on TLC silica gel 60 F^{254} aluminium sheets (Merck). The Standard Capsaicin at concentration of 1mg/ml was spotted at references in the TLC plates. The aliquots of all the samples were applied onto the plates. After drying the plates was visualized under UV 254 nm and UV 366 nm and photographs were documented [31]. The Plates was dipped in vanillin sulphuric acid reagent and kept in oven at 105°C till the colour of the spots appears clearly. The following ratio Petroleum ether: Chloroform: Acetonitrile (4.5:4.5:1.0) was used as mobile phase. The Standard Capsaicin RF value was calculated and compared with the extracts.

2.5 Quantification by UV spectrophotometer

The simple linear regression curve was plotted using standard capsaicin purchased from Sigma Chemical. A stock solution of one milligram capsaicin per millilitre of ethanol was dissolved and different concentrations were prepared from

the stock solution 10 µg to 100 µg. The optical density was recorded at 280 nm. The linear regression curve was developed by using the Statistics. The Capsaicinoids extracted from the solvents were estimated by UV visible spectrophotometer (Hitachi-U1800). The crude extract was diluted to 300X using respective solvent. The Capsaicinoid concentrations in samples were calculated using capsaicin linear regression equation and it was expressed as microgram of capsaicin per millimetre and finally converted to Scoville Heat Unit [32].

2.6 Estimation of Scoville Heat Unit (or) Value (SHV)

The heat level of pepper is measured in Scoville units or valued named by Scoville who developed common Scoville scale. The Habenaro pepper 100.000-350.000 SHV while the pure Capsaicin is rated at 16.000.000 SHV [33]. The conversion of Scoville heat units was done by multiplying the capsaicin content in pepper dry weight by the coefficient corresponding to the heat value for pure capsaicin. The content of Capsaicinoid was converted from parts per million to SHU by multiplying the parts per million by 16 [34]. The Capsaicin and dihydrocapsaicin contents and their corresponding pungency level measured in SHV are the main indicators for the hotness taste of the pepper and the same factors were evoked elsewhere [35]. The habenaro variety has the pungency ranging from 200.000 to 350.000 (Thomas *et al.,*). The Scoville heat unit (SHU) were calculated by using following formula:

Total SHU= [CAPS (ppm) + DHCAPS (ppm)] × 16.1

Whereas, CAPS= Capsaicin, DHCAPS= dihydrocapsaicin

2.7 Estimation of Carotenoids and Total chlorophyll content

Carotenoids are fat-soluble antioxidants found in many fruits and vegetables and are required for human epithelial cellular differentiation. Carotenoids are terpenoid compounds formed by the condensation of eight isoprene units [36]. For extraction and estimation of chlorophyll and Carotenoids – method of Arnon (1949) has been followed. Fresh material of 0.5 gm was ground with 10ml of 80 % acetone using mortar and pestle. The homogenate was centrifuged at 800 for 10 min. The supernatant were collected. The pellet was reextracted with 5ml of 80 % acetone each time until it become colourless. All the supernatant were pooled and made upto with 80 % acetone and utilized for chlorophyll estimation. The absorbance was read at 480 nm, 645 nm and 663 nm in Spectrophotometer using 80 % acetone as blank and chlorophyll content was calculated using the formula of Arnon (1949) and the carotenoid content was calculated using the formula of Kirk Allens (1965) expressed in milligrams per gram fresh weight [37]. .

Total chlorophyll (mg/ml) = 0.0202 ×ΔA645 + 0.00802 × ΔA663

Carotenoid = ΔA 480 + (0.114 × ΔA 663) – (0.638 × Δ A645)

Where, ΔA = Absorbance at respective wavelength

2.8 Antimicrobial Activity

2.8.1 Test Microorganisms

Bacterial Strains - *E.coli, Klebsiella pnuemoniae, Salmonella typhi, Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus.* Fungal Strains – *Candida albicans, Aspergillus flavus,*

2.8.2 Assay of antimicrobial activity using Agar well diffusion method

The 20 ml of sterilized Muller Hinton Agar was poured into sterile petriplates, after solidification, 100 µl of fresh culture of human pathogens were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher at various concentration (20, 30, 40, 50 and 60) of each plant extract were added to the wells. The plates were incubated for 24 hours at 37°C. After incubation the diameter of inhibitory zones formed around each discs were measured (mm) and recorded [38].

2.8.3 Antimicrobial activity of commercially available antibiotics

The antimicrobial activities of selected plant extracts on human pathogenic bacteria were compared with the commercially available antibiotics. The discs (6mm in diameter) were impregnated with 25 µl of the extracts with the concentration of Sample 1 with 25 mg/ml and Sample 2 with 50 mg/ml was placed on the inoculated agar with flamed forceps. Sterile Muller Hinton Agar plates were prepared and the test organisms were swabbed over the surface of agar plates using sterile cotton swab. The antibiotic (Standard) such as Amikacin for bacteria and Ketakonazole for fungi were placed on the surface of the plates. The acetone and acetonitrile extracts were dissolved in the DMSO (Dimethyl sulphoxide) to a final concentration of 20 mg/ml. Antimicrobial tests were carried out by Agar Well diffusion method Perez *et al*., was followed. Each disc 50 µl of the sample was added. The solution was allowed to diffuse for 2 hrs. The plates were incubated at 37ºC for 24 hrs for bacteria and 48 hrs for fungi. Using 100 µl of suspension containing 108 CFU/ml 0, 30, 40, 50 and 60 µl). of test bacteria spread on MHA (Muller Hinton Agar).. Control - Water. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms [39-40].

3. Experimental design and Statistical analysis

The experiment was carried out in a completely randomized factorial design. All the experiments were repeated thrice and the reading were noted and calculated. Data's were statistically analyzed using the SPSS software and One way analysis of Variance (ANOVA) indicated significant treatment effects.

4. Results and Discussion

The results show the presence of some phytochemical components in the *Capsicum chinense* samples (Table 1). Phytochemicals such as carbohydrates, tannins, saphonins, flavonoids, phenols, alkaloids, terpenoids and volatile oils are present. Phytochemical polyphenols have received great attention because of their biological activity. Many flavonoids have shown strong antioxidant properties [41]. These results indicates that the pepper containing high phenolics provides a source of antioxidants and in addition it improves flavour to food hence it is used as a value added ingredient for stabilizing food against lipid peroxidation reactions.

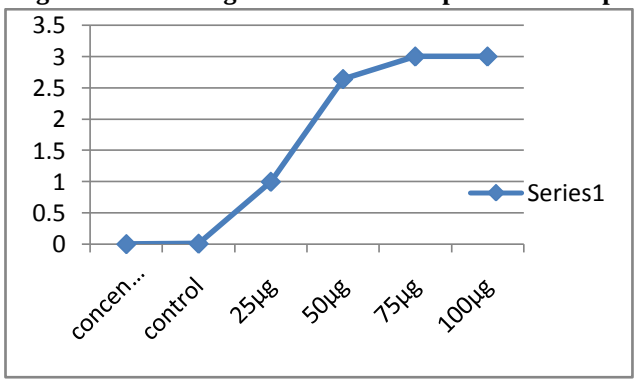
Table 1: Results of phytochemcial screening of acetone and acetonitrile extract of *Capsicum chinense*

Phytochemicals	Callus	Leaf	Shoot	Fruit	Seed
Carbohydrates	–	+	++	+	–
Tanins	–	+	+	–	–
Saphonins	+	–	–	++	++
Flavonoids	+	+	+	++	++
Cardiac glycosides	–	–	–	–	–
Reducing sugar	–	–	–	–	–
Phenols	++	+	+	++	+++
Steroids	–	–	–	–	–
Alkaloids	++	+	+	+++	++
Terpenoid	+	+	+	++	+
Amino acids	–	–	–	–	–
Proteins	–	–	–	–	–
Anthroquinones	–	–	–	–	–
Volatile oils	+	+	–	+	+

+++ = abundance, ++ = moderate, + = Present, – = absence.

The simple linear regression curve was plotted for standard capsaicin purchased from Sigma Chemical. The linear regression curve was developed by using the Statistics. The calibration curve was used to determine the reference concentrations for the acetone and acetonitrile extract samples. The Capsaicinoid contents obtained in µg/g were converted to Scoville heat units. In UV estimation the targeted capsaicin was observed a 280 nm, with a prominent peak.

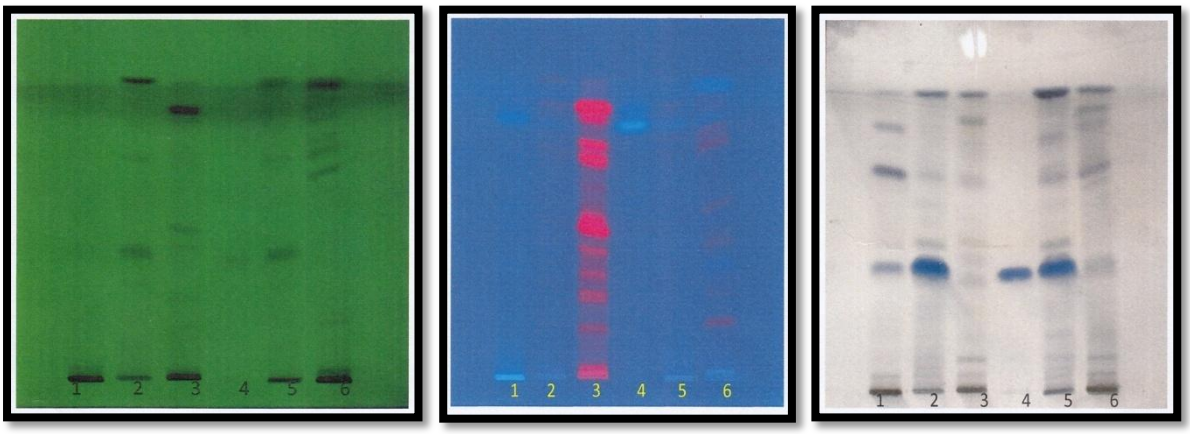
Fig 1.The linear regression curve was plotted for Capsaicin by UV spectrophotometer



Concentration (µg)	Optical density at 280nm
Control	0.007
25µg	0.997
50µg	2.639
75µg	3
100µg	3

Capsaicin and dihydrocapsaicin are the two major compounds responsible for the pungency of *capsicum* plants [42]. The Capsaicinoid content obtained in µg/g were converted to Scoville heat units in order to classify them according to their various pungency levels. The standard capsaicin Rf Value 0.39 coincided with the spot observed in the extracts. The Thin layer chromatography profile of acetone and acetonitrile extract has Capsaicin present in all the samples of *Capsicum chinense.* The standard capsaicin Rf value 0.39 is very much related to the fruit and seed at UV 254 nm and the standard capsaicin Rf value 10.84 at UV 366 nm is very much related to the fruit, seed, callus leaf of *Capsicum chinense*. TLC photo documentation shows the clear identification of the presence of capsaicin in callus, leaf, fruit and seed (fig 2).

Fig 2: TLC photo documentation of *Capsicum chinense* plant extracts such as callus, leaf, shoot, fruit and seed.



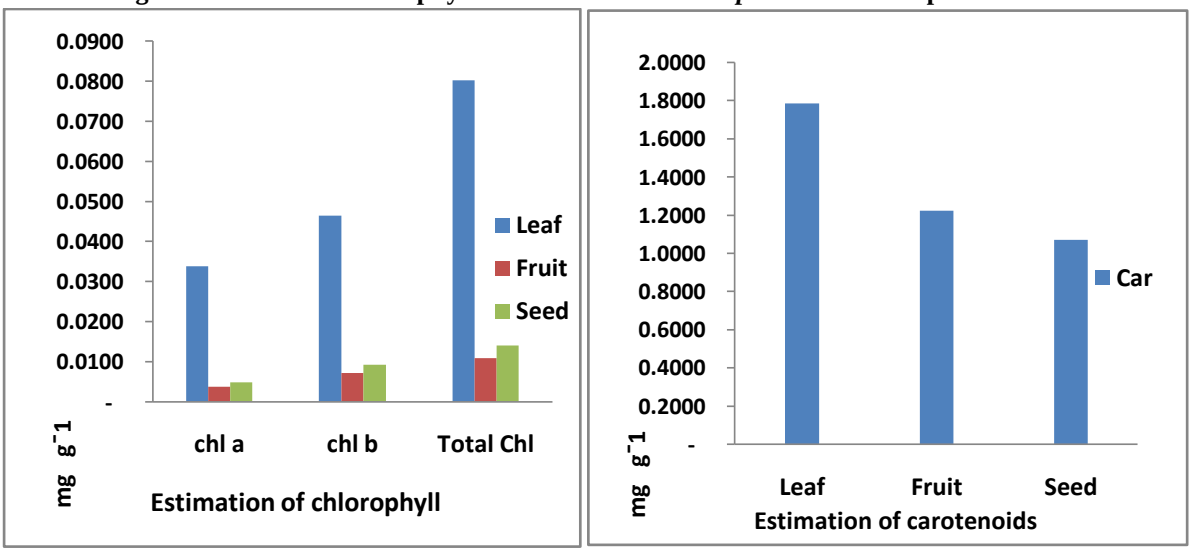
254 nm **366 nm** **After derivatization Vanillin-sulphuric acid**

Track-1. 15 µl (Callus), Track-2. 15 µl (Fruit), Track-3. 15 µl (leaf), Track-4. 15 µl (marker), Track-5. 15 µl (Seed), Track-6. 15 µl (Shoot)

The Scoville heat unit and total Scoville value was determined by summing the concentration of CAPS expressed in Scoville heat value (1529500 SHU). The Capsaicin and dihydrocapsaicin contents and their corresponding pungency level measured in SHV are the main indicators for the hotness taste of the pepper and the same factors were evoked [43]. *Capsicum chinense* is recognised as having the most pungent fruit [32]. The chilli pepper can be analysed and qualified as high hottest pepper in according to Scoville scale that ranks the highest pungency pepper in the range of 100.000-350.000 SHV [44].

The total chlorophyll and carotenoids present in the *Capsicum* plant species were estimated. The concentration of Carotenoid and Capsaicinoid increased as the peppers reached maturity, whereas the concentration of phenol decline [45]. The total phenol does not contribute to the pungency of the chilli [46]. In the comparative study on the UV spectrophotometer, total capsaicin estimation revealed that acetone and acetonitrile solvents were efficient to extract the high amount Capsaicin. The ethanol and acetonitrile are the ideal solvent for the extraction of capsaicin from the sample [47]. Results with the solvents used for the extraction showed diverse solubility of Capsaicinoids. Even pepper from the same plant can vary in their Capsaicinoid profiles attributable merely to differences in post harvest ripening condition [48]. The result shows that total chlorophyll is present abundance in leaf and seed and Carotenoids is present more in leaf as well as fruit (fig 3). The source of Carotenoids (natural colorants) and Capsaicinoids (pungent principle) which are very important application in food and pharmaceutical industry respectively [49].

Fig 3: Estimation of chlorophyll and Carotenoids in *Capsicum chinense* plant extract.



Carotenoids are substances present abundance in pepper and are considered as antioxidant [50]. Epidemiological data reveals that the possible role of antioxidant compounds in the prevention of numerous chronic diseases, such as certain types of cancer, cardiovascular and neurodegenerative diseases [51].

The results show the presence of antimicrobial activity in the *Capsicum* plant. Antimicrobial activity of the extracts obtained from *Capsicum chinense* against the tested organism are shown in the table 2, all the extract tested showed antimicrobial activity. However, the plant differs in their activities against the microorganism tested. Higher antibacterial activity was observed with acetone and acetonitrile extract of *Capsicum chinense* against *Staphylococcus aureus, Klebisella pneumonia and Streptococcus pyrogens* (21 mm & 20 mm) respectively. While minimum antibacterial activity was observed in *E.coli, Salmonella* typhi (5mm, 6mm, & 6mm). The study revealed that the higher antifungal activity was observed in *Aspergillus flavus* (13mm) in the fruit extract and minimum antifungal activity was observed *in Candida albicans* (6mm) (Fig 3). Results obtained in the current investigation revealed that *Capsicum* species extract possess potential antimicrobial activity against entire tested organism, acetone and acetonitrile extract was found to be strongest and broadest spectrum. Tannins are known for their astringent property and antimicrobial activity [52].

Antimicrobial activity of *Capsicum chinense,* the acetone and acetonitrile extracts shows that both the callus and fruit extract which shows higher zone of inhibition (mm). Finding of Bomoniri *et al*, who find that the gram negative bacteria are usually more resistant to antibacterial agents than gram positive bacteria. According to Hemalatha *et al*, the chloroform extract of *Capsicum* chilli showed less antimicrobial activity against all the test pathogens. According to Soetarna *et al* and Zhang *et al* also reported that the acetone and acetonitrile extract which show moderate antibacterial activity against *E.coli* but in our investigation both the solvent extracts of capsaicin were found to be effective against *E.coli* from this result it is clear that capsicum fruits may serve as a natural bactericidal agents. The study confirms that the *Capsicum chinense* species includes the most pungent chilli peppers known to the date [53-54].

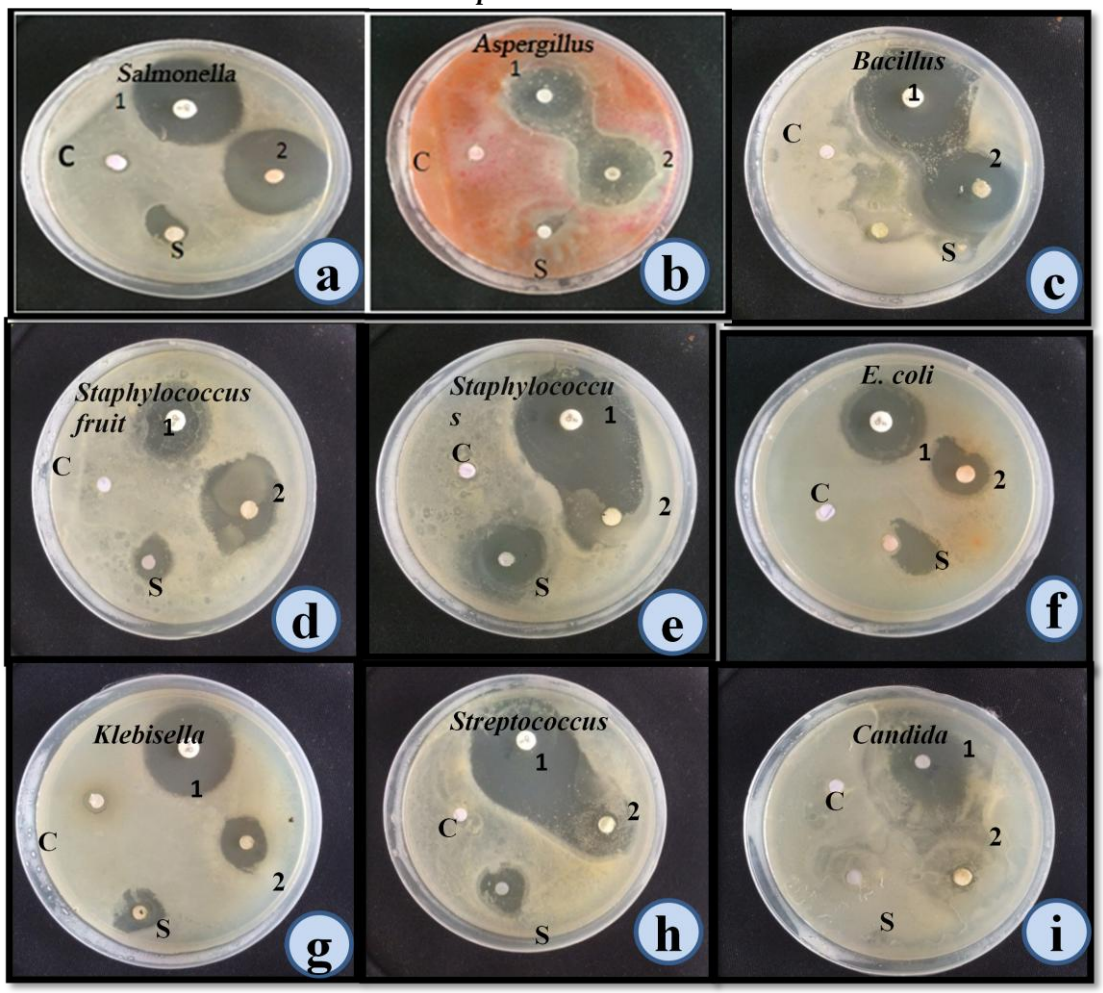
Table 2: Antimicrobial activity of *Capsicum chinense* of acetone and acetonitrile extracts against antibacterial and antifungal activity

S. No	Test Microorganisms	Zone of inhibition (mm)					
		Callus	Leaf	Shoot	Fruit	Seed	Standard
1.	***E.coli***	13	10	9	14	6	16
2.	***Klebisella pneumonia***	11	6	9	15	8	16
3.	***Salmonella typhi***	18	9	16	11	6	16
4.	***Staphylococcus aureus***	19	15	15	21	9	18
5.	***Streptococcus pyrogens***	15	20	19	8	12	18
6.	***Bacillus cereus***	9	11	15	9	8	15
7.	***Aspergillus flavus***	6	13	15	R	13	17
8.	***Candida albicans***	5	6	10	8	12	21

5. Conclusion

Acetone and acetonitrile solvent has the best ability to extract the Capsaicin. The study revealed that *Capsicum chinense* has a great source of potential bioactive compound. Capsaicin present in *Capsicum chinense* fruit is very effective in the prevention of a lot of disease [32,39]. The secondary metabolites identified in *Capsicum* of the present study might be responsible for antimicrobial activity exhibited by this plant against tested pathogen. From this study, it seems important to identify and characterize the compounds isolated for their use in the food, pharmaceutical, medicinal and therapeutic industries. We conclude that the extracts of the *Capsicum* varieties studied are applied as a natural preservative in the cosmetic and food industries or as an accessible and safe alternative to synthetic antimicrobial drugs.

Fig 3: Antimicrobial activity of the acetone and acetonitrile extracts such as callus, leaf, shoot, fruit and seed of *Capsicum chinense*



Maximum antimicrobial activity in (a), (b), (c), (d), (e), (h) and moderate activity in (f) & (g) and minimum activity in (i)

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