



Research Article

ANTIBACTERIAL OF VARIOUS FRACTIONS SEED EXTRACT OF BLACK PEPPER (PIPER NIGRUM) AND INVESTIGATION OF ITS BIOACTIVE FUNCTIONAL GROUPS USING FOURIER-TURNFORM INFRARED SPECTROSCOPIC TECHNIQUE

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Abstract

Black pepper is an edible perennial vine which is widely cultivated for its fruit, which is a spice, and used for medicinal purposes and scientific name is *Piper nigrum* and the family is Piperaceae. The active compound of black pepper is piperine, which is 1-piperoylpiperidine. Other minor alkaloids present in *P. nigrum* fruits include the likes of; piperlongumine, piperidine, starch and resin. Piperine is an alkaloid that is present in the fruits and roots of species under the genus *Piper* belonging to the Piperaceae family. Piperine is the cause of pungency in *P. nigrum* together with chavicine, which is an isomer of piperine. These include the action of phytochemical, namely piperine, which is seen to influence the pain reducing process of pepper. It is also having anti-inflammatory and antioxidant activity. In the present research, the secondary metabolites of the extract of the seeds of *Piper nigrum* are also studied and the antibacterial activity of the obtained fractions is determined using in vitro experiments. 25mg of powdered seeds samples were weighed and transformed into pellets with KBr and also a thin layer of the pellets were pressed as a preparation for FTIR test. Intensity (wavenumber cm^{-1}), (Nature of intensity, identification of bond/functional group) were alkyl halides, Alkenes, Aromatic, Aldehyde, Alkane and Amide. According to the type of extract (methanol, Ethyl acetate fraction, and Ethanol fraction) recorded 17.06 ± 0.29 , 14.73 ± 0.25 , and 20.00 ± 0.29 respectively in *Klebsiella pneumoniae*. While recorded 19.70 ± 0.27 , 15.00 ± 0.26 , and 22.95 ± 0.30 for *Enterococcus faecalis*. At the same time record 10.50 ± 0.11 , 13.00 ± 0.16 , and 18.30 ± 0.29 *Streptococcus pyogenes*. While recorded 17.00 ± 0.29 , 10.00 ± 0.11 , and 12.75 ± 0.15 *Staphylococcus aureus* and 12.97 ± 0.15 , 14.50 ± 0.25 , and 14.00 ± 0.20 *Bacillus cereus* in comparison with KN-Kanamycin 25.00 ± 0.31 and RF-Rifampicin 27.75 ± 0.35 . The metabolites of *Piper nigrum* exhibited significant activity against *Streptococcus pneumoniae* (22.95 ± 0.30).

Keywords: Antibacterial, Black pepper, Bioactive Functional Groups, Fourier-transform.

INTRODUCTION

Black pepper is a spice crop recognized worldwide and is locally referred to as pilipili mwitu. It is a basal angiosperm blooming vine that is mostly dried to be used as a spice fruit. The one that is derived from the family Piperaceae it being a spice. Within the climbing shoot there are internodes Known as the 'main shoot,' and laterals that branches off the main shoot have shorter internodes and the spike. Spice is one of the most valuable goods it controls the world trading market. Black pepper is known as 'the king of spices' and locally it is known as 'Golmarich' in the Bangladesh. It is originated in Kerala at South-western India, Malaysia, Indonesia, West Indies and South America. Black Pepper has also been used in cooking and as traditional medicine for centuries after the dried berries have been ground into a spice. In the present time the method of in vitro cultivation is known to the science breeders as one of the most frequently used practices of growing plants that are not well portable but possess economical importance to the world market. Spicy fruits, resinous oils and essential oils are used as flavours in confectionery, food specification and preservation and cosmetics and perfumery industries [1, 2]. There is Piperine which is an essential oil, which constitutes a pungent alkaloid, responsible for the spiciness. Research has shown that piperine can enhance the availability of many medicines into the body. Safrole, sabinene, pines, limonene, linaonol and caryophyllene at a low percentage are present.

Santra et al. [3-5] stated that black pepper has anesthetic, antimicrobial, antimutagenic, free radical scavenging, immunomodulatory, antitumor, antidepressant, anti-apoptotic, and ant-metastatic effects, antithyroid, antitumor, antidepressant, anti-apoptotic, ant metastatic, anti-thyroid, hepatoprotective, immune. Black pepper fruit is also used commonly for pulmonary diseases, cold extremities, intermittent fever, stomach disorders, colic disorder, and diarrhea. This paper aimed to examine the phyto-compounds that can be obtained from black pepper as a potential solution since the COVID-19 is still an alarm to humanity. Black pepper has shown evidence of insecticidal action against the pests. Nerolidol has its secondary metabolite for the management of mites. Essential oils are constituted with another odor called Pipene. The pepper plant is an evergreen, woody climber that can grow up to 4 meters on the support that is secondary such as trees or poles as well as trellis. Black pepper plant is a woody climber plant, which has the minimum height or length of about 10 meters [6, 7]. After reaching a certain age it has many lateral branches and becomes a bushy column in the centre. Their arrangement on the above stalks is opposite while the underside of the leaves is light green; the top of the leaves are dark green and glistening. Above and or side of it, dark green and shiny while below it is a light green. Oval, narrower at the end, dark shining green on the upper side, lighter green on the under part. Margins of the leaf are entire or with small teeth and the leaves are 5–20 cm long and 3–6 cm wide. I agree with a help of Shango et al. that the leaves blade, which is 17.9 cm, the leaf petiole which is 2.8 cm, and wider leaves 12.9 cm, all belong to Tanzania. Kidney-

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shaped, elongate, ovate-lanceolate or ovate-elliptic are the types of shapes that the leaf laminae may possess. The stems of black pepper can grow to a height of 10 meters with a diameter of about 6 cm. The stem is herbaceous, fragile and of green color, Its height vary from 8 to 11 centimeters [8-11]. The stem carries the leaf the blossom and even the fruit. Depending on the function, the structures on a pepper plant stem include the primary stems, runner shoots, and fruiting stems. According to the penetration degree of access to the pot, a single stem can support 20–30 fruiting spikes. Berries are most frequent and include round drupes, measuring 6 mm in diameter and known as peppercorns. Initially the fruits have a green coloration, as they ripen they change to a red, red coloration. In general, a 50 to 60 fruits are obtained from each spike [12, 13]. The objective of this research therefore is given as innovation and analysis of the bioactive functional group present in the compound through FTIR Technique and secondly determination of the compound's antibacterial activity.

MATERIALS AND METHODS

Plant Material Black pepper seeds of *Piper nigrum* L. were purchased from local stores Hilla station, Babylon, Iraq.

Report on analysed Black pepper plant using Fourier transform infrared spectroscopy (FTIR)

Many spectra of FTIR data that were obtained previously from an FTIR instrument were calibrated and newly processed in the laboratory with the help of mainly PC-based software so as to obtain these FTIR spectra for each of the GLVs. This was actually achieved for the purpose of obtaining the FTIR spectra of both these categories of GLVs. To prepare for the experiment FTIR analysis, to obtain a homogeneous and suitable small amount of laboratory crushed leaf samples, it was made into pellets using KBr and at the same time, a suitable thin layer of the studied mixture was formed by physically compacting the sample. Thus, while data was actually obtained on the transmission of infrared light, studied and valid information was also gathered in the range of wave numbers $4000\text{ cm}^{-1} - 500\text{ cm}^{-1}$. Here all experimental samples were exposed to three different tests and the KBr pellets that were not treated in any way were used as a control.

Assessment of antimicrobial activities

The antibacterial activity of Black pepper was carried out through Agar well Diffusion Method. Bacterial cultures are prepared by inoculating nutrient agar medium and dispersing the bacterial isolates and the suspensions are poured in petriplates. Small wells of 5mm diameter were created into the agar media with the bacterial inoculum with the help of sterile borer. 2mg of crude Black pepper (*Piper nigrum* L.) was dissolved to its entirety in 2ml of methanol, ethyl acetate and ethanol. Antibacterial activity was determined by the addition of 0.05 ml from the extract with other concentrations being 0.5, 0.7 and 0.9% into the wells and allowed to diffuse for one hour [14] before incubation at the suitable temperature for the specific period of time. After the incubation period the microbial growth inhibition zones were measured else recorded for comparison.

Statistical Analysis

Statistical analysis was achieved using the analysis of variance (ANOVA test) with the comparison of the average mean

values obtained by the use of odds ratio at a 95% or 99% confidence interval, and employing SPSS 19.0 statistical analysis based on the software 'IBM, New York, NY, USA' and Tukey's HSD test. The evidence obtained is statistically significant if the p-value is less than 0. Therefore, the value of 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Black pepper still holds a position of the most frequently used spice in culinary and sometime in food distribution and in perfumery. Economically it is the most important and the most utilized spice crop globally. The quality of a spice depends on the odor, color, and Pungency of peppercorn as stated in [15-17]. Anandaraj's view on this concerning all parts of the plant under controlled condition on nutrient media is that in vitro cultivation of all parts of plant on nutrient media develops under controlled conditions. In folk medicine black pepper is used as a flavoring agent as well as to correct appetite, absorption, dyspepsia, and the fleshiness of the body. Of the reoccurring diseases, CVDs, diabetes mellitus, inflammation disease, and the emergence of cancer are some of the health challenges facing mankind today [18, 19]. Several ethical animalistic studies have explicated the benefits that black pepper has regarding the prevention of diseases because per 100 grams contain much energy, protein, carbohydrate, fibre, etc. It is a rich source of tryptophan, isoleucine, threonine, leucine, lysine, cystine, methionine, tyrosine, phenyl alanine, valine, histidine, arginine, glycine Some of the key aspects that demonstrate the health promoting possibility of the bioactive compounds that are present in black pepper are mentioned below. It has the ability to reduce inflammation and has antioxidant effect. In the present work, bioactive compounds isolated from seed of *Piper nigrum* are examined along with the extract against bacterial system in vitro. As for the FTIR analysis a small amount of the powdered seeds sample was converted into pellets using KBr and applying pressure in order to form a thin film. Peak (Wave number cm^{-1}), (Type of Intensity, Bond and Functional group assignment) included alkyl halides, Alkenes, Aromatic, Aldehyde, Alkane and Amide. According to the type of extract (methanol, Ethyl acetate fraction, and Ethanol fraction) recorded 17.06 ± 0.29 , 14.73 ± 0.25 , and 20.00 ± 0.29 respectively in *Klebsiella pneumoniae*. While recorded 19.70 ± 0.27 , 15.00 ± 0.26 , and 22.95 ± 0.30 for *Enterococcus faecalis*. At the same time record 10.50 ± 0.11 , 13.00 ± 0.16 , and 18.30 ± 0.29 *Streptococcus pyogenes*. While recorded 17.00 ± 0.29 , 10.00 ± 0.11 , and 12.75 ± 0.15 *Staphylococcus aureus* and 12.97 ± 0.15 , 14.50 ± 0.25 , and 14.00 ± 0.20 *Bacillus cereus* in comparison with KN-Kanamycin 25.00 ± 0.31 and RF-Rifampicin 27.75 ± 0.35 (Figure 1-5). The metabolites of *Piper nigrum* exhibited significant activity against *Streptococcus pneumoniae* (22.95 ± 0.30).

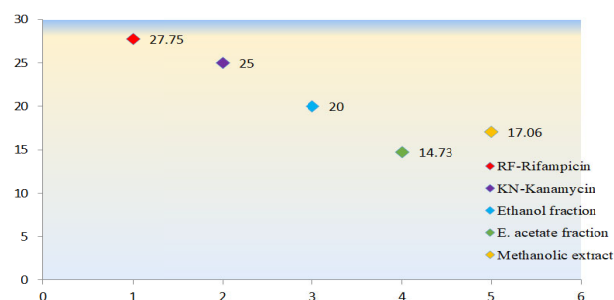
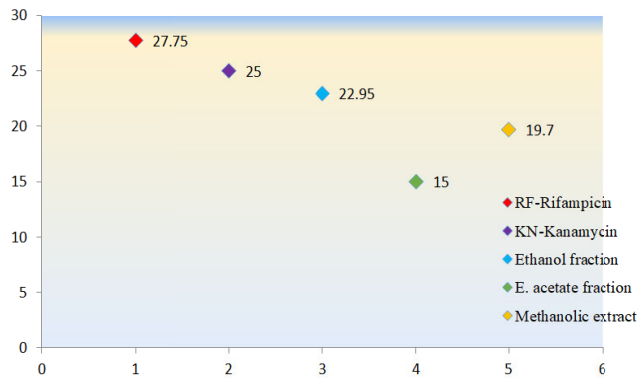
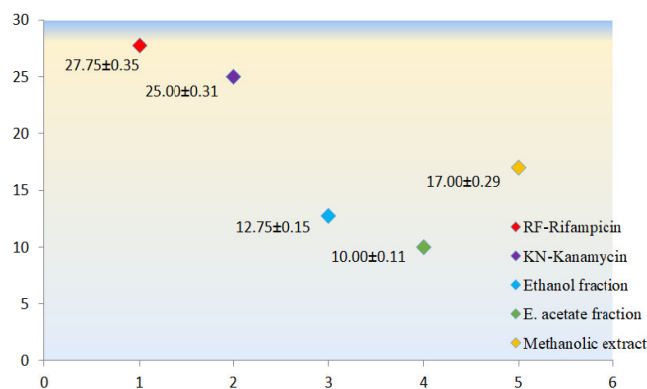
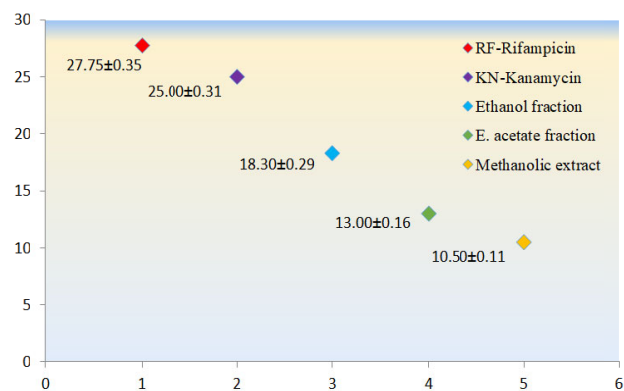
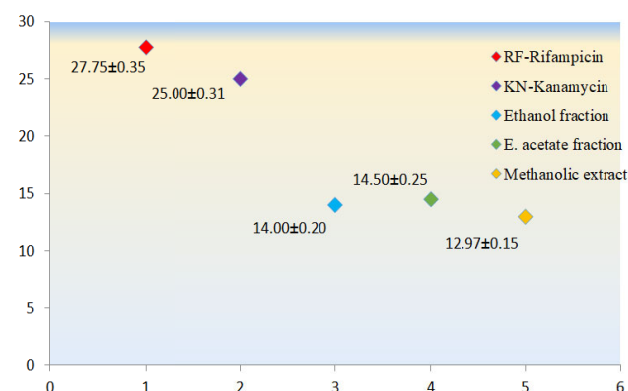


Figure 1. Inhibitory efficiency of *Piper nigrum* against *Klebsiella pneumoniae* compared with standard antibiotics

Table 1. Fourier-transform infrared spectroscopic profile solid analysis of seed extract of black pepper (*Piper nigrum*)

No.	Peak (Wave number cm^{-1})	Intensity	Corr. Intensity	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.37	63.602	1.458	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	873.75	75.464	2.486	Strong	=C–H	Bending	Alkenes	650-1000
3.	921.97	76.037	0.115	Strong	=C–H	Bending	Alkenes	650-1000
4.	1026.13	54.832	19.670	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1139.93	72.360	1.874	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1234.44	79.518	0.208	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1317.38	80.927	1.224	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1379.10	78.194	0.230	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1415.75	75.384	1.784	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1519.91	83.275	0.580	Medium	C=C	Stretch	Aromatic	1400-1600
11.	1598.99	75.718	0.635	Medium	C=C	Stretch	Aromatic	1400-1600
12.	1740.72	87.747	6.874	Strong	C=O	Stretch	Aldehyde	1720-1740
13.	2852.72	86.395	3.584	Strong	C-H	Stretch	Alkane	2850-3000
14.	2922.16	81.542	6.382	Strong	C-H	Stretch	Alkane	2850-3000
15.	3223.05	81.889	0.120	Bending	N-H	Stretch	Amide	3100-3500
16.	3265.49	80.746	0.173	Bending	N-H	Stretch	Amide	3100-3500

**Figure 2. Inhibitory efficiency of *Piper nigrum* against *Streptococcus pneumoniae* compared with standard antibiotics****Figure 4. Inhibitory efficiency of *Piper nigrum* against *Bacillus cereus* compared with standard antibiotics****Figure 3. Inhibitory efficiency of *Piper nigrum* against *Streptococcus pyogenes* compared with standard antibiotics****Figure 5. Inhibitory efficiency of *Piper nigrum* against *Staphylococcus aureus* compared with standard antibiotics**

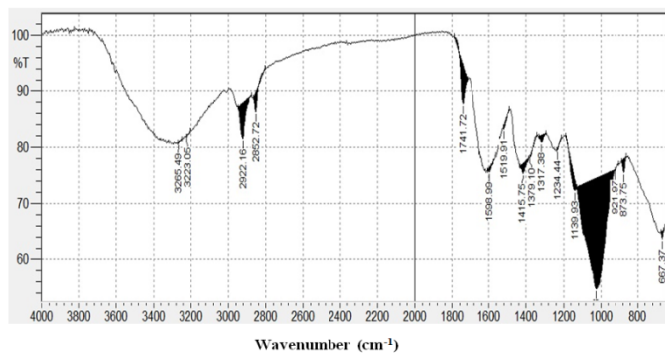


Figure 6. Fourier-transform infrared spectroscopic profile solid analysis of seed extract of black pepper (*Piper nigrum*)

In order to determine the antibiotics effect on carbapenem-resistant enterobacter hormaechei (CREH), vanillic acid mediated changes in the level of intracellular ATP concentration, intracellular pH and membrane potential were investigated. Furthermore the result observed was that concentration dependent alterations in the growth media by adding of vanillic acid (500 µg/mL vanillic acid 65%) also had a strong influence on biofilm formation and virulence of *S. marcescens* ATCC 14756 and MG1. *Cronobacter* spp. can possibly be controlled by vanillin, ethyl vanillin, and vanillic acid. the inhibitory effect to CREH during preparation and storage of foodstuffs as well as interference with the cell membrane of CREH.

On the experimental evaluation of antibacterial activity, it was found that black pepper which belonged to the family Piperaceae contained some metabolites which are effective in destroying bacteria. Moreover, in the ethanolic extract, chlorogenic acid content was identified to be very high in a tall plant, belonging to the Asteraceae family, namely *A. gmelinii*. It has been found in contemporary investigations that chlorogenic acid docks to the outer membrane and reduces its integrity and the intracellular negative value, besides releasing macromolecules in the cytoplasm and eliciting cell death. Microorganisms may contaminate food products; therefore, these products should be protected during storage. There are many comer health hazards with synthetic preservatives if used frequently. Therefore, consumers today pay their attention to try to apply new natural food additives according to the references, 20-23. Foods have been flavored and enriched with spices and herbs by many cultures for the benefit of the countries. Further, they employed in the preservation of foods and as natural antibiotics. Pepper is frequently used while prescribing ailments of Ayurveda as well as other systems of medication. It was further seen that the oleoresin of black pepper varied between the range of 10-15 per cent and its components include volatile oil, non -volatile oils, resins, fixed oil, colours, sugar etc. The quantity of volatile oil found was between 15 to 35 ml/100g.

Secondary metabolites synthesised by the black piper facilitate body metabolism and are a form of defence [24-27]. Academics are conducting a survey of many biological fields on Piper plants for various kinds of second metabolites to use for creating drugs and biocontrol products. Anti-apoptotic, antibacterial, anti-Colon toxin, anti-depressant, anti-fungal, analgesic, anti-diarrhea and anti-inflammatory, anti-mutagenicity, antimetastasis [28], antioxidation, antipyresis, immunomodulatory effect, anti-spermatogenic effect, hypothyroidism, antitumor, ciprofloxacin synergistic, colic, cold limbs

Oxidation is an irreversible process which takes place in the cells of the human body, as a result of which come undesirable reactions [29, 30]. ROS is generally considered as the factor being responsible for a number of diseases such as cancer, inflammation, atherosclerosis, and aging. Oxidative anxiety in the body overpowers the defensive capabilities by increasing the generation of ROS. An even greater number of spices grown and fragrant plants have been discovered. Most of the bioactive compounds include antioxidant capacity molecules in them. With 0. 43mm of black pepper ½ inhibition of free radicals. In another research, Chavarria et al. , discovered that water and ethanol portion of black pepper at seventy five g/ml inhibit lipid oxidation by 95%. 5% and 93. 3%, 50% and 53% respectively in the previous experiments and total phenolics content should be affected and must decrease, which is 54%. 3 and 42. 8 g/mg, respectively.

Polyphenol contents were slightly higher at 191 mg/100g Polyphenol content has been followed by qualitative estimation of antioxidant activities by in-vitro methods which gives promising results. It was also proposed that normal antioxidants available in the diet might be found in black pepper. Thus, due to these functional components present in black pepper it can be concluded that black pepper has the potential for reducing oxidative stress. The high power of antioxidant, potential to chelate metal ions and free radical trapping capacity also play their role in these effects. Pepper helpful in disease preventive approaches that contain ROS and kindred molecules. Similarly, in another study, piperine given at a dose of 10 mg / kg / day intraperitoneally corrected diabetes induced changes in the Sprague-Dawley rats through an experimental model of oxidative damage using diabetes mellitus. The benefits are due to antioxidant properties that prevent LDL oxidation through the modulation of prostaglandin and leukotriene formation [31, 32]. For this reason, black pepper can contain antioxidant activity but that has to be examined in subsequent randomised condone trials. Thus, the outcomes of such studies will help to provide that black pepper with its bioactive compounds will be utilized as a natural antioxidant.

It is noteworthy that plants have been used for a long time as antibacterial agents. The use of natural items, particularly spices, as antimicrobials has two benefits: a better health status and prevention of spread of infectious diseases. Concerning the antibacterial action of phytochemicals incorporated in these items, there has been significant research in the recent past. Of all these, it has been ascertained that phenolics possesses antibacterial effects. Potential antibacterial properties of black pepper and piperine will help minimize the colonization by *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Salmonella typhi*. At the same time extracts from black pepper demonstrated efficient antagonistic effect towards *Staphylococcus*, *Bacillus*, and *Streptococcus* bacterial strains; These findings are in partial agreement with the study by Weerakkody et al. Thus, with the MIC of black pepper extract being 125, 250, and 500 ppm, it was concluded that the extracts prevented the growth of bacteria like *Staphylococcus*, *Bacillus*, and *Streptococcus*. Brahma et al. found that black pepper alkaloids exercised possibility to act as insect- and rodent-repellent in this context. This spice a number of animals have major reactions to and avoid any fresh ground peppers including black pepper. It is also said to be effective against house flies as well as possess or ability to inhibit eggs or laying of eggs by antiparasitic activity against *Giardia* and

Entamoeba species. Black pepper essential oil can also be used to act as a safeguard for cereals from insects thus guard against worm attack. Some of the black's bioactive pepper compounds have been identified because of their allelopathic role in managing insect infestations and their efficiency. The use of Black pepper oil is also beneficial and has proven applicability with immense antibacterial effects on microbial pathogens belonging to animals. Altogether, black pepper possesses a respectable antibacterial effect in opposition to a vast array of bacilli that can be applied for multiple purposes, including food preservation and Infections that are prevented. Also, it possesses anti-inflammatory activity. Black pepper has shown ability to attend the body's neuro-protection against depression and Alzheimer's disease. Black pepper contains antibacterial and antiviral compounds, that might help cure COVID-19 patients. The significance of a cooperated effect of black pepper in the increase of minerals and medicinal compounds absorption cannot be overestimated [33, 34]. In terms of nutraceuticals, health-care consultants should consider black pepper in the following manner. Further steps will be needed to evaluate its proper usage in the range of goods, and the outcomes of such studies will introduce the scrupulousness into concerns.

Conclusion

Black pepper seed is one of the most vital spices in the global markets. This spice is rather picky when it comes to the climate conditions. Its varieties are applied not only in cooking or thermal food material but also serve as medicine or biological product in the world since it comprises bioactive compounds. Taste components of black pepper include piperine, oleoresin, and essential oil; it also aids in the control of digestive system. Therefore, and according to the results obtained in the present work and to recent bibliography, it is a possibility to conceive the usage of new therapeutic protocols for the resistant uneven infectious diseases by employing the natural extracts from pepper.

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