WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 124-133. Research Article ISSN 2277– 7105

IN VITRO ANTI- BACTERIAL ACTIVITY OF ETHANOL EXTRACT OF *ZINGIBER OFFICINALE* (ZINGIBERACEAE) COMBINED WITH AMOXYCILLIN AND CEFIXIME

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Article Received on
01 August 2018,

Revised on 22 August 2018,
Accepted on 12 Sept. 2018,

DOI: 10.20959/wjpr201817-13384

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ABSTRACT

Ginger (*Zingiber officinale*) is used in traditional medicine in management of many conditions including bacterial infections. Usually, many people use traditional medicinal plants in combination with many antibiotics for treatment of many bacterial infections, this may lead to herbal- drug interactions, this herbal- drug interactions may cause a decrease or an increase of antibiotic action, as well as it may be no herbal- drug interactions, so our study was held to test possible interactions between *Z. officinale* rhizomes and some selected antibiotics (amoxicillin and cefixime). The study was conducted *in vitro* by using well diffusion method against *Staphaylococcus aureus* and *Eischerichia coli*. The results revealed that, the ethanolic extract of *Z. officinale* has good anti- bacterial activity against *S. aureus* (mean growth inhibition zone 20.35 mm) and no activity against *E. coli*. The anti- bacterial activity of cefixime against *S. aureas* and *E. coli* was good with mean growth inhibition zones 34.35 mm and 40.2 mm respectively. When *Z. officinale* extract was combined with cefixime, the growth inhibition zones were decreased (when compared with the activity of cefixime alone) against *S. aureas* and *E. coli* (antagonistic effect), the growth inhibition zones were 29.95 mm and 35.05 mm respectively (P- value < 0.01). The mean growth inhibition zones of amoxicillin against *S. aureas* and *E. coli* were 15.15 mm and 30.05 mm respectively, while the combination of *Z. officinale* extract with amoxicillin gave no change on the mean growth inhibition zone against *E. coli* (30.05 mm), but the same combination against *S. aureas* showed no anti-

bacterial activity which was considered as antagonistic effect (P- value < 0.01). We concluded that *Z. officinale* rhizomes should better be avoided in combination with anti-bacterial agents. Also we recommend that researchers should enter the field of herbal- drug interactions researches, for testing the combination effects between many medicinal plants that are used traditionally with known therapeutic agents used in treatment of various diseases.

KEYWORDS: *Zingiber officinale,* amoxicillin, cefixime, *Staphylococcus aureas, Eischerichia coli,* herbal- drug interactions.

INTRODUCTION

Herbal medicines and their preparations are now becoming the most favorable medicinal products used internationally. More than 80% of populations (specially in developing countries) are using herbal medicines for many purposes, often they are used traditionally for improving the health status and in treatment of common illnesses such as colds, microbial infections, inflammations, heart diseases, diabetes, central nervous system diseases etc.[1,2]

Usually, many people are using herbal preparations accompanied with other conventional therapeutic drugs; these may cause an elevation of the incidence of herbal- drug interactions, which may have variable clinical consequences.[3,4] There is a lack of confirmation of efficacy and insufficient toxicological data of combining herbal products with therapeutic drugs;[4,5] that is why our study was conducted to attempt to see any possible herbal- drug interactions between *Zingiber officinale* ethanolic extract and some selected anti- bacterial agents widely used in treatment of bacterial infections (amoxicillin and cefixime), we selected *Z. officinale* because it is widely used in traditional medicine in treatment of many respiratory tract infections,[6] some people are taking it in combination with many anti-bacterial agents.[7]

Generally, herbal- drugs interactions are classified according to various visions; one may classify regarding pharmacokinetics aspects (effects on metabolism, absorption, excretion, etc.), other classification may regard pharmacodynamics aspects (effects on organs and/or systems), whereas another might focus on the physiological systems affected by the interactions, additionally, one might view the interactions from the perspective of antagonism (which means that the effect of two combined chemicals is actually less than the sum of the effect of the two drugs taken independently from each other), or synergism (which means that

the effect of two chemicals taken together is greater than the sum of their separate effect at the same doses).[8]

Ginger rhizomes (*Zingiber officinale*, Zingiberacae) is a medicinal plant part that is widely used in Chinese, Ayurvedic and Tibb- Unani herbal medicines, it is mainly applied for pains, sore throats, constipation, indigestion, vomiting, pregnant motion sickness, hypertension, dementia, infectious diseases, helminthiasis, arthritis, rheumatism, muscular aches, anti-migraine, anti- thrombotic and hypo- lipidaemic properties.[9] In addition, many pharmacological studies proved that it possess anti- diabetic,[9] anti- microbial,[9,10] antioxidant,[9,11] antihypertensive,[10] anti- inflammatory,[11] hepatoprotective,[12] anti-emetic,[13] anticancer,[14] lypoletic and anti- diarrheal activities.[15]

MATERIALS AND METHODS

The plant material

The Rihzomes of *Zingiber officinale* (Zingiberaceae), were brought from local market during March 2018, the rhizomes were authenticated by the taxonomist in Medicinal and Aromatic Plants Research Institute (MAPRI) at National Center for Research in Khartoum, Sudan.

The test micro- organisms

Staphylococcus aureas and *Escherichia coli* bacterial strains were obtained from department of microbiology in Medicinal and Aromatic Plants Research Institute, Khartoum. They were isolated from Sudanese patients and were then incubated under standard conditions.

Extraction process

Z. officinale rhizomes (ginger) were washed well and allowed to dry at room temperature, they were crushed into coarse powder and 100 gm. was weighted, 300 ml of ethanol 80% was added to the plant material and was macerated for 3 days, the extract was filtered (Whatman No. 2) and the method of extraction with the same plant material marc was repeated again (three times), the solvent of all combined ethanolic extract after filtration was evaporated under reduced pressure, the extract was keep in closed container and was stored at 8^{o}C till experimental use.

Preparation of plant extract for anti- bacterial assay

10 mg of the *Z. officinale* ethanolic extract was dissolved in 1 ml of distilled water; further dilutions were made till final concentration of 100 µg/ 30 µl, which was used for anti-bacterial assay.

Preparation of amoxicillin and cefixime for anti- bacterial assay

Amoxicillin and cefixime were used as positive control and as anti- bacterial agents combined with the plant extract separately, they were kindly provided as standard powders from local pharmaceutical factories, they were prepared by dissolving 1 gm. of each in 10 ml sterile water, then the solutions were diluted till final concentration of 100 µg/ 30 µl.

***In vitro* anti- bacterial activity by well diffusion method**

Anti- bacterial activity by well diffusion method was carried out according to the method described by Ghaleb A. and Mohammad M.[16] with some minor modifications. Briefly, 10.2 gm. of Mullor- Hinton Agar (MHA) was weighted, dissolved in 300 ml of distilled water, and then put in autoclave for 30 minutes for sterilization, it was poured in sterile plates (30 ml of agar in each plate) and left to be solidified, then bacterial suspensions of *S. aureus* and *E. coli* strains were prepared and were swapped by cotton on agar media, 6 mm diameter wells was punched on agar by using sterile cork borer (no. 6) and was filled with 30µl of antibiotic alone (either amoxicillin or cefixime), 30 µl of plant extract and with 30µl of antibiotic plus 30 µl of plant extract in case of combinations. The experiments were done as duplicate. The plates were incubated at 37^{o}C for 24 hours. After 24 hours the inhibition zones around the wells were measured, the average of two replicates for each antibiotic, extract and their combinations were then calculated.

Statistical analysis

The obtained results were analyzed through student's t- test by using SPSS statistical package application (IBM® SPSS® V. 20). The p- values less than 0.05 were considered statistically significant.

Preliminary phytochemical screening tests

The ethanolic extract of *Z. officinale* was screened for the presence of some active phytochemical constituents. These screened active phytochemical constituents were flavonoids, coumarins, saponnins, tannins, anthraquinones, cardiac glycosides, alkaloids, steroids, triterpenes and carbohydrates.

RESULTS AND DISCUSSION

The plant extract was screened for anti- bacterial activity against standard organisms of *S. aureus* and *E. coli*, also standard beta lactam antibiotics (amoxicillin and cefixime) were also screened separately for their anti- bacterial activity against the two micro- organisms, then a combinations of the plant extract with either amoxicillin or cefixime were screened for anti- bacterial activity against the two micro- organisms. All experiments were done as duplicate. The average of the diameters of the growth inhibition zones obtained is shown in "**Table 1**" and "**Fig. 1**". According to National Committee for Clinical Laboratory Standard, any growth inhibition zone resulted from 20 mm or more was considered active, while any growth inhibition zone resulted between 14 and less than 20 mm was considered moderate activity.

Table 1: Anti- bacterial activity of *Z. officinale* ethanolic extract alone and when combined with amoxicillin and cefixime.

No.	Extract/ antibiotic	Conc.	E. c.	S. a.
1	Extract	100 µg/ 30 µl	Non	20.35 ± 0.5 mm
2	Cefixime	100 µg/ 30 µl	40.20 ± 1 mm	34.35 ± 0.5 mm
3	Amoxicillin	100 µg/ 30 µl	35.05 ± 0.5 mm	15.15 ± 0.5 mm
4	Extract+cefixime	100 µg/ 30 µl + 100 µg/ 30 µl	35.05 ± 0.5 mm*	29.95 ± 0.5mm*
5	Extract+amoxicillin	100 µg/ 30 µl + 100 µg/ 30 µl	35.05 ± 0.5 mm	Non º

(Mean ± SD)(n=2). E. c.: *Escherichia coli*. S. a.: *Staphylococcus aureas*. *Statistical significant (p- value < 0.01) when compared with cefixime. ºStatistical significant (p- value <0.001) when compared with amoxicillin.

"**Table 1**" and "**Fig. 1**" showed that the ethanolic extract of *Z. officinale* rhizomes has good anti- bacterial activity against *S. aureus* with mean growth inhibition zone equal to 20.35 mm, but the plant extract showed no activity against *E. coli*. Cefixime showed good anti- bacterial activity against *S. aureas* and *E. coli* with mean growth inhibition zones equal to 34.35 mm and 40.2 mm respectively. The combination of plant extract with cefixime has caused a decrease of anti- bacterial activity of cefixime against *S. aureas* and *E. coli*, the mean growth inhibition zones were 29.95 mm and 35.05 mm respectively which were statistically significant when compared with the anti- bacterial activity of cefixime alone (antagonistic effect).

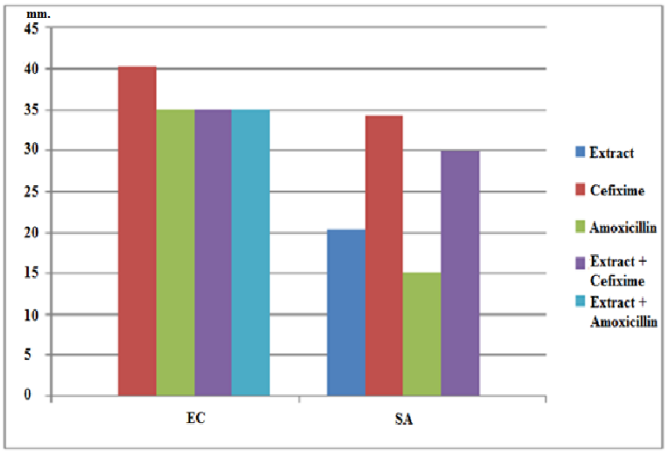

Fig. 1: Anti- bacterial activity of plant extract, cefixime, amoxycillin, plant extract + cefixime and plant extract + amoxicillin against *E. coli* (EC) and *S. aures* (SA).

On the other hand, amoxicillin showed moderate anti- bacterial activity against *S. aureas*, the mean growth inhibition zone was 15.15 mm, this moderate anti- bacterial activity may probably due to that this strain of *S. aureas* is resistant to amoxicillin, when the plant extract was combined with amoxicillin against *S. aureas* there was no growth inhibition zone which was considered antagonistic effect between the plant extract and amoxicillin (statistically significant). Also amoxicillin showed good anti- bacterial activity against *E. coli* with mean growth inhibition zone equal to 30.05 mm. The combination of plant extract with amoxicillin gave no change on the growth inhibition zone of the anti- bacterial activity of amoxicillin alone against *E. coli* (30.05 mm).

There is a previous study which was conducted by Joyce Elaine Cristina Betoni *et. al.*,[17] they studied the synergistic effect between some medicinal plants extracted by methanol 70% combined with some known anti- bacterial agents against *S. aureas*, *Z. officinale* rhizomes was one of these selected medicinal plants, it was tested with thirteen known anti- bacterial agents, these were penicillin, oxacillin, vancomycin, ampicillin, cephalothin, cefoxitin, chloramphenicol, gentamicin, netilmicin, tetracycline, erythromycin, co- trimoxazole and ofloxacin, they found that *Z. officinale* methanolic extract had synergistic effect only with tetracycline and netilmycin (p- value < 0.05) while it showed no synergism effect with others.[17] So their findings were matched with our obtained results that generally *Z. officinale* rhizomes alcoholic extract has antagonistic effect with many anti- bacterial agents including our tested drugs (amoxicillin and cefixime).

Qualitative preliminary phytochemical screening tests on *Z. officinale* ethanolic extract have showed the presence of flavonoids, alkaloids, saponins, tannins and coumarins "**Table 2**", all of these constituents are phenolic compounds except alkaloids. Generally, many previous studies stated that most active plant extracts with antimicrobial activity are mainly due to phenolic compounds,[18,19] so, we can say that the anti- bacterial activity of *Z. officinale* extract may probably due to the presence of these phenolic compounds which are soluble in ethanol 80%.

Table 2: Phytochemical screening results of *Z. officinale* ethanolic extract.

Constituents	Test / Reagent	Result
Flavonoids	NH_4OH test	+
	NaOH test	+
Coumarins	Filter paper/ ammonia/ 366 nm UV	+
Saponnins	Frothing properties	+
	Emulsifying properties	+
Tannins	Formaldehyde test	+
Anthraquinones	Borntrager's test	-
Cardiac glycosides	Kedde's test	+
	Baljet's test	+
	Keller Killiani's test	+
	Liebermann Burchard's test	-
Alkaloids	Mayer's test	+
	Dragendorff's test	+
	Wagner's test	+
	Hager's test	+
Carbohydrates	Molisch's test	+
	Barfoed's test (for monosccharides)	+

CONCLUSION

Zingiber officinale rhizomes (ginger) are widely used traditionally in treatment of many bacterial infections especially sore throat, tonsillitis and lower respiratory tract infections. Many people, who believe on traditional medicine, may accidentally take it in combination with many anti- bacterial agents. We concluded that it is better to avoid tacking *Z. officinale* simultaneously with anti- bacterial agents due to herbal- drug interactions (antagonistic effect); we recommend that further studies are required like *in vivo* anti- bacterial studies in order to study and determine the exact causes of this antagonistic herbal- drug interactions, also *Z. officinale* should be tested on many other micro- organisms which are responsible for many microbial infections in which *Z. officinale* is used traditionally in their treatments.

Also there are fewer studies about herbal- drug interactions, especially in places where people are widely using herbal products with known anti- bacterial agents (or any other known agents which are used therapeutically in treatment of many diseases), so we also recommend that researchers should enter this field in order to provide many researches about herbal- drug interactions, this may lead to discovering of many herbal products which may be synergistic or antagonistic with known therapeutic agents.

ACKNOWLEDGEMENT

The authors are so grateful to Department of Microbiology in Medicinal and Aromatic Plants Research Institute for providing us with the bacterial strains used in this study, also we are so grateful to the local pharmaceutical factories which provided us with the standard antibiotics powders used in this study, we are thankful to all members in Department of Pharmacognosy and Department of Pharmaceutical Microbiology in Faculty of Pharmacy, International University of Africa, for their great help and support during the run of this study.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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