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EXTRACTION, ISOLATION AND CHEMICAL IDENTIFICATION OF PIPERINE ALKALOID FROM BLACK PEPPER SEEDS AND ITS ANTIBACTERIALACTIVITY

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Abstract

Black pepper (*Piper nigrum*) is belong to the Piperaceae family. Its a spices crop, which is used as to enhancing the digestion of food and an essential component in commercial medicine. The aim of this study to show the antibacterial activity of black pepper and the active compound of its (piperine alkaloid) and identify the structure by reagents, TLC technique and melting point. Antibacterial activity was checked) against. *E.coli, Staphylococcus aureus, Pseudomonaas, Streptococcus, Aeromonnas, Klebsiellae*, *Acinetobacter*, which were showed a good biological activity significant of piperine alkaloids compared to crude black pepper and synthetic drugs cefuroxime.

Key words : Black pepper, piperine, antibacterial activity, isolation and identifications.

Introduction

Natural products have been a rich source of bioactive compounds, some of these compounds showed significant antibacterial activity (Abdallah and Abdalla, 2018). Black pepper plant botanical name are *Piper nigrum* belongs to the family Piperaceae. The part of plant contain most active compound *alkaloid piperine* usually seeds. It is cultivated forits fruit, which is usually dried and widely used as a seasoning and spices throughout the world, it is known as the king of spices (Abdallah and Abdalla, 2018; Saha *et al*., 2013). Various piper species have been used as spice in folk medicine due to attributed physiological and pharmacological activities and thus reason cause black pepper bear a great medicinal, economic and commercial potential (Aditi *et al*., 2013). Black pepper has been extensively used in improving appetite, also enhancing the digestion, cold, subsidizing the sorethroat breathing and heart muscle problem, diabetes and anemia.

In addition, to treat the eyes problem, also it is applied as an ointment. In traditional medicine Extract pepper used as antispasmodic and anti flatulent in Greek medicine. Some research suggests that piperine used as antimicrobial, centralnervous system depressant and potential in the treatment of vitiligoas it helps increase pigmentation in the skin (Kavitha and Mani, 2017).

Piperine has important role in pharmacotherapeutics as it has been classified as (potential bioavailability enhancer improver) by promoting rapid absorption and also having the effect on inhibiting the enzyme metabolizing, which are responsible for nutrients or biotransformation of drugs and thus preventing their inactivation and elimination (Khan *et al.*, 2017). Black pepper have much pharmacological action such as antifungal, anti-inflammatory, anticancer effect, antioxidant and it is known to have insecticidal activity against flies and mosquitoes (Shingate *et al*., 2010).

Ganesh *et al*. (2014) studied the antibacterial activity of ethanol extract of (*Piper nigrum*) showed against *Salmonella typhi, Proteus* sp, *Escherichia coli* and *Staphylococcus aureus* whereas *Pseudomonas aeruginosa* showed resistant and chloroform extract of (*Piper nigrum*) showed highly antibacterial activity against *Salmonella typhi*, *Escherichia coli*, *Proteus* sp. and *Staphylococcus aureus* whereas, *Pseudomonas aeruginosa* showed resistant (Ganesh *et al.*, 2012).

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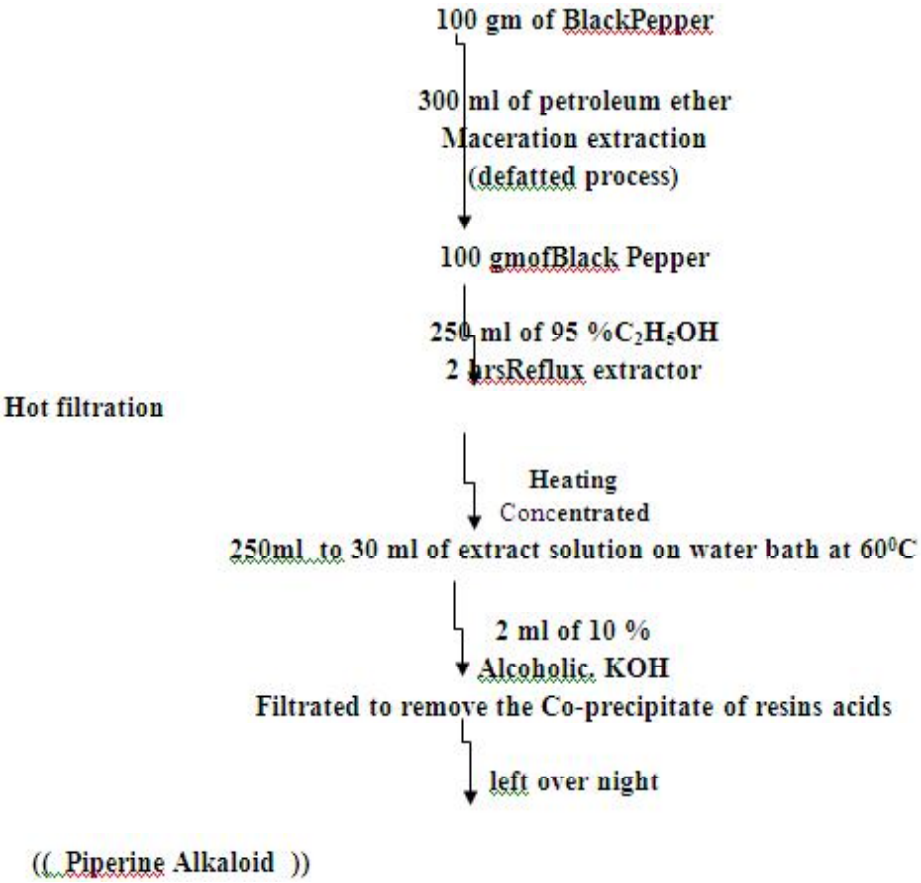


Scheme 1 : Extraction of piperine from black pepper.

Fig. 1 : Yellowish brown needles of piperine.

Materials and Methods

Materials

Black Pepper seeds were purchased from the local market of Nasiriya and grounded to a powder then kept in dry container.

All chemicals obtained from the pharmacy college laboratory. The work was performed at the organic chemistry laboratory.

Methods of extraction and isolation of piperine alkaloids

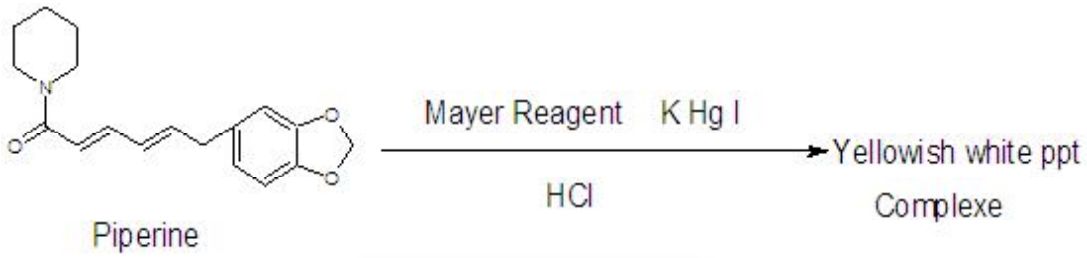
We were extracted and Isolated piperine alkaloids from Black Pepper in this study. The Maceration Extraction was prepared by mixing 100 gm of Black Pepper powder with 300 ml of petroleum ether 1 day for defatted process to removed non polar constituents, which are available in black pepper plant such as aromatic oil, waxes, resins. The ethanol extract was prepared by mixing 100 gm of Black Pepper powder with 250 ml of 95% ethanol. Then the extraction process done by Reflux extractor condenser for 2 hours. Then the extracted solution was collected and then cooled. Then concentrated to 30 ml of extract solution on water bath at 60^{0}C (Shingate *et al*., 2013). Then added 2 ml of 10% Alcoholic KOH to the concentrated solution of Black Pepper to precipitate the acidic resins, then filtrate after 3 hours, finally alcoholic extract is left over night to get Yellowish brown needles with melting point of 124^{0}C are deposited. Yielding 0.8 gm of piperine alkaloids.

Results and Discussion

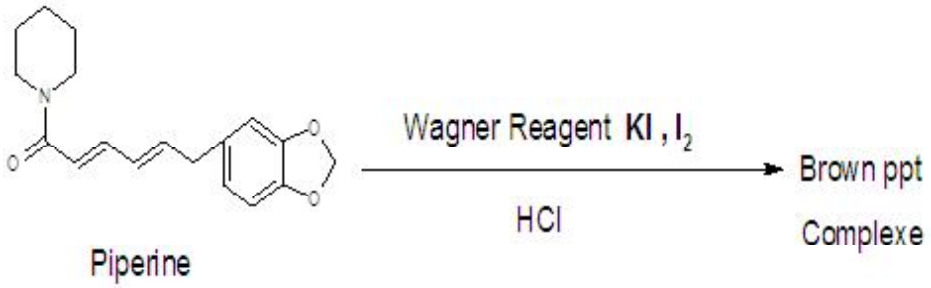
Chemical identification of pure piperine alkaliods

Quantitative Analysis : was done by weighing the crystals of Piperine alkaloid.

Results : yellow needles, yielding 0.8gm of Piperine alkaloids.



Scheme 2 : Mayer test.



Scheme 3 : Wagner test.

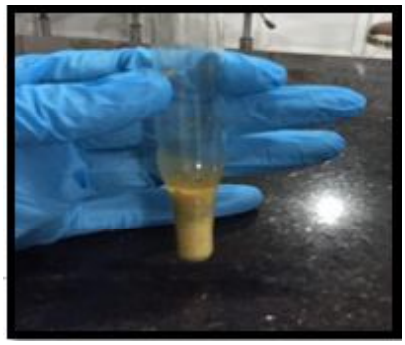


Fig. 2 : Yellowish white formation (Mayer's Test).

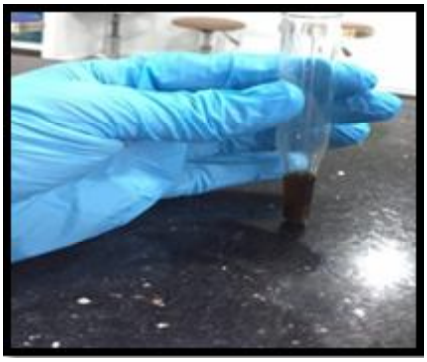


Fig. 3 : Brown precipitate formation (Wagner 's Test).

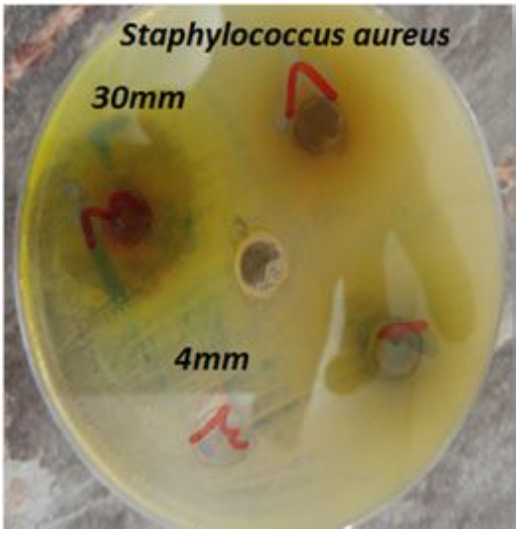


Fig. 4 : Showing zone of growth inhibition of extended spectrum β-lactamase producing bacteria (*Staphylococcus aureus*) by the General Black pepper (no. 3) and piperine (no. 4) extract on Muller Hinton agar (Safronova *et al*., 2015).

Qualitative Analysis (Chemical identification)

After Piperine alkaloids isolated from the acid resins and other polar constituents included in Black Pepper by the method showed in above scheme no.1. It was identified chemically by the followed test and TLC, melting point.

Mayer's test

Mayer's reagent is freshly prepared by dissolving a mixture of mercuric chloride (1.36 g) and of potassium iodide (5.00 g) in water (100.0 ml). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (potassiomercuriciodide solution) to give white to a ceramic colored precipitate.

Procedure

Take few crystals of piperine alkaloid and dissolve in few ml of ethanol, in Petri dish then add 2 drops of HCL. Then add 2 drops of reagent.

Result : a yellowish white precipitate formed.

Wagner's test

Aim to indicate in general the alkaloid as other

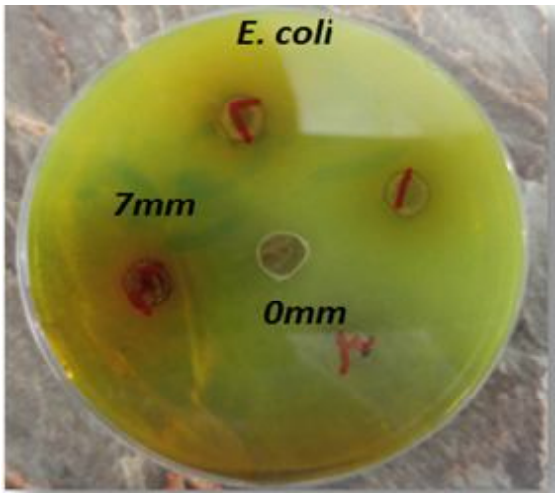


Fig. 5 : Showing zone of growth inhibition of extended spectrum β-lactamase producing bacteria (*E. coli*) by the General Black pepper (Number 3) and piperine (no. 4) extracts on Muller Hinton agar (Jansons and Jurenoks, 2012).

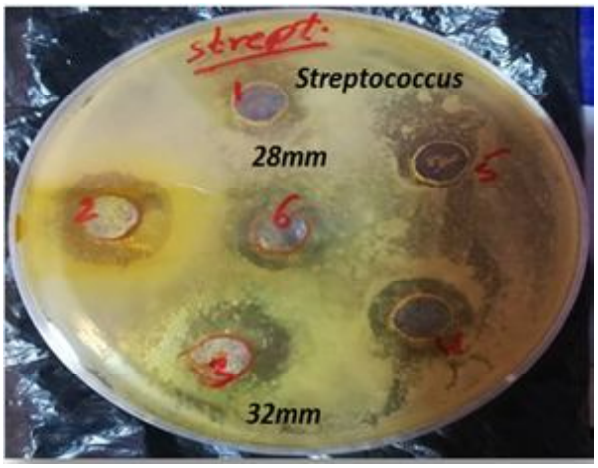


Fig. 7 : Showing zone of growth inhibition of bacteria (*Streptococcus*) by the General Black pepper (no. 6) and piperine (no. 3) extract on Muller Hinton agar (Zhou, 2016).

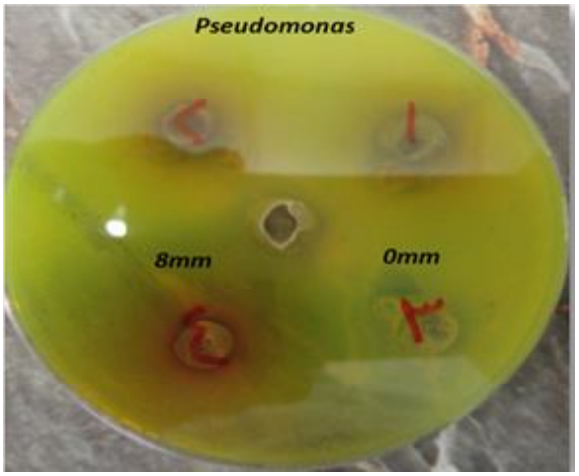


Fig. 6 : Showing zone of growth inhibition of extended spectrum β-lactamase producing bacteria (*Pseudomonas*) by the General Black pepper (no. 3) and piperine (no. 4) extracts on Muller Hinton agar (Zhou, 2016).

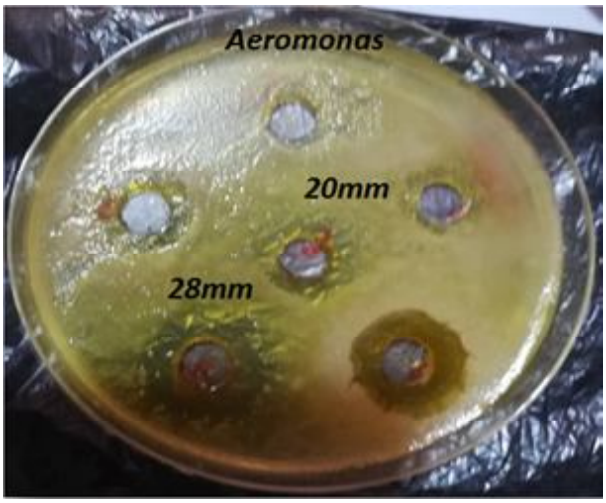


Fig. 8 : Showing zone of growth inhibition of (*Aeromonas*) by the General Black pepper (no. 3) and piperine (no. 6) extracts on Muller Hinton agar.

Table 1 : Diameter zone of inhibition (mm) of bacterial growth by plant extracts.

Types of isolates	CXM*	Antimicrobial agent		
		Plant extracts		
		General Black Pepper	Piperine	Test no.
Staphylococcus aureus	18.7mm	4 mm	30mm	1
E. coli	R	0 mm ®	7 mm	2
Pseudomonas	R	0 mm ®	6 mm	3
Streptococcus		28 mm	32mm	4
Aeromonas		20 mm	28mm	5
Klebsiella		20 mm	42 mm	6
Acinetobacter		40 mm	10 mm	7

* CXM= CEFUROXIME.

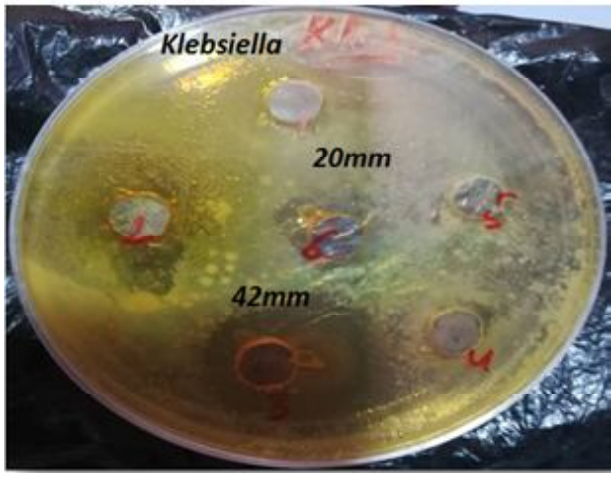


Fig. 9 : Showing zone of growth inhibition of extended spectrum β-lactamase producing bacteria (*Klebsiella*) by the General Black pepper (no. 3) and piperine (no. 6) extracts on Muller Hinton agar.

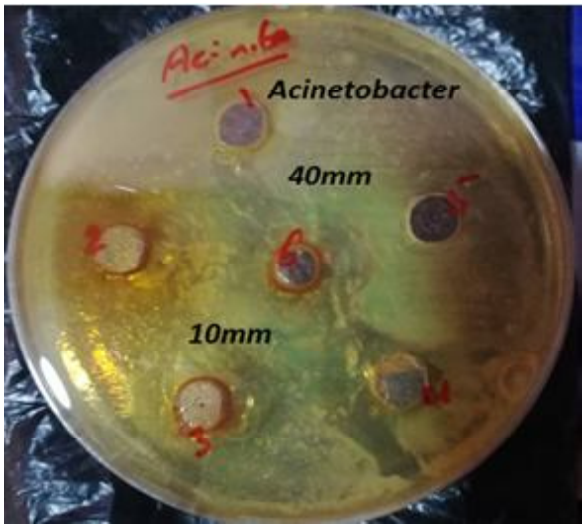


Fig. 10 : Showing zone of growth inhibition of bacteria (*Acinitobacter*) by the General Black pepper (no. 6) and piperine (no 3) extracts on Muller Hinton agar.

alkaloids. Dissolve 2gm of Iodine and 6 gm of KI in 100 ml of water.

Procedure : Take few crystals of piperine alkaloid and dissolve in few ml of ethanol, in Petri dish then add 2 drops of HCl. Then add 2 drops of Wagner's reagent.

Result : Brown precipitate formed.

Chromatography

After isolation of Piperine, it was identified by use thin-layer chromatography (TLC), Silica gel G. used as a stationary phase while the mobile phase = *Ethanol : hexane* (7:3). The spray reagent = dragendorff's reagent. The standard Rf value of Piperinee from the literature was 0.25. The Rf value of purified. Piperine from TLC was found to be 0.23 (Shingate *et al*., 2013).

Result : Orange spot will appear.

Melting point

Melting point of Piperine alkaloid 123^{0}C, which was appeared close to the melting point of piperine 125^{0}C from the literature.

Stock solution preparation

The stock solution of Black Pepper and the active compound piperine For the test No (1-3) done by dissolving 0.1 gm of the Black Pepper and the active compound piperine in 20 ml of ethanol to get a concentration of 5 mg/ml which was the concentration tested while the test No (4-7) done by dissolving 0.4 gm of the Black Pepper and the active compound piperine in 10 ml of ethanol to get a concentration of 40 mg/ml which was the concentration tested as shown in table 1. Sterilization was done by filtration wares through a Millipore 0.45 mm and 0.22 mm.

Biological activity

The anti-bacterial activity of the black pepper and the active compound (piperine) which was extracted and isolated chemically from the black pepper.was determined in avitro using the agar cup method. All the compound were tested for activity against (*Staphylococcus aureus, E. coli, Pseudomonas*, *Streptococcus*, *Aeromonas*, *Klebsiella*, *Acinetobacter* (Deepti *et al*., 2012). From the data of table 1, its indicated that the active compound of black pepper (piperine) were highly active (Alka *et al*., 2017) against the selected pathogens compared to the crude black pepper except the test (no. 7) showed activity of black pepper against *Acinetobacter* more than a Piperine. The result are summarized in table 1.

Conclusion

As discussed previously above, isolated piperine alkaloids from black pepper seeds was identified by chemical test Wagner's, Mayer's, melting point 124^{0}C and TLC. The results of biological activity study suggest that the ethanol extraction of Piperine alkaloids founded very much effective against *E. coli, Staphylococcus aureus*, *Pseudomonas*, *Streptococcus*, *Aeromonas*, *Klebsiella*, *Acinetobacter*. Compared to the crude black pepper as seen in inhibition zone except *Acinetobacter*, which showed good inhibition zone with crude black pepper. The most important in our study the piperine alkaloids more effective than the synthesized drugs CXM = cefuroxime.

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