

# **ANTIBACTERIAL ACTIVITY OF *PIPER CUBEBA* LINN. FRUIT EXTRACTS AGAINST SELECTED BACTERIAL PATHOGENS IN BASRAH CITY**

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## **ABSTRACT**

In this study extracts of *Piper cubeba* fruit were prepared using mechanical method. These extracts include alcoholic, acetonic, chloroformic and water extract. The chemical composition of each extracts were analyzed also. After that the antibacterial activity of these extracts were tested against gram negative ( $G^-$ ) *Escherichia coli*, *Pseudomonas aeruginosa* and gram positive ( $G^+$ ) *Staphylococcus aureus*. All extracts show antibacterial activity on these bacteria, but ethanol and acetone extracts were show the best antibacterial activity *S. aureus*, followed by chloroform extract and then water extract, on the other hand water extract showed inhibition activity against *E.coli* and *P. aeruginosae* followed by ethanol, acetone and chloroform extract. MIC was determined for each extracts against the three pathogenic bacterial strain.

## **INTRODUCTION**

Traditionally plant are used as source of treatment of diseases in different parts of the world (1,2). Many plant-derived molecules have shown a promising effect in therapeutics (3). Among the plant investigated to date, one showing enormous potential is the pepper family otherwise known as Piperaceae (4).

The genus Piper of family Piperacea with over 1000 species, is distributed in both hemispheres *Piper cubeba* Linn., commonly known as cubeb, tailed pepper (due to the stalks attached) jawa pepper (in jawa) and kemukus (in Indonesia) is a climbing perennial plant (5). With climbing stem, round branches, the leaves are from four to six and a half inches long and a half to two inches broad, the fruits of this plant are used as a spice and have medicinal value, being often used for the treatment of abdominal pain, asthma, chronic bronchitis, diarrhea, dysentery, gonorrhoea, enteritis and syphilis (6) and reported to have an inhibitory effect on hepatitis C virus protease

(7). *P. piper* is used also as antibacterial (8), and as gastroprotective (9). Recently, many bacterial pathogens are becoming resistant to existing antibiotics due to their indiscriminate use in the treatment of infectious diseases (10, 11, 12). There is not much work has been done on the antimicrobial activity of their fruits in world generally and in Iraq and Basrah specially. Therefore, the present study has been designed to assess the antibacterial efficacy of fruit extracts of *P. cubeba* against selective bacterial pathogens isolated from infection cases of animals in Basrah city

## **MATERIALS AND METHODS**

**Fruits/** *P. cubeba* were collected from the local market of Basrah. Prof. Dr. Abdul Rada Akber Aloan, (Biology Department), College of Science. Basrah University confirmed the identification of the specimens.

### **Preparation of Fruit extraction:**

The samples were carefully washed under running tap water followed by sterile distilled water, and air dried at 40°C for 5 days and pulverized to a fine powder using sterilized mixer grinder and stored in air-tight bottles (1).

Four different solvent were used in this study ethanol, acetone, chloroform and aqueous solvent for the extraction of plant material. An amount of 10g. of pulverized fruit was separately soaked in 200 ml of acetone, ethanol, chloroform and sterile distilled water in magnetic stirrer (SHMP HO-13) for 24h. Each preparation was filtered through a sterilized whatman No.1 filter paper (13, 14,15).

Filtered extracts were air dried at 40°C for 24 h. The obtained dried extracts were exposed to UV rays for 24 h., then stored in labeled sterile bottles in a deep freeze at -18°C until further use (16).

### **Isolation of Bacterial spp.:**

The species of bacteria include:

*Staphylococcus aureus* as G<sup>+</sup>

*Escherichia coli* , *Pseudomonas aeruginosa* as G<sup>-</sup>

They were clinically isolated from infected animal (cow, dog and chicken) in the Veterinary Hospital in Basrah City. Bacterial cultures were maintained on nutrient agar slant (Difco) at 4°C, re-identified by biochemical test (17,18) and subcultured on to nutrient broth and incubated aerobically at 37°C for 24h., prior to testing.

### **Preliminary Qualitative Chemical Tests for *Piper cubeba* Linn.:**

Four extracts were tested chemically to identify their chemical compounds by following tests:

- 1- Ninhydrin test: for peptides and free amino groups (19).
- 2- Benedict test: for glycosides (20).
- 3- Myer's reagent: for alkaloids (21).
- 4- FeCl<sub>3</sub> (1%): for phenols (22).
- 5- Alcoholic KoH: for flavonoids (20).
- 6- Lead acetate (1%): for tannins (23).
- 7- HgCl<sub>2</sub> (5%): for saponins (24).

#### **Antibacterial activity:**

Antibacterial activity of the 4 crude extracts was determined by the agar diffusion method (25).

In this method, pure isolate of each strain was subculture on the nutrient agar plates and incubated at 37°C for 24h. a minimum of (4) colonies of each plates were touched with a sterile loop and transferred into normal saline (0.85%) under a septic conditions. Density of each bacterial suspension was adjusted equal to that of 10<sup>6</sup> cfu/ml (standardized by 0.5 MCFarland standard) and used as the inoculums for performing agar well diffusion assay. 0.1 ml of inoculums of each test strain was inoculated on Muller Hinton agar (MH) agar plates with sterile swab. The agar plates were allowed to dry and 6 mm-diameter wells were made with a sterile cork-borer in the inoculated agar plates. The dried fruit extracts were recon- situated in 20% dimethylsulfoxide (DMSO) for the bioassay analysis.

A 100 µl volume of each extract was propelled directly into the wells on plates for each test strain. The plates were allowed to stand for 10 min. for diffusion of the extract to take place and incubated at 37°C for 24h. (26), sterile DMSD served as the negative control and ciprofloxacin served 5 mg. as the positive control. The antibacterial activity indicated by the formation of an inhibition zone surrounding the well containing the extract, was recorded if the inhibition zone was greater than 8mm (27).

#### **Determination of Minimum Inhibitory concentration (MIC):**

The MIC for the acetonc, ethanolic, chloroformic and aqueous extract was determined by the following modified agar well diffusion method (28).

A twofold serial dilution of each extract was prepared by first reconstituting the fruit extract in 20% DMSO followed by dilution in sterile distilled water to achieve a decreasing concentration range of 25 mg/ml to 0.1 mg/ml. A 100 µl volume of each dilution was introduced into wells on the MH agar already seeded with 0.1 ml of standardized inoculum ( $10^6$  cfu/ml) of the test bacterial strain. All test plates were incubated aerobically at 37°C for 24h. and observed for the inhibition zones. The lowest concentration of each extract showing a clear zone of inhibition (>8mm.) considered as the MIC, was recorded for each test bacteria (29).

## RESULTS AND DISCUSSION

The results under this study revealed in table (1) the ethanolic, chloroformic and acetic extracts of *P. cubeba* fruits extract contain several chemical compounds like glycosides, alkaloids, phenols, flavonoids, tannin and saponins but without free amino group as compared with aqueous extract contain glycosides, phenols, Flavonoids and tannins only. These results agree with (30, 31) who recorded that dried fruits of *P. cubeba* contain up to 10% essential oil composed of monoterpenes (sabinene 50%, carene (alkaloid),  $\alpha$ -thujene (alkaloid), 1,4-cineol and 1,8-cineol (phenols) and sesquiterpenes (copaene, alkaloid,  $\alpha$  and  $\beta$ -cubebene (alkaloid),  $\delta$ -cadinene (alkaloid), caryophyllene (alkaloid), germacrene (alkaloid), cubebol (phenols).

(1) mention that the essential oil has antimicrobial activity by the action of destroyed the bacteria cell membrane. In addition, (32, 33 and 34) found that two groups of secondary metabolites, alkaloids (piperine) and lignans (cubebin) .

The result of antibacterial activity of crude extract against the tested bacteria were represented in table (2), which compared with the reference standard ciprofloxacin (5 mg/disc), while, the ethanol and acetone extracts higher activity against gram positive bacteria *S.aureus* (the inhibition zone was 16 mm. and 15 mm.) respectively and lowest against gram negative bacteria *E. coli* (10.10) mm. and *P. aeruginosa* (13.13)mm.

(35) found among the tested fruit extracts of *P. cubeba* that the acetic extract showed a greater antibacterial activity against *S. aureus* (18.96 mm.) and ethanolic extract (17.32 mm.). While chloroform extract has antibacterial activity on both gram positive and gram negative at effect (12 mm.) on *E. coli* , (10) mm. on *P.*

*aeruginosa* and (11 mm.) on *S. aureus* found closer results (36) and this agree with results under this study .

The fruit extracts of *P. cubeba* have antibacterial activity against all the tested strains in this study may be due to the presence of the alkaloid (piperine and cubebin) in the berries with phenol, flavonoids and tannins.

These types of compounds have reported with antibacterial activity (37). An Alkaloid in the fruits of *Piper* sp. is responsible for the possess antinflammatory, antiamebic, antiasthmatic, anticonvulsant and antibacterial activity (38, 39). Also Alkaloid has ability to link with bacterial DNA leading to kill it (40). While phenol compound has precipitate activity on microbial enzyme and leading to inhibit and lost their function (41).

(42) reported that hydroxyl group in flavonoids have ability to composite with cell wall proteins and break down the cell membrane of bacteria.

The aqueous extract showed higher antibacterial activity on gram negative *E. coli* (15 mm) and *P. aeruginosa* (15mm) and a lowest activity on *S. aureus* (8mm.). Absence of alkaloid in aqueous extract lead to decrease the antibacterial activity on gram positive bacteria while presence phenol and flavonoids increase the antibacterial on gram negative bacteria.

MIC of *P. cubeba* fruit extracts against bacterial pathogens determined by (mg/ml) are presented in table (3) acetonic and chloroformic extracts on *E. coli* survived up to 0.5 mg/ml while ethanolic and aqueous extract were 1 mg/ml. *S. aureus* having on MIC of 1 mg/ml for all extracts, but *P. aeruginosa* having an MIC of 0.5 mg/ml to all extract. This may be due to the rang of MIC between 0.5-1mg/ml without much variation in the different solvents activity.

For all the result above and the investigate that the crude extracts obtained from the fruits of the *P. cubeba*, may be used to treat bacterial pathogen like *S. aureus* as they produced larger inhibition zones that closer from ciprofloxacin.

In conclusion, the four extracts were use in this study show have good antibacterial activity but the differ got them depending on the type of bacteria were the best affect appear in the acetonic and ethanolic extract specially on *S. aureus*. While, aqueous extract effect on *E. coli* and *P. aeruginosae*.

**Table (1): chemical testes for some compounds of fruit *P. cububa* :**

Type of extract	Peptides free amino group test	Alkaloids test	Flavonoids test	Tannins test	Phenol test	Saponin test	Glycoside test
Acetonal	-	+	+	+	+	+	+
Chloroformal	-	+	+	+	+	+	+
Ethanoal	-	+	+	+	+	+	+
Aqueus	+	-	+	++	+	-	++

**Table (2): antibacterial activity of *P. cububa* fruit extracts against bacterial pathogens determined by diameter of inhibition zone (mm.):**

Type of extract	Diameter of zone inhibition (mm)		
	<i>E.coli</i>	<i>S.arus</i>	Pseudo.
Acetonal	10	16	13
Chloroformal	12	11	10
Ethanoal	10	15	13
Aqueous extract	15	8	15
Ciprofloxacin (positive control)	10	25	20
20% DMSO (negative control)	—	—	—

**Table (3): MIC of *P. cububa* fruit extracts against bacterial pathogens determined by (mg/L):**

Type of extracts	Minimum inhibitory concentration (mg/L)		
	<i>E.coli</i>	<i>S.arus</i>	Pseudo.
Acetonal	0.5	1	0.5
Chloroformal	0.5	1	0.5
Ethanoal	1	1	0.5
Aqueous extract	1	1	0.5

## الفعالية ضد البكتيرية لمستخلصات نبات *Piper cubeba* على ممرضات بكتيرية معزولة في مدينة البصرة

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### الخلاصة

في هذه الدراسة تم تحضير أربعة مستخلصات لثمار نبات الكيابة (*Piper cubeba*) وهي مستخلص كحولي واسيتوني وكلورفورمي ومائي بارد بطريقة الإذابة الميكانيكية. تم الكشف عن ما تحتويه هذه المستخلصات من مركبات كيميائية بعد ذلك تم اختبار الفعالية ضد بكتيرية لهذه المستخلصات في تثبيط نمو ثلاثة انواع بكتيريا اثنين سالبة لصبغة كرام *Escherichia coli* و *Pseudomonas aeruginosa* والثالثة موجبة لصبغة كرام وهي *Staphylococcus aureus* اظهرت النتائج ان المستخلصات الاربعة كان لها تأثير مثبتاً على البكتريا قيد الدراسة ، حيث كانت الافضلية للمستخلص الايثانولي والاسيتوني و الكلورفورمي والمائي ضد البكتريا *S. aureus* بينما كان تأثير المستخلص المائي على بكتريا *E.coli* و *P. aeruginosa* الاكثر تأثيراً وبعده المستخلص الايثانولي والاسيتوني و الكلورفورمي و تم تحديد التراكيز المثبطة الدنيا (MIC) لكل نوع من المستخلصات ضد الانواع البكتيرية الممرضة الثلاثة.

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