Isolation, Characterization and Identification of a Flavonoid from *Capsicum annuum* Fruits and its Antibacterial Activities Against Clinical Isolates

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Abstract
Plants have been used in traditional medicine for thousands of years, and their therapeutic properties have been studied extensively since synthetic medications are not always an option. Antimicrobial resistance and the obvious negative effects of synthetic medications have sparked a renewed interest in the hunt for novel agents to treat both new and old infectious illnesses. This study aimed to contribute to this effort by extracting and characterising potential therapeutic compounds from Capsicum annuum fruit and then testing those compounds' efficacy against various clinical disease-causing bacteria. The plant material under study was reduced to a powder and then extracted using a soxhlet apparatus using n-hexane and ethyl acetate, respectively. The hexane extract underwent phytochemical analysis, the results of which indicated the presence of alkaloids, flavonoids, steroids, phenols, tannins, and saponins. Using GC-FID, we were able to determine the concentrations of these phytochemicals; the results indicated a cyanogenic glycoside (9.3843 ppm) to have the highest concentration and a cardiac glycoside (1.6804 ppm) to have the lowest. The results of the proximate analysis showed that the concentration of carbohydrate was the greatest (at 40.636%), while the concentration of ash was the lowest (at 2.214%). Methanol was added little by little while the extracts were being separated on a silica gel column in ethyl acetate. The calculated fractions were analysed. TLC, UV, and IR spectroscopy were used to evaluate the resulting fractions, and from there, 50 fractions were aggregated into 10 combined fractions for NMR (1D and 2D) analysis to determine the structures of their components. Hesperetin, a kind of flavonoid, was identified as JLS-3's chemical makeup. High susceptibility of bacteria to the isolated chemical in the antimicrobial assay suggests the isolated natural flavonoid may be useful as a pretreatment for illnesses caused by these pathogens.

INTRODUCTION

Our quest for a healthy, disease-free existence is made easier by nature's provision of therapeutic plants. Plants have been utilised for medicine by humans and other animals for a long time because of their versatility in this regard. Medicinal plants have evolved with African civilization, and now they are widely seen as a representation of Africa's rich scientific and cultural past. Products derived from medical plants are in high demand because of the wide variety of phytochemicals found in plants. These phytochemicals include flavonoids, alkaloids,

phenol, saponins, glycosides, terpenes, carotene, and steroids (Abbas et al., 2019). The pharmaceutical industry's previous disinterest in making herbal health care formulations and herbal nutritional supplements has been reawakened by this. Capsicum annuum, also known as the Christmas ornaments of the vegetable world due to its delicate taste, pleasant flavour, different colours, nutritious value, and beautiful shaped form (Sharma et al., 2019), is among the eminent domesticated species or perennial herbaceous plant that is grown all over the world for cooked vegetables, pickles, salads, and processing purposes. It is highly valued in many Nigerian diets due to the large variety of nutritious components and pharmacologically active metabolites it contains. Coughs, toothaches, parasitic infections, rheumatism, sore throats, and wounds have all been treated with it, according to research (Singletary, 2011). Antiseptic, appetite stimulant, counterirritant (Pawar et al., 2011), antioxidant, and immunomodulatory are just few of the many beneficial uses for this plant. In addition, it has anticancer and antimicrobial properties (Singletary, 2011).

Sample collection and preparation

An April 2022 purchase of Capsicum annuum from the Bori Market in the Khana Local Government Area, Rivers State, Nigeria, was verified by a Botanist from the Reference Laboratory Section of Conig-Simonne Laboratories in Awka, Anambra State, Nigeria. After two weeks of air drying, the sample was crushed into powder using a mortar and pestle before being stored in the herbarium. Then, it was extracted and put under the microscope in a glass jar.

Extraction of Sample

A thimble containing 10g of the powdered sample was weighed and inserted in the thimble chamber of the soxhlet apparatus. A 250 mL volume of n-hexane (boiling point: 75 °C - 80 °C) was added to the round-bottom flask, and the soxhlet apparatus was built, heated with a heating mantle, and left to reflux for 6 hours. The n-hexane in the round-bottom flask evaporates in the heat and drips into the condenser, where it eventually returns to the sample thimble. When the liquid level reached the Syphon arm, it was redirected back into the round-bottomed flask. In order to achieve a concentrated sample, the soxhlet apparatus was disassembled at the conclusion of the procedure, and the mixture was recycled.

Phytochemical screening

Alkaloids, flavonoids, steroids, polyphenols, tannins, and saponins were identified in capsicum annuum extract by chemical analysis using established protocols (Aiyelaagbe & Osamudiamen, 2009; Edeoga et al., 2005).

Proximate Composition

Association of Analytical Chemists'recommended methods were used to determine the *Capsicum annuum's* moisture, ash, fibre, protein and carbohydrate contents (AOAC, 1990).

Chromatographic separation

Silica gel was used as the stationary phase in a chromatographic column to separate the filtrate. N-hexane and ethyl acetate were both components of the solvent system. About 25 mL of fractions were extracted using gradient elution, and the elutes were analysed using thin layer chromatography with an ethyl acetate to hexane solvent ratio of 3:7.The Rf-corresponding fractions were then assembled after being visualised with a UV laser. The obtained fractions were validated through thin layer chromatography after being sprayed with anisaldehyde-sulphuric acid. The retention factors for each sample were calculated. For NMR analysis, fractions with similar Rf values were combined, while those with a large number of spots were purified further using sephadex column chromatography.

Spectroscopic characterization

The pure coded fraction was also sent in for melting point, IR, and spectroscopic (Proton and Carbon NMR) analysis of the chemical. The 1H and 13C NMR spectra were validated in chloroform (CDCl3) at the University of Strathclyde in Glasgow, Scotland, United Kingdom, using a Bruker Avance 3 spectrophotometer.

Antimicrobial Activity of Extracts against some Selected Test Bacteria

The organisms came from Conig-Simonne Laboratories' Reference Laboratory Section in Awka, Anambra State, Nigeria. Nutrient Broth was used to feed the organism for a whole day. Using a sterile wire loop, 3–5 pure cultures of the test microorganism were selected and emulsified in 3–4 ml of sterile physiological saline to achieve standardisation. The absorbance of the 0.5 McFarland standard was measured using a spectrophotometer at 540 nm, and the turbidities of the test organisms were corrected using physiological saline to match the absorbance of the 0.5 McFarland standard at the same wavelength.

Antimicrobial Susceptibility Test

Extracts were tested for their antibacterial properties against the test microorganisms using modified disc diffusion techniques (Agu et al., 2013; Adindu et al., 2016). The test bacteria (1.5x108 cfu ml-1) were diluted to 0.5 McFarland and 25 l of the solution was poured onto Mueller-Hinton plates for growth. The 6mm filter paper discs were impregnated with exactly 50 l of the extracts and put on two sections of the agar plate. Each plate's inhibition zone diameter was reported in millimetres. There were three of everything in the tests. Sterile physiological saline was used for the negative controls, whereas 50 ng/ml was used for the positive controls. Ciprofloxacin

Determination of Minimum Inhibitory Concentration (MIC)

This research built on the work of Pallota et al. (2007) and Agu et al., (2013). To find out what the MIC was, we diluted the broth with the microorganisms in it. Bacterial and fungal inocula of known concentrations (0.5 McFarland adjusted cultures for bacteria and yeast; and dilution factor of 10-2 for fungus) were added to Nutrient Broth and Sabouraud Dextrose Broth containing 20%, 40%, 60%, and 80% of the extracts, respectively. We also established both negative and positive controls. The tubes were placed on a metabolic rotary shaker (220rev/min) and incubated at room temperature for 24 hours for bacteria and 48 hours for fungus. After 24 hours for bacteria and 48 hours for fungus in the incubator, the test tubes were subcultured onto sterile newly prepared plates. After the plates had been incubated, the total number of CFU/ml of bacteria present was reported. The minimal inhibitory concentration was

found on plate 2. The minimum inhibitory concentration (MIC) is the concentration at which further microbial growth is prevented after incubation for either 24 or 48 hours.

Results and Discussion

Table 1. Qualitative Phytochemical screening of *Capsicum* annuum

S/N	Phytochemical components	Remark
1	Alkaloids	+
2	Flavonoid	+
3	Steroid	+
4	Phenol	+
5	Tannins	+
6	Saponins	+

Table 2. Quantitative Phytochemical screening of *Capsicum annuum*

S/N	Phytochemical	Composition ppm
1	Lunamarin	5.2274
2	Cardiac glycoside	1.6804
3	Anthocyanin	2.6683
4	Spartein	4.4838
5	Rutin	3.6003
6	Cyanogenic glycoside	9.3843
7	Flavonone	2.7896
8	Steroid	5.4124
9	Kaempferol	1.8177
10	Epicatechin	9.2404
11	Phytate	7.1889
12	Flavone	2.3911
13	Oxalate	5.5012
14	Catechin	2.4736
15	Resveratrol	2.5997
16	Tannin	3.0368
17	Sapogenin	5.2669
18	Epihedrin	5.6647

Table 3: Proximate Composition

S/N	PARAMETER	% Yield
1	Moisture	32.792
2	Ash	2.214
3	Fat	13.172
4	Fibre	6.286
5	Protein	4.9
6	Carbohydrate	40.636

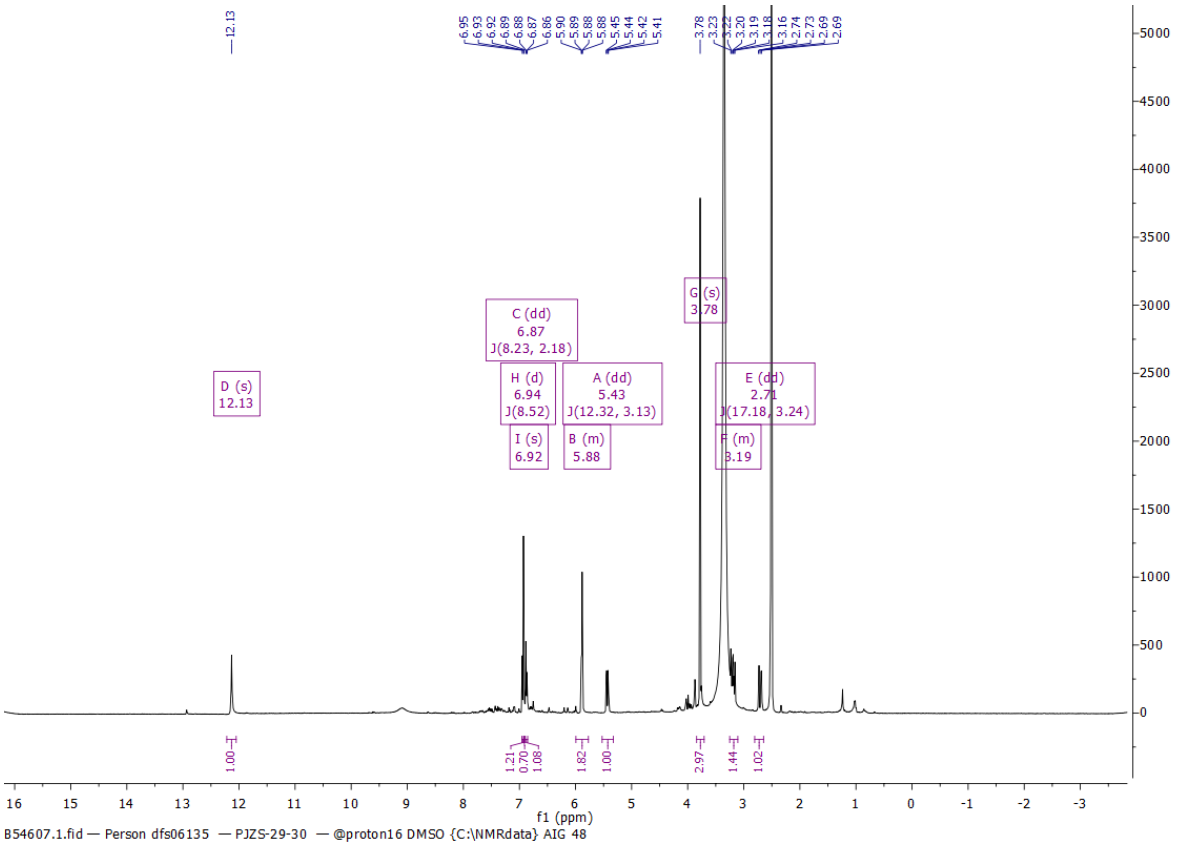
Table 4: ^{1}HNMR and ^{13}C NMR Chemical Shifts for JLS-3

Spectroscopic technique	Data
Rf [Hex: EAC (7:3)]	0.63
Mp	226-228 ^{0}C
^{1}HNMR(DMSO)	δ 5.41(s), 2.72, 3.20 (d), 5.88, 5.89, 6.93, 6.87, 6.92, 3.78
^{13}C NMR(DMSO)	δ 56.2, 78.7, 42.5, 196.6, 163.9, 96.3, 167.2, 95.5, 163.2, 102.3, 146.9, 148.5, 112.5, 118.1, 114.5, 56.1.

Table 5: Comparison of NMR data of JLS-3 with Literature Report

Positions	Experimental		Literature Shankar and Kirti (2014)	
	^{1}H (δ)	^{13}C (δ)	^{1}H (δ)	^{13}C (δ)
1		56.2		55.7
2	5.41	78.7	5.40	77.98
3	2.72, 3.20	42.5	2.37, 3.20	41.89
4		196.6		195.72
5		163.9		162.54
6	5.88	96.3	5.89	95.65
7		167.2		166.4

8	5.89	95.5	5.91	94.81
9		163.2		163.25
10		102.3		101.65
1’				
2’		146.9		146.36
3’		148.5		147.7
4’	6.93	112.5	6.92	112.0
5’	6.87	118.1	6.89	117.38
6’	6.92	114.5	6.94	113.89
OCH_3	3.77	56.1	3.78	55.6



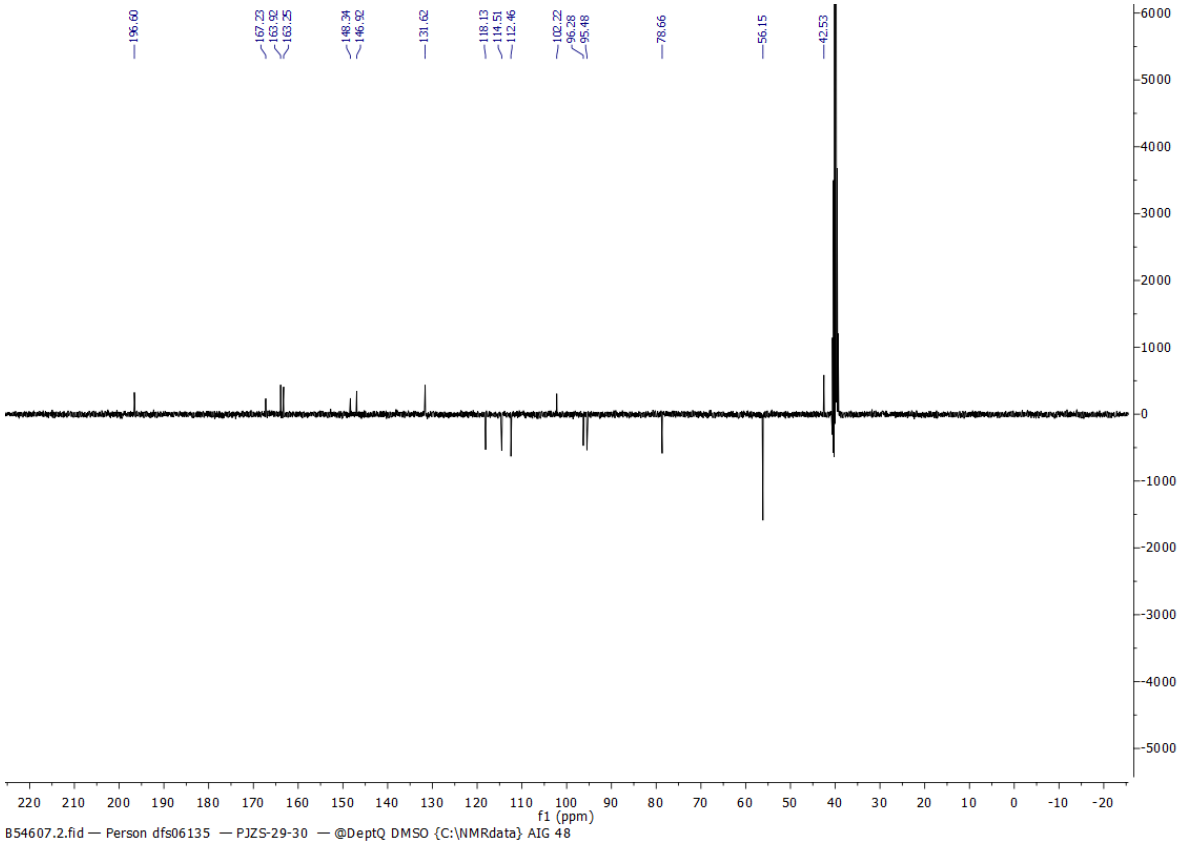


Table 6: Preliminary Antimicrobial Susceptibility Screening of *Capsicum* annuum extract

Test Bacteria	Cayenne extract				MEAN INHIBITION ZONE DIAMETERS (IZD) IN MILLIMETRES± STANDARD DEVIATION							
	+ve ctrl								-ve ctrl			
	ϰ	y	Z	Mean	ϰ	Y	Z	Mean	K	y	z	Mean±SD
Pseudomonas aeruginosa	24	23	21	22.667	50	48	46	48.000	-	-	-	-
Staphylococcus aureus	23	26	22	23.667	52	56	53	53.667	-	-	-	-
Salmonella enterica	22	20	22	21.333	43	44	46	44.333	-	-	-	-
Citrobacter murliniae	19	18	20	19.000	40	35	37	37.333	-	-	-	
Bacillus licheniformis	21	21	20	20.667	40	44	40	41.333	-	-	-	
Micrococcus roseus	21	20	20	20.333	40	39	37	38.667	-	-	-	

Bacillus subtilis	21	22	18	20.333	41	39	45	41.667	-	-	-

Table 7: Minimum Inhibitory Concentration (MIC) of the *Capsicum* annuum extract on the various test organisms

	extract				+ve ctrl ($mgml^{-1}$)				-ve ctrl ($mgml^{-1}$)			
Test Bacteria												
	500	250	125	62.5	500	250	125	62.5	500	250	125	62.5
Pseudomonas aeruginosa	NT	NT	T	T	NT	NT	NT	NT	T	T	T	T
Staphylococcus aureus	NT	T	T	T	NT	NT	NT	NT	T	T	T	T
Salmonella enterica	NT	NT	NT	T	NT	NT	NT	NT	T	T	T	T
Citrobacter murliniae	T	NT	T	T	NT	NT	T	T	T	T	T	T
Bacillus licheniformis	NT	NT	T	T	NT	NT	T	T	T	T	T	T
Micrococcus roseus	T	T	T	T	NT	T	T	T	T	T	T	T
Bacillus subtilis	NT	T	T	T	NT	NT	T	T	T	T	T	T

Key: T= turbidity NT = No turbidity

Discussion

Alkaloids, flavonoids, steroids, phenol, tannins, and saponins were found in the crude extract of Capsicum annuum during early qualitative phytochemical examination (Table 1). Antibacterial, antiviral, hepatoprotective, anti-carcinogenic, antioxidant, and anti-inflammatory properties of flavonoids and other phytocompounds have been described (Shashank & Abhay, 2013). As can be seen in (Table 2), eighteen different phytochemical substances were identified and measured in Capsicum annuum. Cyanogenic glycoside was found to have the greatest content (9.3843ppm), followed by cardiac glycoside (1.6804ppm). It was found to contain the following phytochemicals: lunamarin (5.227 ppm), cardiac glycoside (1.680 ppm), anthocyanin (2.668 ppm), spartein (4.483 ppm), rutin (3.603 ppm), cyanogenic glycoside (9.3843 ppm), flavonones (2.789 ppm), steroids (5.4124 ppm), kaempferol (1.8177 ppm), epicatechin (9. The anti-amoebic and free radical scavenging effects of lunamarin are well-known. (Rahmani and Sukari, 2010; Sohni and colleagues, 1995). Cyanogenic glycosides, lunamarin, ephedrine, and spartein are the four alkaloids found in varying amounts, with cyanogenic having the highest (9.3843ppm) and spartein having the lowest. (4.4838ppm). Because they protect plants against herbivores, pathogens, and insects, alkaloids are essential to plant viability despite possessing antifungal and antibacterial properties. It was found (Molyneux et al., 1996). Alkaloids, compounds found in plants, have been used as poisons, dyes, and medicines since the dawn of civilization.

Many indole alkaloids have pharmacological effects, including those against cancer, hypertension, malaria, and hypertension. There is a lack of evidence demonstrating the importance of this group of plant components to business success. The intoxicating effects of alkaloids, which are employed in analgesics and antimalarials, are comparable to those of caffeine, morphine, nicotine, and quinine (Wink et al., 1998).Examples of flavonoids include anthocyanin, rutin, flavones, kaempferol, flavonone, catechin, epicatechin, etc. The longevity of flavonoid use suggests they were integral to the first successful medicinal interventions. Many flavonoids produce conjugated forms with sugar in a natural process.

Carbohydrates include sugars like D-glucose and L-rhamnose as well as the more complex sugars like galactose, gluco-rhamnose, arabinose, and gluco-rhamnose. Glycosidic linkages often occur at either position 3 or 7. In particular, flavonoids and phenol The many pharmacological and biological properties of resveratrol have attracted a lot of interest lately. Multiple biological properties, such as cytotoxicity, antimicrobial activity, anti-inflammatory effects, and tumour suppression, have been ascribed to flavonoids and phenols (Shirsat et al., 2012; Teiten et al., 2013). But it's clear that flavonoids of almost every kind may protect the body against free radicals and volatile oxygen species (ROS) thanks to their powerful antioxidant properties.In addition to their widespread presence in plants, tannins have potential medical use as hemostatic, antidiarrheal, and antihemorrhoidal agents. They help keep gastritis, enteritis, esophagitis, and irritating bowel problems at bay due to their anti-inflammatory properties. Internally, tannins may treat wounds and prevent further bleeding, burns, and infections (Praveen & Kumud, 2012).

Cocoa, persimmon, grapes, blueberries, and tea are just few of the many fruits that contain tannins. Tannins may also be found in legume forages, legume trees like Acacia spp. and Sesbania spp., and grasses like sorghum and maize (Giner-Chavez, 1996). Epidemiological studies have identified some correlations between tannin consumption and a decrease in the prevalence of chronic illnesses, among other advantages (Serrano et al., 2009). Recent scientific interest in tannins has been sparked by the increasing incidence of deadly illnesses like cancer and AIDS. Since it was discovered that tannin-covering plant extracts were physiologically active, the quest for such compounds to use in the development of new treatments has taken on greater importance (Mueller-Harvey, 1999).One kind of saponin is called a sapogenin. It is well knowledge that saponins protect plants against mould and insects thanks to their anti-microbial characteristics. Saponins are a class of compounds present in plants that act as a defence mechanism. They are also known as phytoprotectants or phytoanticipins. Reference: (Lacaille-Dubois & Wagner, 2000). There is a wide range of biological effects caused by the presence of different combinations of saponins in plants and plant products. The properties of saponins, such as their ability to break down membranes, stimulate the immune system, lower cholesterol, and prevent cancer, have been the subject of a great deal of research. These chemicals are also known to have significant effects on animal growth, feed intake, and reproduction. When exposed to these structurally varied chemicals, mollusks and protozoa have been observed to die. It possesses antiviral and antifungal effects, suppresses viral and fungal growth, produces hypoglycemia, and disrupts protein digestion (Takechi et al., 1999). Antibacterial phytochemicals have been identified, and they may be found in steroids, flavones, and tannins (Sodipo et al., 1991). They found that phytochemicals inhibit bacterial growth by depriving bacteria of protein via a process called precipitation. Tannins, particularly when paired with digestive enzymes, may speed up the breakdown of some proteins (Abara, 2003). According to research (Odugbemi, 2006), plants' antibacterial

effects come from bioactive compounds such alkaloid, saponin, steroids, and tannins. This provides more evidence for the effectiveness of Capsicum annuum extract in the treatment of malaria and other comparable disorders, as well as its potential to restrict the development of particular insects. Chromatography of Gases Some of these components were measured using a gas chromatograph–flame ionisation detector (GC–FID).

Carbohydrates, proteins, ashes, fibres, fats, moisture, and cellulose were all found in the proximate composition study. The results showed that the greatest concentration was found for carbs (at 40.636%), followed by the highest concentration found for moisture (at 32.792%). (Table 3). Carbohydrates in the diet play a crucial role in preserving gastrointestinal health and function and in keeping blood sugar levels stable. As long as it's from a variety of sources, eating a lot of carbohydrates isn't bad for your health as eating a lot of fat or protein is. Carbohydrates' primary role is to provide as a source of fuel for the body's cells. Glucose, rather than compounds like fatty acids, is a top choice for many cells when it comes to generating energy. Some cells, like red blood cells, rely entirely on glucose for their metabolic needs. The brain is particularly susceptible to low blood glucose levels since glucose is the sole fuel it needs to create energy and operate (unless in the event of acute hunger). To wit: (Nna & Legborsi, 2022). Carbohydrate content is a standard metric used in determining the commercial viability of fruits like peppers. Carbohydrates are widely acknowledged as a key metric for evaluating the flavour, nutritional value, and technical attributes of both natural and artificial foods. New studies have shown reasonable concentrations of carbs that are within the safe zone (Quartey et al., 2012). The large quantity of carbs may not be nutritionally advantageous, even though the body will presumably excrete them undigested (Rubio et al., 2002). A number of factors, including fertilisation technique, humus and soil complex, nutrient accessibility, and climate, might affect the total quantity of carbs in a given sample. Essential oil may be produced from Capsicum annuum, despite the plant's low fat content (Hubbard & Pharr, 1992).

The metabolic processes that provide you energy and enable your blood to transport oxygen across the body both rely heavily on protein. It also helps the body produce antibodies that can fight off diseases and infections, and it promotes the growth of new cells. Protein has several positive benefits on health. For optimal health, your body needs a steady supply of protein. When you have a cut or scrape, the protein you eat may aid in the healing process. Protein and carbohydrates provide the body fuel and keep it from becoming tired. Protein also has the added benefit of boosting the immune system and protecting the body against sickness. To wit: (Nna & Legborsi, 2022). Protein is crucial to the human diet because it supplies an amino acid that the body cannot make on its own (Hui et al., 2006). Active protein metabolites, like capsaicin, may account for pepper's acceptable protein content. Peppers on their own don't provide enough protein, thus they should be combined with other protein-rich meals. To wit: (Elleuch et al., 2011)

Fibre is an important part of a healthy diet since it helps prevent many different diseases, including diabetes, heart disease, colon cancer, and obesity. The sample's Fibre concentration was well within the allowable range (Quartey et al., 2012). The health advantages of dietary fibre are many. Increased fibre intake is associated with a decreased risk of cardiovascular disease, stroke, high blood pressure, diabetes, obesity, and certain gastrointestinal diseases. Adding more fibre to your diet will help lower your blood cholesterol and blood pressure. Increasing soluble fibre intake improves glycemia and insulin sensitivity in both diabetics and non-diabetics. The capacity of obese people to lose weight is significantly impacted by using

fibre supplements. An increase in fibre intake has been shown to be beneficial in treating a number of gastrointestinal complaints, such as GERD, duodenal ulcers, diverticulitis, constipation, and even haemorrhoids (Nna & Legborsi, 2022). The thixotropic characteristics of semi-solid dosage forms are affected by moisture concentration in the same ways as chemical stability, crystal structure, powder flow, compaction lubricity, dissolution rate, and polymer film permeability are affected by solid dosage forms. Because of this, it affects group activities that must have access to clean water. It is crucial to describe how moisture modifies the characteristics of these particular active components and excipients. It has been shown (Nna & Legborsi, 2022) Ash gourd is a low-cholesterol meal that should be taken daily to maintain and enhance heart health due to its low cholesterol content. After being cooked, the vegetable is used into a variety of traditional Indian dishes with the purpose of promoting healthy blood circulation. Water, which is more prevalent in plant foods, maintains human tissue and cell health by controlling pH and temperature (Martinez et al., 1999). The high moisture content showed that pepper would not be able to be kept for a long time without rotting, since high water activity may promote microbial action, which results in food degradation. Therefore, pepper's storage life is increased with dehydration.

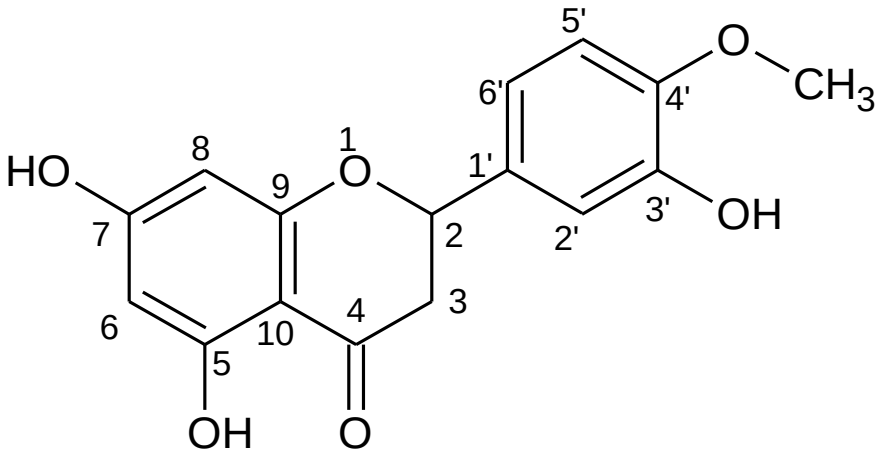
A food's mineral content may be estimated by looking at its ash content. Capsicum annuum may have mineral content due to the presence of ash (Bakkali et al., 2009). Fat is a necessary component of our diets due to its many health benefits. Eating less saturated and trans fats may help reduce your risk of cardiovascular disease. It helps us keep our internal temperature stable, absorbs the nutrients we need, and keeps us going. To be at one's healthiest, fat is a necessary macronutrient. Researchers have observed that the high percentage of unsaturated fatty acids in the oil from the seed improves cardiovascular indicators such as the quantity of high-density lipoprotein (HDL) cholesterol, total triacylglycerol, and LDL cholesterol (Nna & Legborsi, 2022).

Isolation and Characterization of JLS-3 as Hesperetin

Using an ethyl acetate extract of Cayeen pepper, JLS-3 was isolated and purified on a Sephadex column to its current brownish solid form (Sharkar & Kirti, 2014). Absorption maxima at 290, 315, and 345 nm (at a wavelength of 285) were observed in the UV spectrum. Infrared spectroscopy revealed prominent OH bands at 3541 and 3470 cm1, CH (aliphatic) bands at 3077, 2973, 2935, and 2913 cm1, C=C (aromatic) bands at 1601, 1514, 1464, and 1443 cm1, and C=O, C-O, and C=O bands at 1651, 1280, and 1203 cm1. Ethyl acetate: hexane, 3:7 yielded a value of 0.71 for Rf.

There were five signals indicative of aromatic proton H values between 5.88 and 6.93 in the 1H-NMR spectrum, and three signals indicative of aliphatic proton H values between 2.72 and 5.41. Very distinctive of the 5-OH of a flavonoid moiety was the presence of a signal for an intramolecular H-bonded proton at 12.13 ppm (Sharkar & Kirti, 2014). The existence of a tetrasubstituted benzene ring may be deduced from the presence of two metacoupled protons at H5.88 ppm and H5.89 (Sharkar & Kirti, 2014). (Table 4) Protons at 5.41, 2.72, and 3.20 eV of hydrogen. These results suggested that ring A was 5, 7-dioxygenated and ring C was saturated with flavanone. Integrated for three protons representing 2', 5', and 6' for ring B protons, the spectrum additionally indicated signals between H 6.87 and 6.93. This sequence also points to a ring B with 3' and 4' disubstitutions. There were also three hydroxyl groups visible in the spectra, at H8.9, H10.6, and H12.13. The hydroxyl group at carbon position 5 was responsible for the change at H 12.13. Table 4 further shows that there was a singlet at 3.78, which corresponds to three hydrogens in a -OCH3 group.

Sixteen carbon atoms were detected by 13C-NMR, including nine quaternary carbon atoms, five CH atoms, one CH2 atom, and one methoxyl group. (Vesna et al., 2014) found that flavanone moiety often gives out signals around c196.6, 78.7, and 42.5 ppm. Three aromatic carbons with hydroxyl groups attached to them at positions C-5, C-7, and C-21 account for the signal at c 163.9, c 167.2, and c 146.9. Table 4 shows that the carbon-9 and carbon-10 flavonoid ring junctions occurred at c163.2 and c102.3, respectively. As can be seen in (Table 4.1), the newly revealed 13C-NMR spectrum data for compound JLS-3 compares well with the previously reported data. Therefore, Hesperetin (5, 7, 12-trihydroxy-13-methoxyflavanone) was isolated from fraction JLS-3.



Structure of JLS-3.

Table 5 demonstrates that seven different bacterial strains were employed to test the extract of Capsicum annuum for its antibacterial capabilities and confirm that it is effective against the germs listed there. These bacteria included the common ones like Salmonella enterica and Citrobacter murliniae as well as the less common ones like Pseudomonas aeruginosa, Bacillus licheniformis, Micrococcus roseus, Bacillus subtilis, and Staphylococcus aureus. This hypersensitivity has been attributed to the abundance of phytochemicals (Manisha & Shyamapada, 2011). Table 5 shows that the average development of these organisms is greatly slowed by Capsicum annuum extract, from 19.000mm to 23.667mm. Table 5 shows that the typical values for Staphylococcus aureus were 53.667mm, whereas those for Citrobacter murliniae were 37.333mm. Some examples of common infections that have been linked to a broad range of disorders include Salmonella enterica, Citrobacter murliniae, Pseudomonas aeruginosa, Bacillus licheniformis, Micrococcus roseus, Bacillus subtilis, and Staphylococcus aureus (Odoki et al., 2019). Capsicum annuum extract was effective against all of the isolates because of the high content of phytochemicals in the sample (Manisha & Shyamapada, 2011). The extract efficiently suppressed test microorganisms at doses ranging from 62.5 mg ml-1 to 500 mg ml-1, as shown in Table 6.

At 500 mg m-1, all bacteria tested did not grow (there was no turbidity), with the exception of Citrobacter murliniae and Micrococcus roseus.

A concentration of 62.5mgml-1 was only enough for the development of Bacillus licheniformis, Bacillus subtilis, Micrococcus roseus, and Citrobacter murliniae. The turbidity did not increase due to any new growth.

The ability of several bacterial strains to suppress the growth of Capsicum annuum extract was determined by measuring the size of their respective zones of inhibition (in millimetres). The extent of this buffer zone is proportional to the extract's ability to control microbial growth. It's common for zones to expand as antibiotic concentrations are increased.

Conclusion

In the current work, physical and spectroscopic techniques were used to isolate and characterise flavonoid from Capsicum annuum fruit. Testing of the chemical against a variety of clinical infections revealed significant antibacterial activity, lending credence to the plant's traditional medicinal use. This provides support for further research into the isolated chemical as a possible therapeutic candidate for treating various illnesses induced by the pathogens employed in this study. This research, however, is the first to mention the existence of the flavonoid in question.

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