Evaluation of synergistic antimicrobial activity of *Cinnamomum zeylancium, Trachyspermum ammi* and *Syzygium aromaticum*

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Abstract

Antimicrobial assay of acetone and ethanol extract of *Cinnamomum zeylancium*, *Trachyspermum ammi* and *Syzygium aromaticum* was performed using agar well diffusion method against bacterial culture. *(E.coli, P.mirabilis and K.pneumoniae)* the acetone extract of *Cinnamomum zeylancium*, ethanol extract of *Trachyspermum ammi* and acetone extract of *Syzygium aromaticum* were selected to evaluate the synergistic activity. The activities were combined in the ratio of 1:1:1, 1:2:1 and 1:1:2 *(Trachyspermum ammi: Cinnamonum zeylancum: Syzygium aromaticum*). Phytochemical analysis was carried out for the ethanol and acetone extract of *Cinnamonum zeylancium, Trachyspermum ammi* and *Syzygium aromaticum*, to check the present of carbohydrate, proteins, sterioids, resins, tannins, glycosides, flavonoids, saponins and quinines.

Key words: antimicrobial, synergistic and phytochemicals.

Introduction

"A medicinal plant is any plant which, one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs" (Sofowora, 1982). *Trachyspermum ammi* is commonly called Ajowan belongs to the family *Apiaceae*. Its fruits yielded 2% - 4% brownish essential oils, with thymol as major constituent (35%-60%). The plant is used traditionally as a stimulant, carminative, flatulence, atonic dyspepsia, diarrhea, abdominal tumor, abdominal pains, piles, and bronchial problems, lack of apitite, galactogogue, asthma and amenorrhea. It possess various pharmacological activities like anti-fungal, an-oxidant, anti-microbial, antinoceptive, cytotoxic activity, hypolipidaemic, antihypertensive, antispasmodic, bronco-dialatic action, antilithiasis, diuretic, abortifacient, antitussive, nematicidal, anthelmintic and antifilarial activity. (K.jeet et al., 2012)

Clove (*Syzygium aromaticum*) are dried aromatic unopened floral buds of an ever green tree 10-20m in height, belonging to the family *Myrtaceae*, indigenous to India, Indonesia, Zanzibar, Mauritious and Ceylon. Cloves have many therapeutic uses. They control vomiting, cough, diarrhea, stomach distension, gastro-intestinal spasm, relieve pain, causes uterine contractions and stimulate the nerves. (Ambasta, 1986; Chaieb et al., 2007).

Cinnamon (*Cinnamomum zeylanicum*) is a small ever green tree, 10-15 m tall, belonging to the family *Lariaceae*, native to Sri Lanka and South India. The flowers, which are arranged inpanicles, have a green color and have a distinct odour. The fruit is a purple one-centimeter berry containing the single seed. It has also been used to treat diarrhea, and other problems of digestive system. Cinnamon is high in antioxidant activity; the essential oil of cinnamon also has antimicrobial properties, which aid in the preservation of certain food. "Cinnamon "has been reported to have remarkable pharmacological effect in the treatment of type II diabetes. **(**Anupriya Pandey1, et.al 2010).

Antimicrobial activity of some medicinal plant against *E.coli, K.pneumoniae* and *P.mirabilis* were highly inhibited .enteric of diarrheal infections are causes of major concern world wide antibiotic resistance bacteria are on the raise and it becomes important to identify new drugs with antimicrobial activity. *T.ammi, S.aromaticum* and *C.zeylanicum* are common ingredients in conventional formulations used in India for digestive aliment. The present work was aimed to investicate if these indigeneous drugs will have antibacterial pathogens and if they will exhibit synergetic interaction when tested together for antibacterial activity.

Materials and method

Collection of plant

The fruit pods of *Trachyspermum ammi* dried flower buds of *Syzygium aromaticum* and dried bark of *Cinnamomum zeylancium* was purchased during December 2013 from local market in Chennai.

Preparation of ethanol and acetone extract

The plant samples were pulverized into fine powdered substance by a grinder 20g of powder of *Trachyspermum ammi, Syzygium aromaticum* and *Cinnamomum zeylancium* was weighed with the electric balance and transferred into two separate 100ml conical flasks.40ml of ethanol in one flask and 40ml of acetone in another was added .The conical flask were closed by foil paper and placed in dark placed for maximum 7 days. The crude ethanol and acetone extract were then filleted by passing the extract through whatman no.1 filter paper, and then concentrated at 80°c by using heating metals. The residual extracts were stored in refrigerator at 40°c in small and sterile plastic bottles.

Test microorganisms

Stock culture of known bacteria (*E.coli* mtcc2068, P.mirabilis mtcc29906, and *K.pneumoniae* mtcc2883

Determination of antimicrobial activity

The antibacterial activity of ethanol and acetone extracts of *Trachyspermum ammi, Syzygium aromaticum* and *Cinnamomum zeylanicum* was evaluated by using agar well diffusion method. Nutrient agar plates were prepared for ethanol and acetone extract of *Trachyspermum ammi, Syzygium aromaticum* and *Cinnamomum zeylanicum*. 100µl inoculums of each selected bacterium was uniformly spread on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. 50µl, 100µ l, 150µl, and 200µl plant extract were poured into the wells. The plates were incubated at 37cC for 24 hrs.

Synergetic activity

The extracts of Trachyspermum ammi, Syzygium aromaticum, Cinnamomum zeylanicum that exhibited optimum anti-microbial activity were mixed in the ratios 1:1:1, 1:2:1, 2:1:1, 1:1:2 (Trachyspermum ammi: Syzygium aromaticum: Cinnamomum zeylanicum) and tested for their anti-microbial activity using agar well diffusion method. Nutrient agar plates were prepared and 100 l inoculums of each selected bacterium were uniformly spread on agar plates with the help of glass spreader. After five minutes three wells approximately 5mm diameter was bored with the help of borer. 50l and 100 l, of the mixed plant extracts were poured into the wells. The plates were incubated at 37°C for 24 hrs.

Phytochemical test

Test for carbohydrates

- Molish test: to about 2ml of extract few drops of Molish reagent was added. Then about 1ml of concentrated sulfuric acid was added along the side of the test tube. Appearance of reddish violet ring at the junction of the two layers indicates the present of carbohydrates.

Test for protein

- Millon's test: 2ml test solution, add about 2ml of millon's reagent, white precipitate appears which turns red gentle heating.

Test for steroid (Umesh et al., 2010)

- Salkowski's test: 2ml of extract was added to 2ml of chloroform and 2ml of concentrated sulfuric acid was added carefully and shaken gently. A reddish brown color indicated the presence of steroid.

Test for glycosides (Umesh et al., 2010)

- Killer- Killani test: 1ml of glacial acetic acid containing traces of ferric chloride and 1ml of concentrated sulfuric acid were added to the extract carefully. A reddish brown color is formed at the junction of two layer turns bluish green in the presence of glycosides.

Test for tannins (Ciuki, .1994)

- To 2ml of extract was added 2ml of 5% FeCl3. A dark blue or green black color indicates the presence of tannins.

Test for alkaloids (Ciuki, .1994)

- To 2ml of extract were added 2ml of conc. HCl and few drops of Meyer's reagent. A green or white precipitate indicates the presence of alkaloids.

Test for flavonoids (Umesh et al., 2010)

- To 2ml of extract was added to 1ml 2N NaOH. Yellow color appears.

Test for quinone (Umesh et al., 2010)

- 1ml of conc. H2So4was added to 1ml of extract. A red color formed.

Test for terpinoids (Evans 1989, Harbrone 1998)

- 2ml of extract is mixed with 5 ml of chloroform and few drops of concentrated sulfuric acid is carefully added to form a layer. A reddish brown coloration formed in the interference shows positive result for the presence of terpinoids.

Test for saponins (Evans 1989, Harbrone 1998)

- Foam test: The extract is mixed with 5ml of water and shaken vigorously. The formation stable foam is taken a positive result for saponins.

Test for resins (Evans 1989, Harbrone 1998)

- Acetone- H2O test: extract treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.

Result and discussion

Cinnamonum zeylanicum

In this present study, ethanol extract of Cinnamomum zeylanicum was found sensitive to E.coli, P.mirabilis and K.pneumoniae. The acetone extract of C.zeylanicum had a better effect when compared to the ethanol extract.

Trachyspermum ammi

In this present study, ethanol extract of Trachyspermum ammi had a better inhibitory effect against E.coli, P.mirabilis and K.pneumoniae, when compared to the acetone extract , which did not have any effect at 50µl.

Syzygium aromaticum

In the present study Syzygium aromaticum was found to be effective against E.coli, P.mirabilis and K.pneumoniae. Both the acetone an ethanol extract showed similar activity against all three E.coli, P.mirabilis and K.pneumoniae. The ethanol and acetone extract of Syzygium aromaticum showed higher antibacterial activities when compared to T.ammi, C.zeylanicum.

Synergistic activities

The extracts were also evaluated for synergistic interactions. The extract that exhibited maximum antimicrobial activity when tested alone was selected to evaluate the synergistic interactions. The ethanol extract of T.ammi, acetone extract of C.zeylanicum and acetone extract of *S.aromaticum* were selected and mixed the ratio of 1:1:1, 1;2;1, 1:1:2, 2:1:1 (*Trachyspermum ammi: Cinnamomum zeylanicum: Syzygium aromaticum*) an used for antimicrobial activity. The extracts were mixed in the ratio of 1:2:1 exhibited maximum synergistic activity against the bacterial culture tested.

DISCUSSION

In this present study, ethanol extract of *Cinnamomum zeylanicum* was found sensitive to *E.coli, P.mirabilis* and *K.pneumoniae*. Earlier, Masih Usha et al., 2012**,** reported that the ethanol extract of *Cinnamomum zeylanicum* was found to exert antimicrobial activity against *E.coli, Bacillus subtilis* and *Staphylococcus aureus.*They also reported that acetone extract of *Cinnamomum zeylanicum* produced antimicrobial effect against *E. coli* and *Bacillus subtilis.*In this present study, acetone extract of *Cinnamomum zeylanicum* was found to be sensitive against *E.coli, P.mirabilis* and *K.pneumoniae.*

In this present study, ethanol extract of *Trachyspermum ammi* was found sensitive to *E.coli, P.mirabilis* and *K.pneumoniae*. The acetone extract of *Trachyspermum ammi* produced antimicrobial activity against *K.pneumoniae*. Hassanshahian et al., 2014 reported inhibitory effects of essential oil from *Trachyspermum ammi***,** against *E.coli, K.pneumoniae* and *S. aureus*. The results from their study showed that essential oil of *Trachyspermum ammi* had inhibitory effects against *E.coli, K. pneumoniae* similar to our results. MasihUsha et al., 2012, have also reported that the ethanol extract of *Trachyspermum ammi* was found to be sensitive against *E.coli* apart from *B.subtilis and S.aureus.*

In the present study *Syzygium aromaticum* was found to be effective against *E.coli, P.mirabilis* and *K.pneumoniae. Both the acetone and ethanol extract showed similar activity against all the three E.coli, P.mirabilis* and *K.pneumoniae.* The ethanol and acetone extract of *Syzygium aromaticum* showed higher antibacterial activities when compared to the *Trachyspermum ammi* and *Cinnamomum zeylanicum.* Antibacterial properties of *Syzygium aromaticum* have been reported earlier (Chopra *et al*, 1982, Ueda *et al*,

1982, Watanabe *et al.,* 1985, Briozzo *et al*, 1989 and Islam *et al.,* 1990; Hoque et al., 2008). The antimicrobial activities of ethanol, methanol, acetone and aqueous (hot and cold) extracts of *Syzygium aromaticum* buds and *Syzygium aromaticum* oil were studied..

The extracts were then evaluated for synergistic interaction. The extracts that exhibited maximum antimicrobial activity when tested alone were selected to evaluate the synergistic interactions. The ethanol extract of *Trachyspermum ammi*, acetone extract of *Cinnamomum zeylanicum* and acetone extract of *Syzygium aromaticum* were selected and mixed in the ratio of 1:1:1, 1:2:1, 1:1:2, 2:1:1 (*Trachyspermum ammi*: *Cinnamomum zeylanicum*: *Syzygium aromaticum* and used for antimicrobial activity. The extracts when mixed in the ratio of 1:2:1 exhibited maximum synergistic activity against the bacterial cultures tested.

The present study confirmed that *Trachyspermum ammi, Syzygium aromaticum* and *Cinnamomum zeylanicum* exhibited antimicrobial effect against enteric pathogens. Apart from this they also show synergistic interaction when tested in combination. They can be used as a combination in formulations that can be used to treat infections due to enteric pathogens. In future they should be tested on other enteric pathogens to strongly confirm their anti-microbial effects. Further a churna can be formulated and standardized with these three indigenous drugs that can be exclusively used to treat stomach infections. They can also be formulated in combination with standard antibiotics used to treat stomach infections to modulate resistance behavior of certain strains of bacteria.

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Table

Table 1 Antimicrobial activity of Cinnamonum zeylanicum extract against E.coli, P.mirabilis and K. pneumoniae

Extract	Zone of inhibition (in mm)											
	E.coli				*P.mirabilis*				*K.pneumoniae*			
	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl
Acetone	8	12	18	20	2	4	7	9	9	11	15	18
Ethanol	6	10	14	19	-	4	7	10	3	5	6	8

Table.2. Antimicrobial activity of extracts of Trachyspermum ammi against E.coli, P.mirabilis and K.pneumoniae

Extract	Zone of inhibition (in mm)											
	E.coli				*P.mirabilis*				*K.pneumoniae*			
	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl
Acetone	-	5	7	10	-	1	4	6	-	3	5	8
Ethanol	2	5	8	11	2	5	7	11	-	2	7	10

Table.3. Antimicrobial activity of Syzygium aromaticum against E.coli, P.mirabilis and K.pneumoniae

Extract	Zone of inhibition (in mm)											
	E.coli				*P.mirabilis*				*K.pneumoniae*			
	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl	50 µl	100 µl	150 µl	200 µl
Acetone	6	10	12	15	6	12	13	14	6	12	14	18
Ethanol	6	10	13	14	7	13	15	16	7	10	12	15

Table.4 Evaluation of synergistic activity of *Trachyspermum ammi, Cinnamonum zeylanicum and Syzygium aromaticum* against *E.coli, P.mirabilis, K.pneumoniae* by testing their antimicrobial activity

Extract	Zone of inhibition (mm)					
	E.coli		*P.mirabilis*		*K.pneumoniae*	
	50 (µl)	100 (µl)	50 (µl)	100 (µl)	50 (µl)	100 (µl)
Trachyspermum ammi	2	6	2	5	0	2
Cinnamonum zeylanicum	6	10	2	4	8	11
Syzygium aromaticum	6	10	6	10	6	12
1:1:1	7	10	8	10	8	11
1:2:1	9	11	10	13	10	15
1:1:2	8	10	7	10	10	13
2:1:1	5	8	7	11	8	13

Phytochemical test

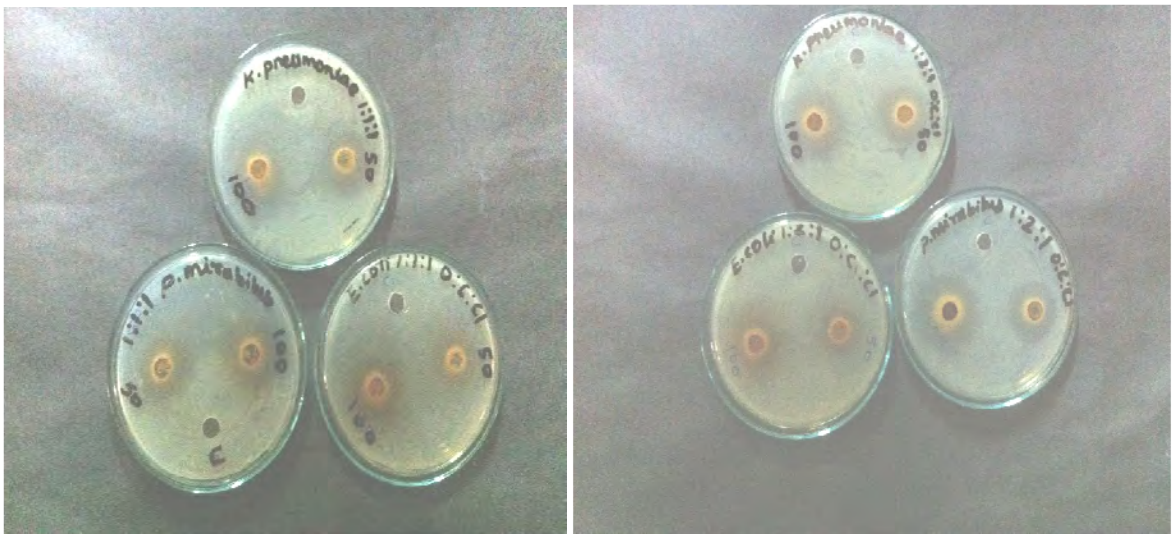
Table 5 Phytochemical screening of *Cinnamonum zeylanicum*, *Trachyspermum ammi* and *Syzygium aromaticum*

Plant name	Text	Acetone	Ethanol
Cinnamonumzeylanicum			
	Alkaloids	+	+
	Carbohydrates	_	+
	Flavonoids	_	_
	Killer killani	+	+
	Millon	_	_
	Quinine	+	+
	Resin	_	_
	Saponin	_	+
	Steroids	+	+
	Tannin	+	-
	Terpenoids	+	_
Trachyspermum ammi			
	Alkaloids	+	+
	Carbohydrates	_	+
	Flavonoids	_	_
	Killer killani	+	+
	Millon	+	_
	Quinine	+	+
	Resin	_	_
	Saponin	_	+
	Steroids	+	+
	Tannin	+	+
	Terpenoids	+	+
Syzygium aromaticum			
	Alkaloids	+	+
	Carbohydrates	+	+
	Flavonoids	_	_
	Killer killani	+	+
	Millon	+	+
	Quinine	+	+
	Resin	_	+
	Saponin	+	+
	Steroids	+	+
	Tannin	+	+
	Terpenoids	+	+

Figures

Fig.1. Evaluation of synergistic activity of *Trachyspermum ammi, Cinnamonum zeylanicum and Syzygium aromaticum* against *E.coli, P.mirabilis, K.pneumoniae* by testing their antimicrobial activity

1:1:1 1:2:1



1:1:2 2:1:1

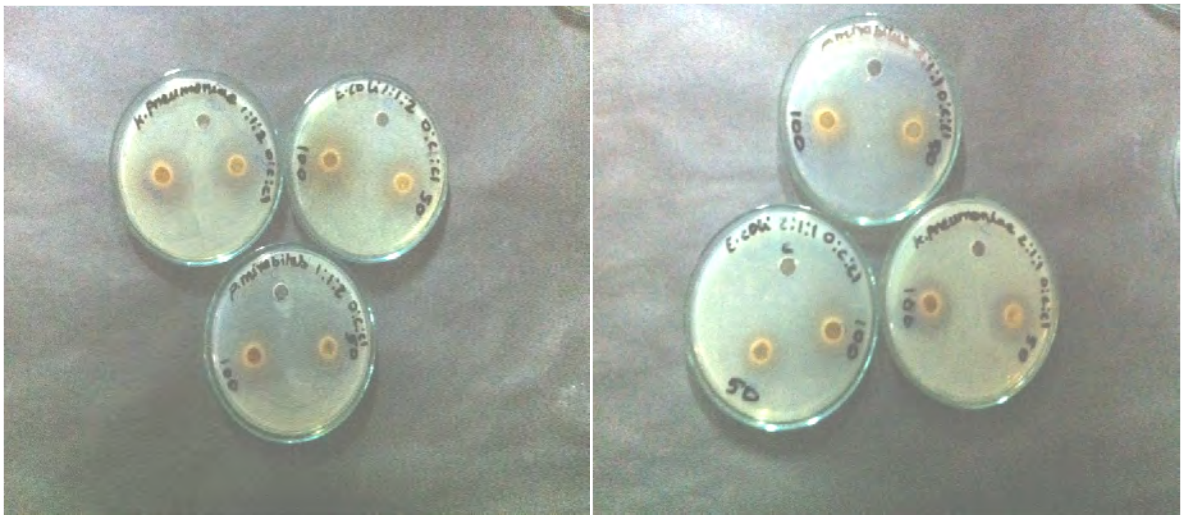


Fig.2. Synergetic activity against *E.coli*

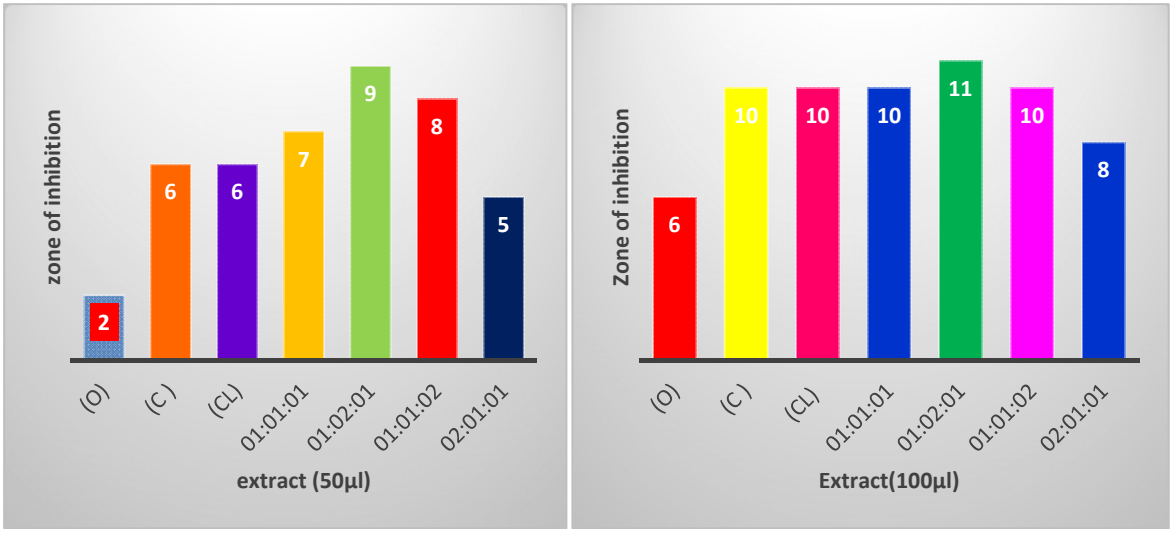
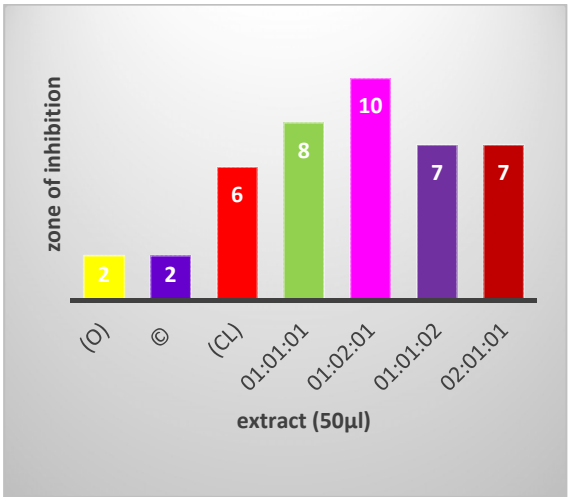


Fig.3. Synergetic activity against *P.mirabilis*



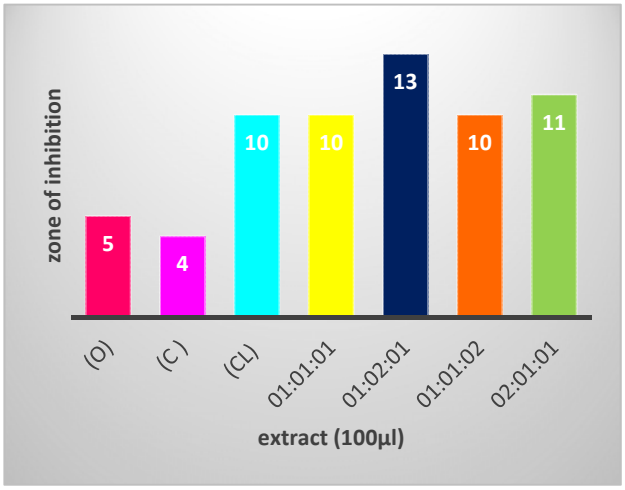


Fig.4.Synergetic activity against *k.pneumoniae*