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# GC/MS Technique for Bioactive Metabolites Analysis of *Capsicum frutescens* and Evaluation of Its Antioxidant [Superoxide, Nitric Oxide Radical Scavenging] and Antifungal of Four Different Yeast and Fungi

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**Abstracts:** Background: Despite the fact most of the medicinal plants can be associated with low mortality, they can lead to severe morbidity such as stunted physical and mental growth, among children when used in treatment of gastrointestinal diseases such as diarrhoea. Native Americans used capsicum fruits for various conditions for centuries and the identification of bioactive compounds in these fruits vindicates this practice. Finding among Capsicum frutescens, our study sought to establish if the plant had antifungal or antioxidant properties on the basis of its bioactive metabolites.

Methods: The fruits were first washed and then put in an oven at a temperature of 60◦C for 48 hours until dried. They were then left to cool and later on a fine powder was made from it. The aqueous extract was obtained after boiling 100mL distilled water with 10g powder at 15 minutes. The preparation of a sample in a heated injector block and then ongoing on the head of a chromatographic column packed with a non-volatile liquid phase is the technique called gas chromatography. The inhibitory zone diameter, in millimeters (mm), is applied as a factor for the evaluation of the result of antifungal efficiency of the phenolic extracts.

Results: Altogether, GC–MS revealed that Juniperus communis contains more than nineteen wished natural and helpful secondary metabolites. This paper describes the anti-fungal and anti-yeast actions of conventional antibiotics and three different ethanolic, methanolic, and ethyle acetate extracts of Juniperus communis fruits. Phytophthora infestans (16.09 ± 0.19, 13.26 ± 0.15, and 21.19 ± 0.24 respectively), P. oryzae (20.07 ± 0.23, 13.03 ± 0.15, and 23.11 ± 0.28 respectively), Microsporum canis (19.08 ± 0.22, 15.37 ± 0.18, and 23.94 ± 0.28 respectively), and C. glabrata (13.83 ± 0.15, 21.19 ± 0.24, and 18.01 ± 0.21 respectively). Posaconazole (PCZ) and Voriconazole (VCZ) as standard anti-fungal activity were (24.09 ± 0.29 and 29.36 ± 0.38) respectively. Capsicum frutescens metabolites was very highly active against Microsporum canis (23.94 ± 0.28). Antioxidant activity (Superoxide radical scavenging and Nitric oxide radical scavenging) of (methanol, Ethyl acetate, Ethanol extract and standards) of Capsicum frutescens. recorded 19.60 ± 1.01, 23.76 ± 1.30, 26.36 ± 1.39 and Quercetin (standard) 39.82 ± 2.07 respectively of Superoxide radical scavenging. 35.00±2.08, 46.51±2.93, 30.28±2.67 and Curcumin (standard) 83.12±4.07 respectively of Nitric oxide radical scavenging

Conclusion: Bell pepper extracts, like Capsicum frutescens, have antifungal properties against a few fungal infections that cause food poisoning. There were determined to be biologically active substances, including alkaloids, flavonoids, polyphenols, and sterols. As a result, Capsicum fruits could be used in food and medicine as a natural antibiotic. This article provides insight into pepper's use and supports the use of Capsicum fruit extracts for antifungal activity. The scientific basis for pepper's usage in cooking and traditional medicine should be strengthened by these findings.

**Keywords:** Capsicum frutescens, GC/MS, Antioxidant, Antifungal, Metabolites.

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## Introduction

The berries or the fruits of the capsicum plant are well utilized in the spicy dishes across the globe. Uses that have been touted against some of the many health benefits that are related to the use of capsicum species include hunger stimulation and digestion enhancement, the relief of numerous common ailments. The consumers and the scientists are in equal measure very concerned with the discovery of inexpensive foods for health and prevention of diseases [1, 2]. Capsicums, commonly known as bell peppers are flowering plants that are grown in many of the Latin American and some Asian countries within the Solanaceae family. Globally, the species of capsicum are cultivated with the purpose of producing seeds for conical aspects and other sauces. Five species of capsicum are available for purchase: The vegetables that are falling under chilli pepper family are bell peppers, cayenne, jalapeno, tabasco, Thai and piri piri and others are habanero, Scotch bonnet peppers of Capsicum chinense, rocoto peppers of Capsicum pubescens and aji peppers of Capsicum baccatum. Their most important role is, of course, culinary but besides that, people cultivate them as a vegetable, use in form of spice, and prepare remedies from. In the combating of human illnesses and their prevention, plant derived compounds have been crucial. For the future pharmaceuticals, they present a substantive base to them and are primary candidates for further chemical modification in the search for small molecule inhibitors from plants. An example of the application of enzyme inhibitors is as a weapon against diseases or for the improvement of chemotherapy. Capsicum is rich in calcium, pro vitamin-A (carotene), [3-5] vitamin C (ascorbic acid). It is this vegetable that occupies the second place in popularity in the world, trailing only behind the potato. Fresh pepper fruits could provide one hundred percent daily value for vitamin C and sixty percent daily value for vitamin A if the consumer takes fifty to one hundred grammes of fresh fruits. The fruits' uses can be traced back as remedies for illnesses such as colds, cough, diarrhoea, and discomfort. They are also employed in traditional medicine in management or conditions associated with high cholesterol and lipids in the blood [6, 7 ]. The bioactive molecules that are pharmaceutically active are vitamins and other secondary metabolites such as flavonoids, phenols, terpenoids, steroids as well as alkaloids. People all over the globe consume an average 3. Yearly, the global population consumes 5 million metric tonnes of dried fruits while pepper remains the most popular spice [8, 9]. Daily Nigerian's vegetables consumption reveals that 40 percent of them are peppers. The current study was conducted to; establish whether Capsicum frutescens had antifungal and antioxidant attributes by having the bioactive metabolites analysed.

## Materials and Methods

### The Method of Storing and Concentrating Plant Materials and Agents

The ingredients included end products from the Capsicum frutescens plant. The selected Capsicum frutescens were dried as that is how they used to be employed. The fruits were first washed and then proceeded to be dried using an oven at 60◦C for the next 48 hours. After that they allowed to cool and then transferred in mortar and ground into a fine powder. When 100 mL of distilled water and 10 g of powder were boiled for 15 minutes, the aqueous extract was obtained. The filtrate was, after that, dried in an oven at 50◦C for 2 hours with the help of Whatman paper No. 2 from Whatman International, Maidstone, England. The extract density after its' concentration was 100 mg mL−1 after trying to re-suspend in distilled water. For the preparation of methanol extract, 10 grammes of the fruit powder was dissolved in 60 millilitres of methanol. Allow the mixture to stand at room temperature for 15 minutes at 22◦C ± 2◦C after shaking. We collected the supernatant. Again, the filtrate was mixed with 60 mL of methanol and shaken, then the bulk of supernatant was once more, separated. This extraction procedure had three versions. Subsequently, the obtained extract was filtered using Whatman paper No 2. The liquid was transferred through a filtering pump into a vial and dried in an oven at 50◦C. The final concentration of the extract was 100 mg mL −1 extract obtained by re-suspension of the dried extract in distilled water.

### Hyphenated technique for chemical analysis such as gas chromatography and mass spectrometry.

The process of depositing the sample in the heated injector block in combination with the process of gas chromatography is the process of vaporising the sample and then putting it onto the head of a chromatographic column containing a non-volatile liquid stationary phase. Since distinct parts of a mixture are soluble related to the density and affinity level of the stationary phase, these aspects spread the components. It is seen that the flow rate and mode of the

carrier gas of the column does affect the elution of the separated components. As for the reasons, to analyze samples of large number of molecules that are not very volatile but thermally stable, GC separation is carried out at high temperatures normally in the range of 150 $C^0$ to 300 $C^0$.

**Methods for Cultivating Plants and Extracting Their Active Ingredients**

The culture medium was autoclave sterilised at 121°C for 15 minutes in a 15pis\inch2 chamber, following the manufacturer's instructions. The fungus have been isolated and cultured using this technique.

**An Investigation into the Antifungal Activity of Metabolites Juices**

The antifungal activity of phenolic extracts at three doses (100, 200, and 300 mg\mL) was investigated using the mixing method in conjunction with Sabouraud dextrose agar (SDA). From each concentration, 0.1 mL was transferred to a Petri dish. After pouring the SDA medium on top, the dishes were let to solidify. Then, using a sterile cork borer, a 5 mL disc was removed from each fungus and deposited on top of the culture medium [10, 11]. The dishes containing the petry are kept in an incubator set at 25°C ± 2 for a duration of 7 days. The phenolic extracts' antifungal activity is ascertained by gauging the inhibitory zone diameter, which is measured in millimetres (mm) using the ruler Mohammed AS. Finding the percentage inhibition of diameter growth (PIDG) Using the data collected from the MFC experiment, the following equation was used to calculate the PIDG values:

**Percentage inhibition of diameter growth PIDG (%) = Diameter of sample – Diameter of control / diameter of the control × 100 …. (11)**

**Antioxidant [Superoxide, Nitric oxide radical scavenging]**

**Superoxide radical scavenging**

This activity was previously measured by lowering NBTs, and the approach that was followed for this was one that has been published before. In the presence of superoxide radicals, the PMS/NADH system reduce NBT to a purple formazan Since the superoxide radical is the primary product of O2.Graded Assignment Page 4 To 1 ml of reaction mixture varying concentrations of sample solution (0-20 µg/ml), 20 mM phosphate buffer (pH 7. 4), sodium NADH (73 µM), NBT (50 µM) and PMS (15 µM) were added. This was measured at 562 nm and compared with a suitable blank after incubation for 5 minutes at room temperature of the amount of formazan that was produced. For all the tests note that we repeated each test six times. Quercetin was used as a positive control for this study purposed [12, 13].

**Nitric oxide radical scavenging**

SNP dissolves in water to create nitric oxide, and at physiological pH it per reacts with oxygen to form nitrite ions. Such ions can be determined by the use of the Griess Illosvoy reaction. In the final volume of 3 ml, the reaction mixture consisted in the phosphate buffered saline, pH 7.4, various concentrations of the test solution (from 0 to 70 µg/ml), and 10 mM SNP. After 150 minutes of incubation of the plates with the solutions at 25°C, to 0. 5 ml of the incubated solution, 1 ml of sulfanilamide (0. 33% in 20% glacial acetic acid) was added and allowed to stand for 5 minutes. After adding 1 ml Of napthylethylenediamine dihydrochloride (NED) (0. 1% w/v) to the mixture the same was incubated at 25°C for 30 minutes. The concentration of the formed pink chromophore arising from the diazotized sulphanilamide coupled with NED was measured at 540 nm against a blank sample [14, 15]. They are all repeated six times each test. A standard was curcumin.

**Data Analysis by Statistic**

Software used was statistical from IBM Company based in New York, NY, USA while Tukey HSD test was used in analysis of variance with mean value differences tested at either 95% or 99% confidence interval. The cut off point used for statistical significance in this study was a p-value of less than 0. 05.

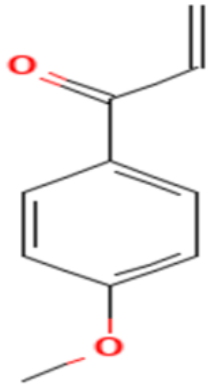
## Results and Discussion

Analysis of the secondary metabolites of *Juniperus communis* It was proven that there are more than nineteen natural and effective compounds using GC-MS technology. These compounds are: N,N-Dimethylhexadecylamine, N,N-Dimethylformamide diisopropyl acetal, beta-Terpinene, 4-Aminomethyl-hepta-1,6-dien-4-ol, 4'-

Methoxyacrylophenone, pentacene-6,13-dione, N,N-Dimethylhexadecylamine, 1'-Acetoxyestragole, alpha-Turmerone, Dimethyl icosanedioate, 9,12-Octadecadienoic acid, ethyl ester, 1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-, n-Octadecane, Heptadecane-2,4-dione, 9,10-Octadecadienoic acid, cis-9-Heneicosene, linoleic acid, and palmitic acid. Bioactivity of the methanolic, ethyle acetate and ethanolic extract of Juniperus communis and standard antibiotics against four fungi and yeast. *Phytophthora infestans* (16.09 ± 0.19, 13.26 ± 0.15, and 21.19 ± 0.24 respectively), *P. oryzae* (20.07 ± 0.23, 13.03 ± 0.15, and 23.11 ± 0.28 respectively), *Microsporum canis* (19.08 ± 0.22, 15.37 ± 0.18, and 23.94 ± 0.28 respectively), and *C. glabrata* (13.83 ± 0.15, 21.19 ± 0.24, and 18.01 ± 0.21 respectively). Posaconazole (PCZ) and Voriconazole (VCZ) as standard anti-fungal activity were (24.09 ± 0.29 and 29.36 ± 0.38) respectively [Figure 1,2,3 and 4]. *Capsicum frutescens* metabolites was very highly active against *Microsporum canis* (23.94 ± 0.28). Antioxidant activity (Superoxide radical scavenging and Nitric oxide radical scavenging) of (methanol, Ethyl acetate, Ethanol extract and standards) of *Capsicum frutescens*. recorded 19.60 ± 1.01, 23.76 ± 1.30, 26.36 ± 1.39and Quercetin (standard) 39.82 ± 2.07respectively of Superoxide radical scavenging Figure 5. 35.00±2.08, 46.51±2.93, 30.28±2.67 and Curcumin (standard) 83.12±4.07 respectively of Nitric oxide radical scavenging Figure 6.

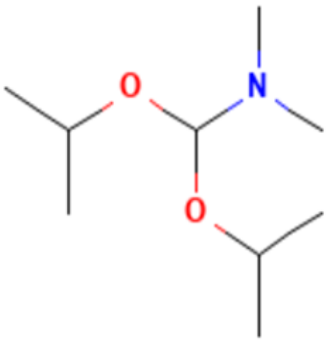
4'-Methoxyacrylophenone

M.W.: 162.18



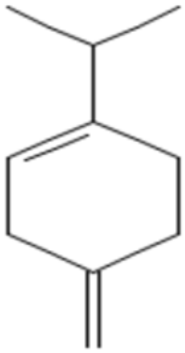

N,N-Dimethylformamide diisopropyl acetal

M.W.: 175.27



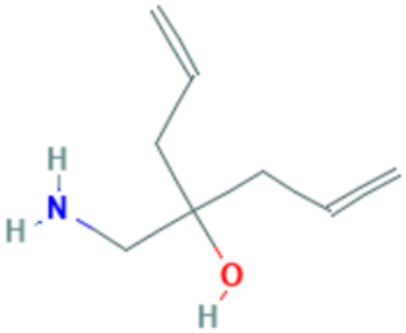

beta-Terpinene

M.W.: 136.23



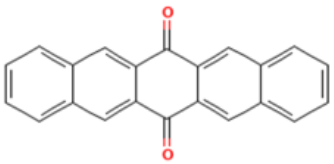
4-Aminomethyl-hepta-1,6-dien-4-ol

M.W.: 269.5 g/mol



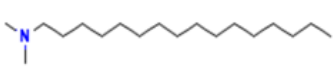

pentacene-6,13-dione

M.W.: 308.3 g/mol



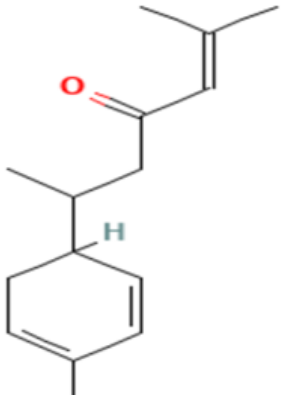

N,N-Dimethylhexadecylamine

M.W.: 269.5 g/mol



alpha-Turmerone

M.W.: 218.33

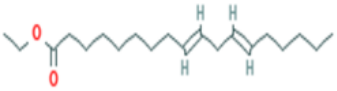


Dimethyl icosanedioate

M.W.: 370.6

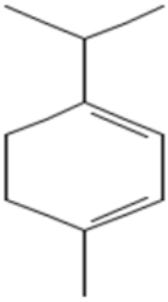
9,12-Octadecadienoic acid, ethyl ester

M.W.: 308.5



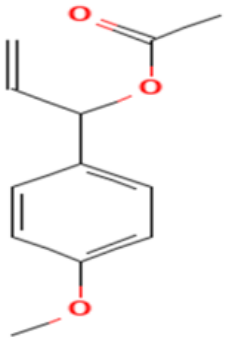
1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-

M.W.: 136.23



1'-Acetoxyestragole

M.W.: 206.24

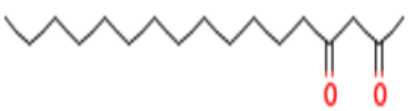


n-Octadecane

M.W.: 254.5

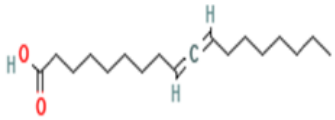
Heptadecane-2,4-dione

M.W.: 280.4



9,10-Octadecadienoic acid

M.W.: 280.4

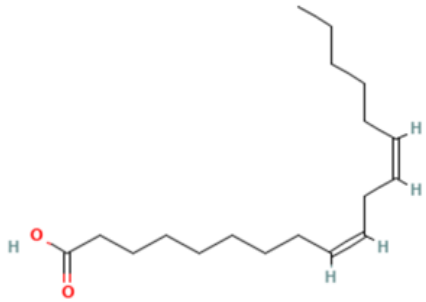


cis-9-Heneicosene

M.W.: 127.5

linoleic acid

M.W.: 280.4



palmitic acid

M.W.: 256.42

**Figure 1. Secondary Metabolites Compounds derived from ethanol, ethyl acetate and methanolic extract of *Capsicum frutescens* as anti-fungal activity against *Phytophthora infestans***

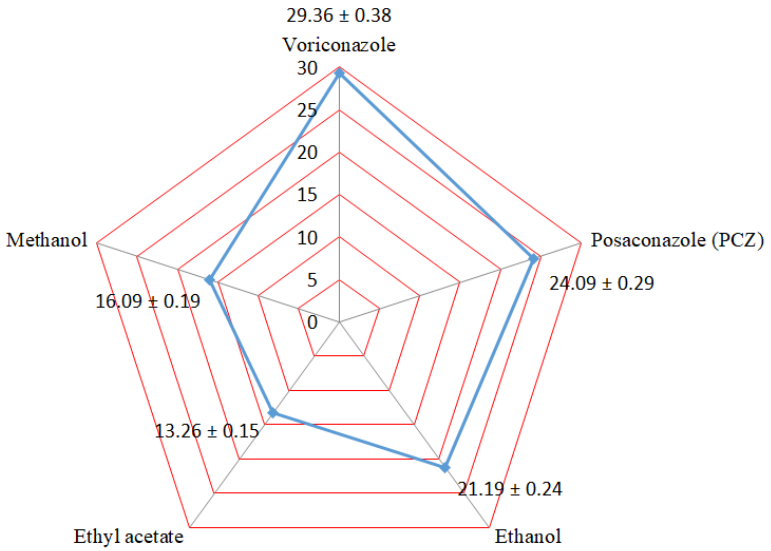



**Figure 2. Secondary Metabolites Compounds derived from ethanol, ethyl acetate and methanolic extract of *Capsicum frutescens* as anti-fungal activity against *P. oryzae***

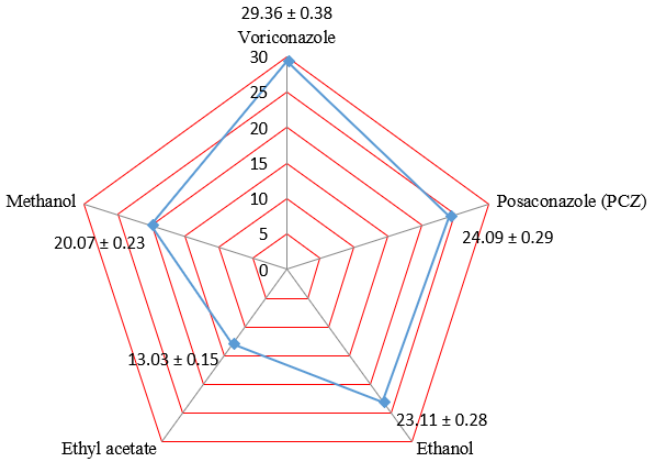



**Figure 3. Secondary Metabolites Compounds derived from ethanol, ethyl acetate and methanolic extract of *Capsicum frutescens* as anti-fungal activity against *Microsporum canis***

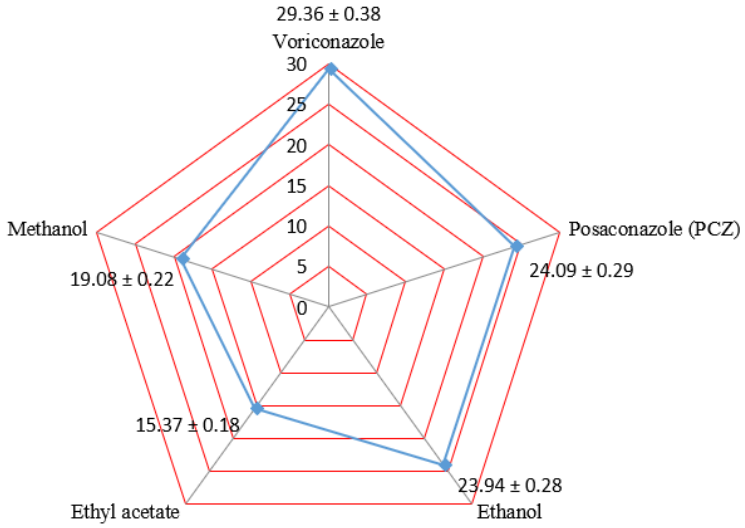


**Figure 4. Secondary Metabolites Compounds derived from ethanol, ethyl acetate and methanolic extract of *Capsicum frutescens* as anti-fungal activity against *C. glabrata***

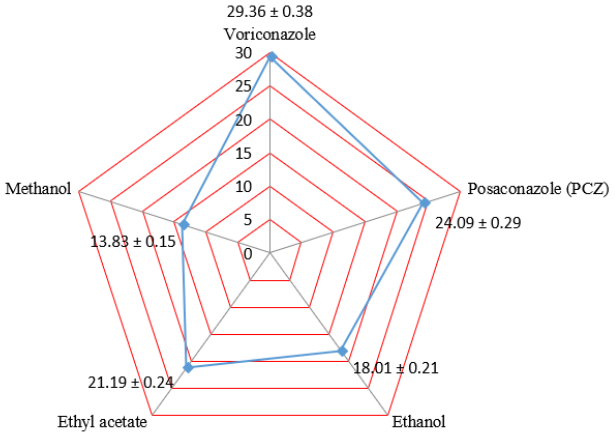



**Figure 5. Antioxidant activity (Superoxide radical scavenging) of *Capsicum frutescens* (extract (Methanol, Ethyl acetate , Ethanol fraction) and Quercetin (standard**

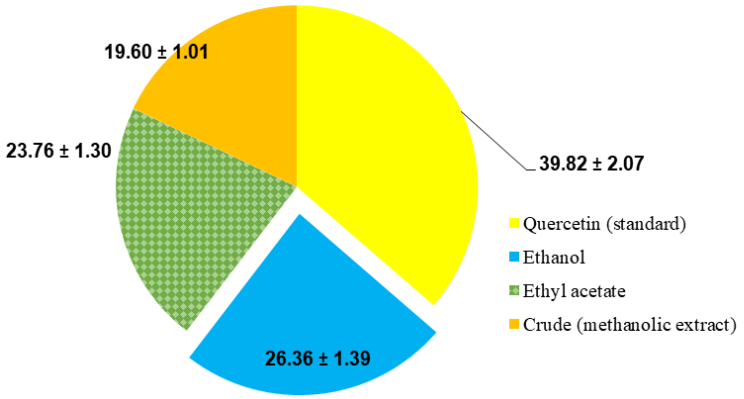
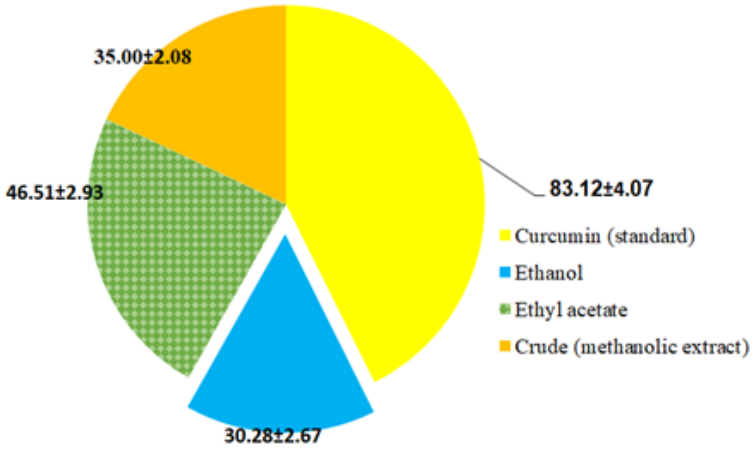



**Figure 6. Antioxidant activity (Nitric oxide radical scavenging) of *Capsicum frutescens* (extract (Methanol, Ethyl acetate , Ethanol fraction) and Curcumin (standard**




The following phytochemicals such as alkaloid was found in all the extracts from the Capsicum pepper and this phytochemical has antimicrobial activity due to several pathways. Cytotoxic manifestations against the cells of an alien organism are inherent to the properties of alkaloids. Since the above actions may contribute to the reduction and

even complete elimination of human cancer cell lines, there is much research on these actions. It is for this reason that alkaloids can alter immunological state in vivo due to anaphylactic, anti-inflammatory, and anti-asthmatic conferences [16, 17]. Moreover, there are powerful opioid analgesic agents which base has its origin in alkaloids. The two tested capsicum varieties being Capsicum annuum var Antillais and Capsicum frutescens var Soudanais have flavonoids in their respectively isolated forms. Apart from their antioxidant, anti-inflammatory, anti-angionic, analgesic and anti allergic properties, flavonoids possess cytostatic and antibacterial properties. These antioxidants are very vital in the prevention of disorders associated with oxidative stress on the DNA, proteins and cell membranes. To try and control the onset of menopausal symptoms, or indeed, reduce the risks of contracting cancer, flavonoids should be included in the diet. All this imply the feasibility of Capsicum fruits as a folk remedy This is due to the fact that Capsicum fruits are easy to come by as compared to some of the remedies that are practiced by men. That and for other reasons, several African tribes use these fruits to treat complaints of all sorts. Moreover, flavonoids exhibit antitrypanosomal and antileishmanial activities other than themselves being antibacterial. Based on several epidemiological researches it has been demonstrated that flavonoids lower the risk of coronary heart disease on intake. Moreover, the evidence of antiviral properties has been identified in some flavonoids, to some extent. Previous findings of steroids and polyphenols with antibacterial properties have been made earlier. Another category member that has been reported to have antibacterial action is the polyphenols. Steroids as a group of secondary metabolites contains large number of compounds and represent a rather heterogenous group [18–21]. The specifics of these investigations are tied to the global increase in antibiotic resistance, which has revived interest in researching plants' antibacterial properties. Preventing the synthesis of cellwalls and cellular aggregation result in exhaustion and this is the well-established bactericidal action of medicinal herbs against microbes. The pc of a bacterium has adhesive material around the cell constituted as a wall and this acts as a barrier to the entry of some substances into the protoplasm. Permeability barrier function of cell membranes helps in solute transport, metabolic regulation and turgor pressure regulation. As a result, the passive diffusion of active principles has to be evaluated alongside with the estimation of bacterial membranes permeability to shed more light on the mechanism of action of antimicrobials at the molecular level. Flavonoidal compounds have been identified to exert a beneficial influence on the wound healing rates of both skin and irritated mucosa and tannins are recognized feats of free radical species [22, 23]. So, the wound is closing and the rate of epithelialization is going up because of both groups of metabolites. We believe this to be the first research to show that MECa, via its antibacterial activity mechanisms, may treat infected wounds in rats.

## Conclusion:

This experimental work aimed to explore several features of the medication, including phytochemical and pharmacological research, bearing in mind the medicinal characteristics of *Capsicum Frutescence*. The combination of gas chromatography and mass spectrometry makes for powerful chemical analysis instruments. Bell pepper extracts, like *Capsicum frutescens*, have antifungal properties against a few fungal infections that cause food poisoning. There were determined to be biologically active substances, including alkaloids, flavonoids, polyphenols, and sterols. As a result, Capsicum fruits could be used in food and medicine as a natural antibiotic. This article provides insight into pepper's use and supports the use of Capsicum fruit extracts for antifungal activity. The results of this study provide scientific evidence that supports the use of pepper in traditional medicine and as a dietary ingredient. Extracted fruits of Capsicum species were tested for phytochemicals, antioxidants, and percentage yields. The extracts of Capsicum species were found to have the maximum yield and concentration of components when ethanol was used. It was in the ethanolic extracts that the antioxidant activity was greatest. According to the results of this study, Capsicum species contain a lot of important natural antioxidants that can be utilised in phytotherapy to fight or slow down the effects of ageing and oxidative stress that comes with it.

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