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Original paper

Pharmacognostic Evaluation, Phytochemical Screening and Antimicrobial Activity of Rhizome of *Zingiber officinale*Roscoe (*cv* Moran)

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Abstract

Pharmacognostic evaluation and phytochemical screening of the rhizomes of *Zingiberofficinale*Roscoe (*cv* Moran) locally known as 'MoranAda', revealed the presence of some bioactive components, which have been linked to antimicrobial properties. Phytochemical screening showed the presence of alkaloid, glycoside, tannins, phenols, saponin and flavonoid when tested on aqueous andmethanolicextracts of *Z.officinale*Roscoe. The effects of methanolic and aqueous extracts on some pathogenic bacterial strains viz.: *Staphylococcus aureus, Enterobacteraerogens, Klebisiellapneumoniae, Bacillus subtilis* and *Escherisia coli* respectively, showed that the plant part can be used to treat infections caused by these bacteria. The aqueous and methanolic extracts also showed antibacterial activity but the significant antimicrobial activity was shown by aqueous extract against all the bacteria, while moderate activity was shown by the methanolic extract. The effectiveness of the crude extract confirmed its use in traditional medicineto treat asthma, cough,common cold, fever, sinusitisand other gastrointestinal diseases.

Keywords: Antimicrobial, Phytochemical,*Staphylococcus aureus, Enterobacteraerogens, Klebisiellapneumoniae, Bacillus subtitis* and *Escherisia coli*

Introduction:

*Zingiberofficinale*Roscoe(*cv*-Moran) locally known as 'Moran Ada' in Assamese,belongs to the family Zingerberaceae is a perennial herb. ThisZingiberaceous plants have strong aromatic and medicinal properties. These plants are characterized by their tuberous rhizomes [1].The cultivation of ginger is known to originate in China which then spread to India, South East Asia, West Africa and the Caribbean [2, 3]. India is the biggest producer of ginger in the world. In India, it is cultivated in almost all the states. Some reports suggest that the climatic conditions of Orissa, West Bengal, Assam and Kerala are more suitable for the growth of ginger in India [4]. The medicinal use of ginger is well known in India for more than 2000 years as one of the most versatile medicinal plants. Ginger has been using both as Ayurvedic and Chinese medicine for curing heart problems, treat stomach upset, diarrheaandnausea [5]. It is also promotes

the release of bile from the gall bladder [6, 7], decrease joint pain from arthritis, useful for the treatment of heart diseases and lungs diseases [8].

Ginger plays an important role in traditional Indian Ayurvedic medicine. It is also used as an ingredient in traditional Indian drinks. Fresh ginger is one of the main spices used for making dishes, both vegetarian and non-vegetarian based foods. Indian traditional medicinal remedies especially for cough and asthma consists of juice of fresh ginger with a little juice of fresh garlic mixed with honey. It is also suggests 1-2 tea spoons of ginger juice with honey is a potent cough suppressant. Besides these ginger is very often used to cure many illness such as indigestion, tastelessness, loss of appetite, flatulence, intestinal, nausea, vomiting, allergic reactions, acute and chronic cough, common cold, fever, allergic rhinitis, sinusitis, acute chronic bronchitis, respiratory troubles, pain, headache, backache or any kind of muscular catch, painful tooth and swelled gum etc[9].

Gingerols are the major active components in the fresh rhizome. The volatile oil components consists mainly of sesquiterpene hydrocarbons, predominantly zingeberene (35%), curcumene (18%) and farnesene (10%) [10].Non-volatile pungent compounds include gingerols, shogaols, paradols and zingerone. Paradol is similar to gingerol and is formed from hydrogenation of shogoal (phenylalkanones). Ginger contains fats, waxes, carbohydrates, vitamins and minerals. Ginger rhizomes also contain a potent proteolytic enzyme called zingibain. The pungent taste of ginger is due to nonvolatile phenylpropanoid-derived compounds, particularly gingerols and shogaols. Supplementing ginger in fish diets may enhance disease resistance by reinforcing host innate immune functions that are necessary for protection against infectious diseases. Ginger may play diverse biological roles in anti-oxidative, anti-inflammatory, hypolipidemic, anticarcinogenic, anti-nausea, antithrombotic, cardiovascular, and antibacterial processes [11, 12, 13, 14, 15].*Z. officinale* Roscoe*cv* Moran found in the Upper Assam regions, named after the place 'Moran' ofDibrugarh districthas tremendous use in the traditional medicine by the local communities of the region. Since not many studies have been conducted, this study focusesin the pharmacognostic, phytochemical and antimicrobial activity of the rhizome.

Material and Methods:

Collection and Identification:

The experimental sample was collected from Moran region of Dibrugarh district of Assam, India in the year 2018. The voucher specimen was submitted to the Department of Herbal Science and Technology, ADP College, Nagaon for future reference. Further, taxonomic name of the plant species was confirmed from online databases namely: The Plant List (http://www.theplantlist.org) [16].

***Microscopic study of plant material*:**

For microscopic evaluation, the rhizome of *Z.officinalecv* Moranwas freshly collected and thin section i.e., T.S, L.S, starch grain and powder crude drug were studied under Foldscope microscope. Foldscope- an origami based paper microscope provides upto 140X magnification with submicron resolution. It is operated by inserting a sample mounted on a microscope slide, turning on the LED, and viewing the sample while panning and focusing with one's thumbs. The sample is viewed by holding the Foldscope with both hands and placing one's eye close enough to the micro-lens so one's eyebrow is touching the paper. [17].

***Preparation of plant extract*:**

***Drying and pulverization*:**

The plant materials were washed thoroughly with running tap water to remove the earthy material or adherent impurities. The plant material was allowed to shade dry. The rhizome was cut into pieces and dried in hot air oven at 45^{0}C and pulverized in a mixer grinder. Then the material was subjected to maceration.

Preparation of plant extract (rhizome) by maceration method:

10gmof sample were soaked in 100ml of distilled water in a conical flask and other 10gms were soaked in 100ml of methanol in a conical flask The extraction was done for continuously shaking for 6hrs in Shaker at 140rpm then it was allowed to stand still for 18hrs.The extracts were then filtered through filter paper The filtered extracts weremade into more concentrated volume at room temperature by using water bath at 40 ^{0}C. The extract obtained was kept in petridish, wrapped by aluminum foil and kept in the desiccator.

***Physiochemical Studies*:**

Physical constants were determined by following Indian Pharmacopoeia[18].Physicochemical analysis of the crude drug of *Z. officinale*Roscoe*cv* Moran has been done such as ash value (i.e. total ash, acid insoluble ash, water soluble ash), extractive value (i.e. water and alcohol soluble extractive value) and moisture content by following standard protocols [18].

***PreliminaryPhytochemical Analysis*:**

The phytochemical analysis were carried out for aqueous and methanolicextracts to determine the presence of following bioactive compounds using the standard qualitative procedures [19,20].

***Anti-microbial activity of the plants*:**

The aqueous and methanolicextracts were evaluated for antibacterial assay against two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and three gram negative bacteria *Escherichia coli*, *Enterobacteraerogenes* and *Klebsiellapneumoniae*. The organisms were purchased from MTCC, Chandigarh, (Punjab),India and maintained in nutrient agar slants at 4^{o}C.The antibacterial evaluation of different bacterial strains was tested by Agar Well Diffusion method[21,22].The diluted bacterial culture was streaked over the nutrient agar plates by sterile bud. Three wells of 6 mm diameter for each extract were made by sterile cork-borer in agar plate aseptically. The wells were then filled by each extract of different concentrations (30mg/ml, 50 mg/ml and 100mg/ml in DMSO) and loaded (40, 60 and 100 µl) and allowed to dry and incubated at 37^o C for 18 hours. The inhibition zones were recorded in the test, standard as well as control. The assay was repeated twice.

Result and discussion:

Macro-morphological and microscopic study of plant material:

Macro-morphological characteristics:

Zingiberofficinalecv Moran is a specific variety of ginger found in specially Assam only. The plant is a biennial or perennial, creeping rhizome, and an annual stem, which is cylindrical, erect, and enclosed in an imbricate membranous sheathing. The leaves are lanceolate, acute, and smooth and stand alternately on the sheaths of the stem (Fig: 1 (A). The flowers are of a yellow color, and appear two or three at a time between the bracteal scales. The rhizomes are comparatively thin then other varieties and reddish brown in color (Fig: 1 (B).

Fig: 1. *Z.officinale* Roscoe (A) Aerial part, (B) Rhizome

Microscopic observation of Rhizome:

Transverse section of rhizome shows distinct outer cork cells, followed by outer brown parenchymatous cortex tissue followed by endodermis region. The vascular bundle containing xylem and phloem fiber are distinctly observed in inner cortex. Trachea vassals and oleoresin cell is present in inner cortical region (Fig. 2 A-B).*Ali et al*.,2016reported that, the T. S of rhizome have outer black cork followed by colorless inner cork cell.The parenchymatous tissues are polygonal structured, scattered conjoint, collateral vascular bundle containing xylem and phloem and surrounded by sclerenchymatous fiber.Tracheids and yellow polygonal oleo-resin cell are also present in the cortical region [23].

The starch grain is observed in inner cortex region.Morphologically, the starch grains are typical rectangle to oval shape. *Sharma Y*, 2017 and *Mehta S*, 2016 reported that, the cortical region of the rhizome of *Z. officinale* contain plenty of simple, fairly large, flattened and ovoid starch grain [24, 25].

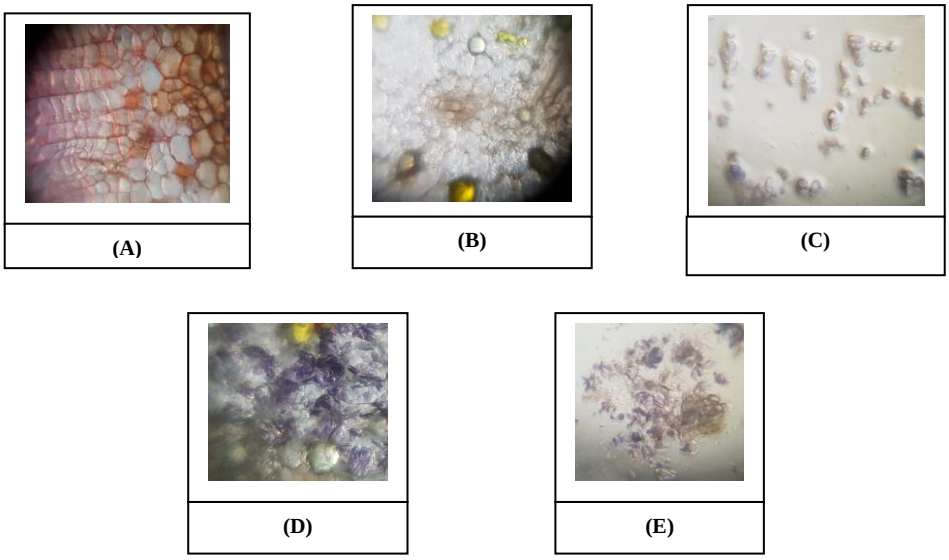


Fig:2. *Z.officinale* Roscoe (A) Outer cortex region, (B) Vascular bundle and oleo resin deposition, (C) Starch grain (unstained), (D) Starch grain in inner cortex region (iodine stained), (E) Powder drug microscopy

Physicochemical study of *Z.officinale*Roscoe*cv* Moran (rhizome) extract:

The physicochemical parameters (Table: 1) of dry rhizome powder of. *Z. officinale*Roscoe*cv* Moran, analyzed ash value, extractive value and moisture content. In our observation, the total ash value (9.5% w/w), water soluble ash (1.2% w/w) and acid insolubleash (1.7%) were recorded.Extractive value determination using maceration method for 24 hours in rotary shaker, showed 0.90% w/v water soluble extractive value and0.60% w/v alcohol soluble extractive value.Moisture content was recorded to be 14.68% w/w.*Mianet al.* 2019, revealed that, water soluble extractive value (11.233± 0.4651)is higher than alcohol soluble mater (8.55± 0.1923). They also reported about the ash value parameter from rhizome, total ash (7.6083± 0.1491), acid insoluble ash (1.900± 0.0577) andwater soluble ash (3.366± 0.3609) [26].From the previous study, we conclude that, the physicochemical parameters of this local variety ginger are almost same. The total ash value and acid insoluble ash value is quite different with local variety, but the value of water soluble ash is comparatively high than our examined species. The moisture content of crude drug is also one of the important parameter of quality of a drug. Because of excessive amount of moisture can deteriorate the medicinal activity of particular drug. In our present study, the content of moisture in dry ginger powder is high (14.68 %) than the previous report (10.00%) [27].

Table1: Physiochemical parameters of crude powder prepared from *Z.Officinale*Roscoe *cv* Moran:

Sl. No	Parameter		Result
1	Ash value	Total ash	9.5% (W/W)
		Water soluble ash	1.2% (W/W)
		Acid insoluble ash	1.7% (W/W)
2.	Extractive value	Alcohol soluble extractive value	0.60% (W/V)
		Water soluble extractive value	0.90% (W/V)
3.	Moisture content		14.68% (W/W)

Preliminary Phytochemical analysis:

The preliminary phytochemical analysis shows the presence of alkaloids, carbohydrate, flavonoid, phenol, protein, saponin, tannin and glycoside in aqueous extract and alkaloid, flavonoid, saponin and glycosides in methanolic extract (Table: 2).Presence of bioactive compound in aqueous extract is highest than methanolic extract. According to the *Mian et al.* 2019, the aqueous extract of *Z. officinale* Roscoe*cv* Moran revealed the presence of alkaloids, carbohydrates, flavanoids, proteins, glycosides, saponins, fats and oils, terpenoids and starch [26]. Beside this, *Osabor et al.* 2015 reported that, the aqueous

extract of *Z. officinale* revealed the presence of cardiac glycoside, alkaloids, saponins, flavonoids, polyphenols, and reducing sugars [28].

Table2: Preliminary phytochemical studies of *Z.officinale* Roscoe *cv* Moran:

Sl.No	Plant Constituents	Test	Extracts	
			Methanol	Water
1	Alkaloid	Dragendroff's test	+	+
		Mayer's test	+	-
2	Carbohydrate	Benedict's test	-	-
		Fehling's test	-	+
3	Flavonoid	Alkaline reagent test	+	-
		Lead acetate test	-	+
4	Phenol	Ferric chloride test	-	+
5	Protein	Xanthoprotien test	-	+
6	Saponin	Foam test	+	+
7	Tannin	Lead acetate test	-	+
8	Glycoside	Conc. H2SO4 test	+	+
		Killer- killani test	+	-

Where (-) signs indicate absence of the constituent and (+) signs indicate presence of the constituents.

Determination of Antibacterial activity:

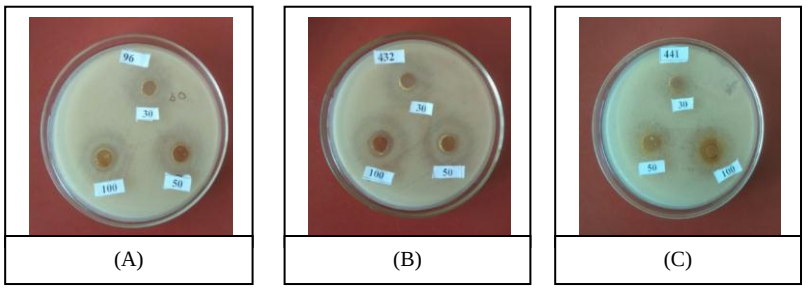
The antibacterial activity of aqueous and methanolicextracts were evaluated against two gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and three gram negative bacteria *Escherichia coli*, *Enterobacteraerogenes* and *Klebsiellapneumonia*e in concentration of30mg/ml, 50mg/ml and 100mg/ml.The aqueousextract exhibited a stronger antibacterial activity against all pathogenic bacterial strains (Table 3);*Escherichia coli* (24±1) followed by *Enterobacter aerogenes* (24±1.33),*Bacillus subtilis* (23±1.66), *Klebsiella pneumonia*e (22±1), and *Staphylococcus aureus* (20±1.55) at a concentration of 50mg/ml. Whereas, *E. coli* (14±0.43), *K. pneumoniae* (14± 0.67), *S. aureus* (13±0.43),*B. subtilis*(12±0.67) and *E. aerogenes* (12±1) diameter zone of inhibition (mm) was seen in methanol extract against above pathogenic bacteria at the concentration of 50mg/ml . From the above results, it is revealed that, the anti-microbial activity of aqueous extract showed more effective results compared to themethanolic extract.

According to the previous report, *Islam et al.* 2014, the antimicrobial activity of the ginger was found lowest activity against *Escherichia coli*. *Staphylococcus aureus* showed lower sensitivity to ginger extract as compare to the most other Gram-negative bacteria [29]. In the present study, the antimicrobial activity of aqueous extract of *Z. officinale* Roscoe *cv* Moran, showed the highest inhibition against*E. coli* (24±1) and *E. aerogenes* (24±1.33) and lowest against *S. aureus*(20±1.55). In 2012

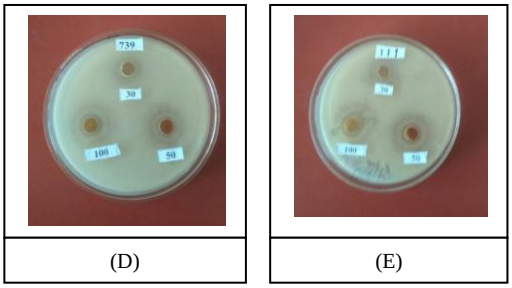
PonmuruganKaruppiah et al., reported that, the antimicrobial activity of ethanolic extracts showed highest inhibitory effect against *B. subtilis* (16.55±0.25) followed by *E. coli* (15.50±0.30), *S aureus* (13.55±0.20), *Klebsiella spp.* (7.50±0.50) and *Enterobacter spp.* (5.50±0.40) [30]. In the present study, the methanolicextract shows the highest inhibitory effect against *S.auerous* (15±0.67), *K.pneumoniae* (15±0.64), *E. coli* (15±1), followed by *B. subtilis* (14±1.67) and *E. aerogenes* (13±0.43)at the concentration of 50mg/ml.From the above result, we found that the antibacterial activity of both aqueous and methanolic extracts highly inhibited all the pathogenic bacterial strains.

Table3: Antibacterial activity of *Z.officinale*Roscoe*cv* Moran extracts against pathogenic bacterial strains:

Test of microorganisms	Diameter of inhibition (mm)								
	Methanol extract			Aqueous extract			Standard		
	30mg/ml	50mg/ml	100mg/m	30mg/ml	50mg/ml	100mg/m	30mg/ml	50mg/ml	100mg/m
Staphylococcus aureus	11±0.67	13±0.43	15±0.67	19±1.33	20±1.55	23±1.33	31	32	34
*Klebsiella pneumonia*e	13±0.44	14±0.67	15±0.64	21±1	22±1	23±1	32	33	34
Bacillus subtilis	11±0.44	12±0.67	14±1.67	22±1.66	23±1.66	24±1.66	34	35	37
Escherichia coli	13±1	14±0.43	15±1	24±1.33	24±1	25±1	31	32	34
Enterobacteraero genes	11±0.67	12±1	13±0.43	23±1.66	24±1.33	25±1.66	32	33	34



(A) (B) (C)



(D) (E)

Fig. 3: Antimicrobial activity of methanolic extract of *Z.officinale*Roscoe (A) *Staphylococcus aureus*,(B) *Klebsiella pneumonia*,(C) *Bacillus subtilis*, (D) *Escherichia coli*,(E) *Enterobacter aerogenes*

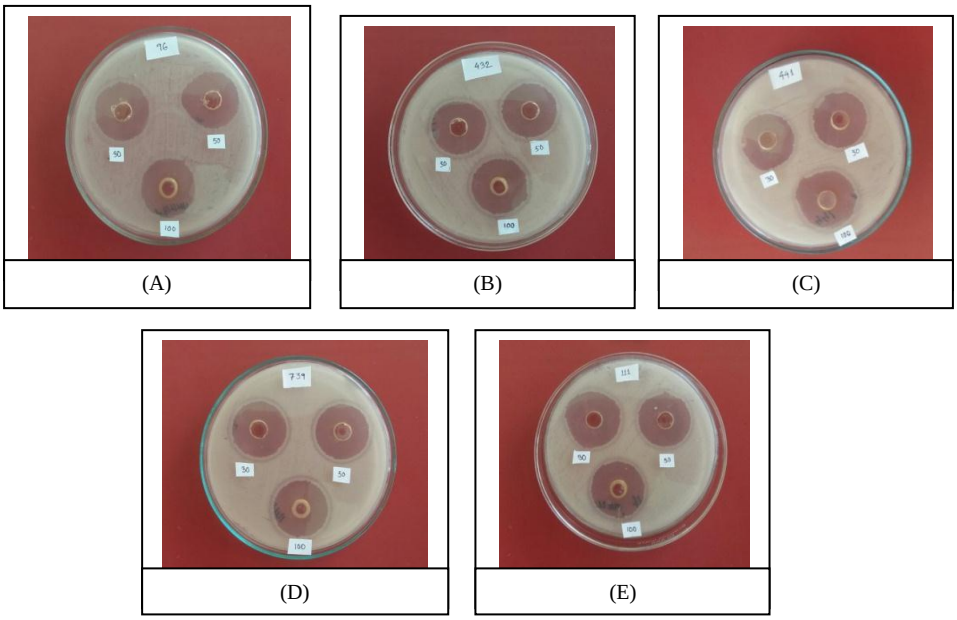


Fig. 4: Antimicrobial activity of aqueousextract(A)*Staphylococcus aureus*, (B) *Klebsiella pneumonia*, (C) *Bacillus subtilis*, (D) *Escherichia coli*, (E) *Enterobacter aerogenes*

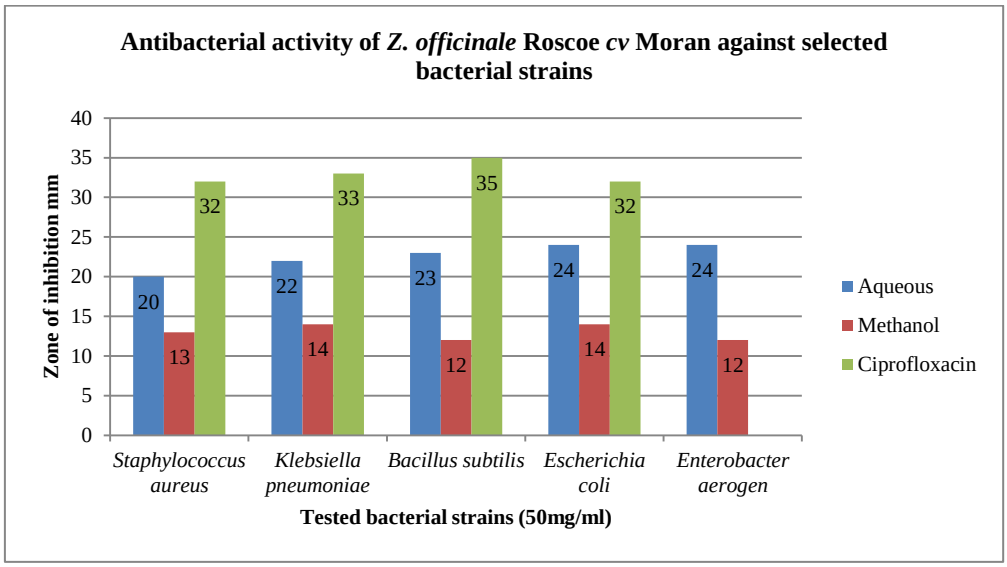


Fig. 5: Antibacterial activities of aqueous extract of *Zingiber officinale*Roscoe*cv* Moran against selected bacterial strains

Conclusion:

In conclusion, the antimicrobial activities displayed by the aqueous extract of *Z.officinalecv* Moranrhizomes are significant, while the methanolic extract also shown moderate antibacterial activity. Preliminary phytochemical screening showed the presence of alkaloids, carbohydrate, flavonoid, phenol, protein, saponin, tannin and glycoside. The physicochemical characteristics of crude drug determined the quantitative parameters like ash value, extractive value and moisture content. Aqueous and methanolic extract shown considerable activity against the bacterial strains and also the presence of secondary metabolites was confirmed in the both the extract of *Z.officinalecv* Moranrhizomes, so it might be concluded that the activity may be due to the secondary metabolites present in this plant. After the overall observation, we can conclude that, this local ginger variety have potential antimicrobial activities and it will assure that, the use of ginger as a spice in our daily life will act as a good antimicrobial agent which can protect from common bacterial infections.

Conflict of interest:

Authors declares no conflict of interest

Compliance with Ethical Standards

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors

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