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REVIEW ARTICLE

Antimicrobial Efficacy of Olive Leaf Extract Antimicrobial Efficacy of Olive Leaf Extract: A systematic review of *in vitro* study

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ABSTRACT
*To evaluate the antimicrobial efficacy of olive leaf extract against bacterias, viruses and fungies . We searched articles with keywords in Pubmed , Scopus, Embased, Cochrane, and Medline Since 1978 to 2015.After screening and excluding papers, including articles were 15 papers .Studies which evaluate antibacterial effect of OLE with outcome measure like zone of inhibition and minimum inhibitory concentration were included for this research .**T**his study shows that OLE demonstrated good result as antimicrobial agent Further study could evaluate antimicrobial efficacy of OLE in greater depth and vivo clinical testing is essential to confirm the in vitro results.*
KEY WORD: *Olive leaf extract , antibacterial ,antiviral , antifungal efficacy ,systematic review ,in vitro study*

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INTRODUCTION

Nature is full of miracle .Therapeutic properties of herbs are incredible . Nowadays ,patients are trend to use natural medicine because of side effects of synthetic drugs [1]. Many reports have shown that plants have an antioxidant, antithrombotic , anti inflammatory , antidiabetic , anti blood hypertensive , anti obesity , anticancer ,hepatoprotective , gastroprotective and cardioprotective activity [2]. Several studies demonstrated antimicrobial activity of herbs by inhibition of the growth of a wide variety of bacteria [3] ,fungi [4, 5] and viruses [6].Olive tree is a sacred plant which is Allah swears in the Holy Quran . potential beneficial effects of components from olives (*Olea europaea*. L.) has been demonstrated in many studies[7].Leaves from olive tree are rich in biophenols such as oleuropein , verbascoside , ligstrosides ,tyrosol or hydroxyl tyrosol [8].The objective of systematic reviews play an important role in clinical decision making in medicine .To our knowledge , there is no systematic review available which summarized studies of OLE activity against microorganisms.

METHODS

Steps of search were done in Pubmed, Scopus, Embased, Cochrane, Medline for key words. The detail of searching keywords was as follows:
1-olive leaf extract [Mesh] or olea europaea [Mesh]
2- antimicrobial [Mesh] or antibacterial ,antiviral ,antifungal activity[Mesh]

These items were searched from 1973 to 2015. First, two researcher's independently screened and checked articles by title. The articles imported into endnote program. Then, duplicate, irrelevant articles and non-in vitro studies were excluded. The remaining paper studied according to abstract and full text. Finally, selected articles entered for this study. Data analysis were done by ANOVA with percentage and frequency . Analysis was applied by Microsoft excel (version 2015).

RESULT

Searching in Pubmed, Scopus, Embased, Cochrane, and Medline with mesh terms found 778 papers. We excluded the duplicate and irrelevant articles .The remaining articles were 80 papers .Then, the abstract and full texts of papers were studied for eligibility. Finally, 26 studies were included (figure 1)21 studies evaluated the antibacterial efficacy

3 studies evaluated the antiviral efficacy and 7 studies evaluated the antifungal efficacy of OLE (table 1).

Publication year of included studies ranged from 1985 to 2015. The number of studies had an increase in the recent years.

Outcome parameter like difference in diameter of zone of inhibition and minimum inhibitory concentration were assessed.

All of studies conducted in vitro. 15 Of 26 Included studies measured zone of inhibition and 13 of 26 measured MIC as outcome measure (figure 3,4).

The zone of inhibition was determined by standard diffusion method . The MIC was determined by dilution method. .Different inhibition zone was detected against variety of microorganism. The activity of OLE are low in some microbial strains and high in others . the mean diameters of inhibition zones against microorganisms tested in different studies were mentioned in figure 3.

Inhibition zone with diameter less than 12 mm were consider as having low antibacterial activity ,Diameters between 12-16 mm were consider as moderately active and these with >16 mm were consider as highly activity [9].Majority of them reported a great antimicrobial activity (figure 5) .most of them demonstrated that different solvent and concentration presents a significant effectiveness against microorganisms(figure 6).

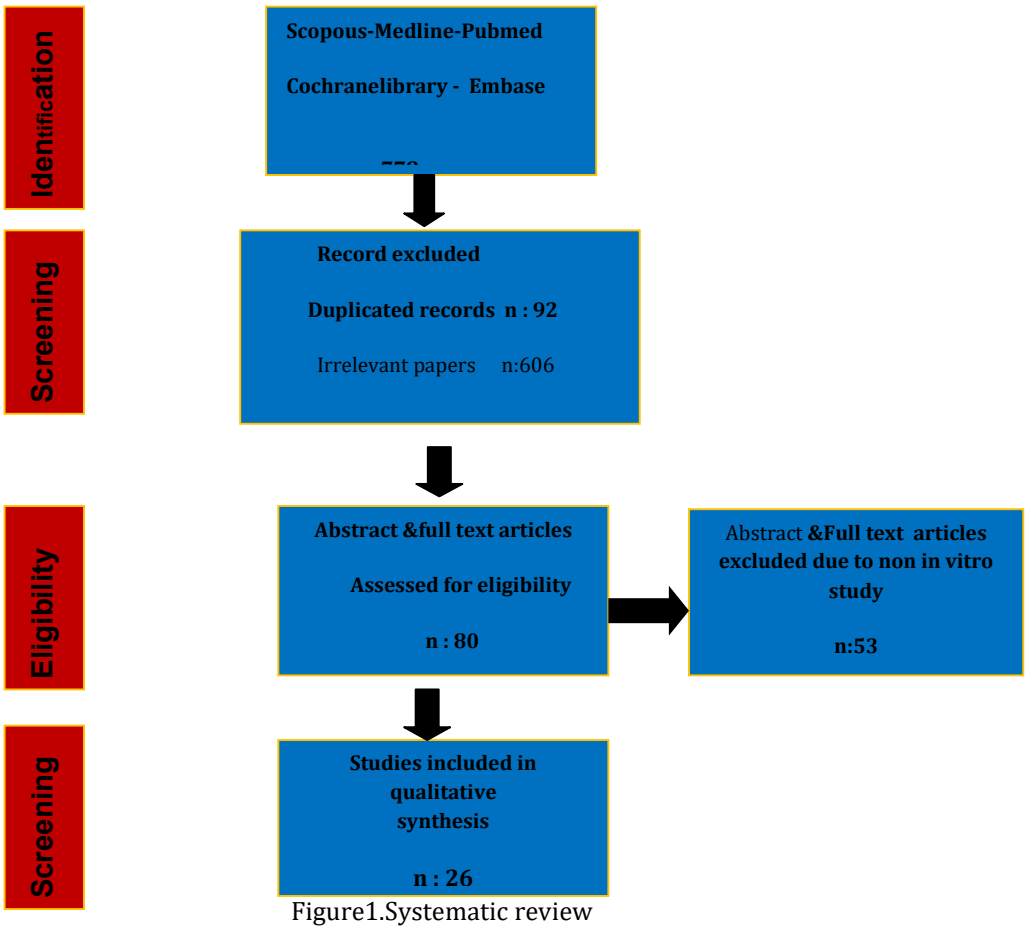

Figure1.Systematic review

Table 1: characteristics of studies

Author	Method	Material	Result	Conclusion
Fleming *et al* 1973	OLE tested by paper disc bioassay	Solvent: ethyl acetate - chloroform Concentration:4 mg/ml -1mg/ml Micro : bacteria and yeast	The acid hydrolysate of the extract was inhibitory to all of gram positive bacteria and was not inhibitory on growth of 7 species of yeasts tested	Oleuropein is degraded to antibacterial compounds when unheated olives are brined
furneri *et al* 2002	In vitro minimum inhibitory concentration was determined by a broth microdilution assay	Solvent: water Concentration:20-320mg/ml Microorganism: *mycoplasms*	Oleuropein is more toxic for gram positive bacterias than for gram negative bacterias	Oleuropein was found to be effective against mycoplasmas
Markin *et al* 2003	In vitro scaning elctron microscopic observation for *candidia albicans*	Solvent: water Concentration :0.6 %,1.25%,15% Microorganism : bacteria*(E.coli)*,fungi : *candida albicans*	Deramato phytes were inhibited by 1.25 %(w/v) plant extract following a 3 day. *Candida albicans* was killed following a 24 h in the presence of 15 % (w/v) plant extract	Olive leaves extract shows antibacterial and antifungal properties
Sylvia lee *et al* 2003	In vitro anti HIV activities were examined by CDNA micro assay analysis	Solvent :water concentration :5mg/ml Microorganism:HIV1	OLE caused a dose dependent inhibition of syncytia formation with an EC50 about 0.2 mg/ml	OLE may modulate the host response to infection rather than solely by effects on viral pathogenesis
Battinelli *et al* 2005	MIC and MFC (minimum fungicidal concentration) was determined	Solvent: DMSO Concentration: 109-125 microgram/ml Microorganism :bacteria-fungi	Study showed a different spectrum of anti microbial activity and MFC values were the same of the MIC ones	OLE could be useful agents in the topical treatment of fungal cutaneous infection
Micol *et al* 2005	The inhibitory effect measured as the number of foci formation . 50% inhibitory concentration evaluated as IC50 . Viral titer determination was done	Concentration : 0.6-1 mg/ml Microorganism: viral haemorhagic septicaemia virus(VHSV)	OLE showed inhibitory action against VHSV and reduce the progression of VHSV	OLE can be considered a potential source of antiviral agents for aquaculture
Korukloglu *et al* 2006	In vitro MIC & (MBC) determination were made	Solvent : aceton , ethyle alcohol Concentration :0.1,0.3,0.5 mg/ml Microorganism :fifteen bacteria including food-born pathogenic were used in this study	All the aceton ethyle alcohol & diethyle ether extracts of OL showed antibacterial sensivity . Aqua OLE had no antibacterial against test bacteria	Antibacterial activity of OLE has been observed against lactic acetic acid and human pathogenic bacteria
Motamedi Far *et al* 2007	Virucidal effect and viral replication in vero cell line were studied in the presence of various concentration of OLHE applied at different time intervals using a standard plaque assay method .the 50% cytotoxic concentration (CC50), IC50 & therapeutic index of OLHE were determined	Solvent: water , ethanol Concentration : >1 mg/ml Microorganism : HSV1	OLHE showed virucidal effect on HSV1	OLHE has anti HSV 1 activity likely due to the prevention of virus entry into the cells .
korukluoglu *et al* 2007	Using the disc diffusion method	Solvent :water ,aceton,methanol ,ethyle acetate Concentration:30mg/ml Microorganism :fungal strains	ZOI ranged from 7-21mm	Aqueous extract showed the most prominent activity.diethyle ether extracts of the olive leaves showed poor antifungal activity
Pereira *et al* 2007	In vitro MIC for the inhibition of microbial	Solvent : water Concentration : 0.05 -5mg/ml	At low concentration OLE showed an unusual	OLE showed great potential as nutra

	growth obtained	Microorganism :human intestinal and respiratory bacterial pathogen and fungi	combined antibacterial & antifungal activities	ceuticals particularly as a source of phenolic compounds
Aliabadi *et al* 2009	In vitro zone of inhibition were used to determine the antibacterial activity of OLE	Solvent : water Microorganism:bacteria Concentration:10-15-25-30-50 mg/ml	Olive leaf aquaes extract showed good antimicrobial abilities and highest inhibition of 11.5 against salmonella typhi	OLE is potent source of polyphenols having antimicrobial properties.
sudjana *et al* 2009	Using agar dilution and broth microdilution techniques . MIC , MBC & MFC were determined .	OLE purchased from local health store Concentration: 4.4 mg/ml Microorganism : bacteria	OLE may have a role in regulatory the composition of gastric flora by selectively reducing level of *H.pylory* and *C.jejuni*	Olive leaf extract was not broad spectrum in action showing appreciable activity only against *H.pylory, S.aureus , C.jejuni & MRSA*
Turhan *et al* 2009	Agar diffusion method was done	Solvent : water Concentration : 129 mg/ml Microorganism *: S.aureus*	MC film with 1.5% OLE decreased the count of *s.aureus*	OLE was effective against *staph.aureus*
Ok lee *et al* 2009	In vitro antimicrobial activities were examined by measuring the zone of inhibition	Microorganism: bacteria	ZOI = 23.5	Antimicrobial activities of the combined phenolics showed similar or better effects than the individual phenolics
Erdohan *et al* 2011	OLE concentrate on the film properties and the antimicrobial efficiacy of films against bacteria by agar diffusion method was investigated	Solvent :chloroform, methanol Concentration :3 gr/100ml Microorganism : *s.aureous*	OLE concentrate in the MC film discs changed within range of 0.6-3.6 mg and increasing concentrate caused a significant increase in ZOI	OLE have a great potential in antimicrobial food package to reduce post-process growth of bacteria
Faiza *et al* 2011	Agar disk diffusion method and measurement of diameters of inhibition zone were used	Solvent:ethyl acetate , aceton Concentration : 10-15-20-30-50 mg/ml Microorganism: bacteria and fungi	All of extract showed good inhibitory effects toward *E.coli & bacillus cereus* as compared to other bacteria . Ethyle acetate and aceton extracts were more effective against fungi	Olive is a potent source of polyphenols having antibacterial and antifungal properties
Paudel *et al* 2011	The capacity of the extracts were evaluated as ZOI	Solvent : methanol ,pet roleum ether , chloroform ,water Concentration :250 mg/ml Microorganism : bacteria	Effectiveness of OLE varied from one species to other with ZOI of 7-21 mm	Extract obtained with methanol appeared to be the most effective against all pathogenic bacteria . best
Keskin *et al* 2012	In vitro zone of inhibition measured in mm	Solvent :water Concentration 200g/l Microorganism:bacteria	Aqueous extract of OLE showed antimicrobial activity against some of the test microorganisms with the exception of *B.cereus ,E.aerogenes , E.cloacae*	Significant antibacterial activity against opportunistic pathogens was found in OLE obtained from west Anatolia
Bisignano et al 2014	In vitro minimum inhibitory concentration (MIC) of 3,4 DHPA –EA were determined by the broth microdilution method and zone of inhibition (ZOI)	Solvent :methanol, DMSO Concentration:5mg/ml Microorganism:pathogenic bacterial strains of American type culture collection were used*(ATCC*)	3,4 DHPEA-EA was effective against *ATCC* and clinical isolates of *staphylococcus aureus* . no significant difference between the two solvent	OLE would be novel therapies for the treatment of skin infection
gumgumjee *et al* 2014	Using agar diffusion assay method and antimicrobial activity was determined by measuring the inhibitory zone	Solvent :petroleum ether ,ethanol,ethyl acetate Concentratin : 200mg/ml Microorganism: 3 gram negative and 3 gram positive bacteria	Ethanol extract is the most effective against all bacteria .Petroleum ether extract of the leaves and stem showed no activity against	The optimal effectiveness of the OLE may not be due to one main active constituent but may be due to the

			B.subtilis MRSA & *S.aureus* and low activity against *E.coli ,K.pneumonia and P.aeruginosa*. Ethyl acetate of leaves showed a moderate effective ness against all bacteria	combine action of different compounds originally present in the plant .
Altaf Hussain *et al* 2014	Agar disc diffusion method was used and zone of inhibition (ZOI) was measured	Solvent : methanol ,ethanol chloroform ,water,ethyl acetate Concentration : 15mg/ml Microorganism : bacteria	OLE have a potential antimicrobial activities against some of gram-positive and gram-negative bacterial strain	Leaf extract is a cheap and effective antibacterial agents that can be used as alternative to purified oil
lamprini *et al* 2014	The MIC assay and MBC assay were applied	Solvent : aceton , water Concentration : 10 mg/ml Microorganism : bacteria and *candida albicans*	OLE showed a milder inhibitory effect against oral pathogen	OLE were active against the tested pathogens specially gram- negative anaerobic bacteria
Buddhini *et al* 2014	Colonies of bacteria were counted after 18-24 h of incubation .	Solvent :water Concentration: 1%,3%,5 % Microorganism :*E.coli*	Reduction in *E.coli* population were observed with all three concentration of olive extract by day 3	Olive leaf extract demonstrated antimicrobial activity against *E.coli*
Abbasvali *et al* 2015	In vitro zone of inhibition were used	Solvent :aceton ,ethanol,methanol Concentration :10,20 mg/ml Microorganism:*salmonella typhimurium*	Acetonic extract at 10 mg/ml inhibited *s.typhy* .the percentage of the area under the growth curve of acetonic extract of 4 cultivares at 10 mg/ml was lower than 5%	Antibacterial effect of the extracts varied in response to the solvent used .acetonic extract had stronger antibacterial effect against *s.typhy* in comparison with ethanolic& methanolic extract
Salma malik *et al* 2015	In vitro zone of inhibition in mm were measured and MIC was determined	Solvent : methanol Concentration: 500,1000,1500,2000 mg/ml Microorganism :2 gram positive bacteria : *S aureus & B.cereus*	OLE were effective against 2 gram positive strains but low activity was observed against gram negative strain	Use of OLE as nutraceuticals may lower the risk of microbial infection in the intestinal and respiratory tract
Gokman *et al* 2015	ZOI and MIC was determined	Concentration: 50 mg/ml Microorganism:bacteria	The diameter of ZOI and MIC was evaluated against 5 gram positive and 5 gram negative bacteria	OLE presented the highest antibacterial activity against *B.cereus* & the lowest antibacterial against *S.typhy*

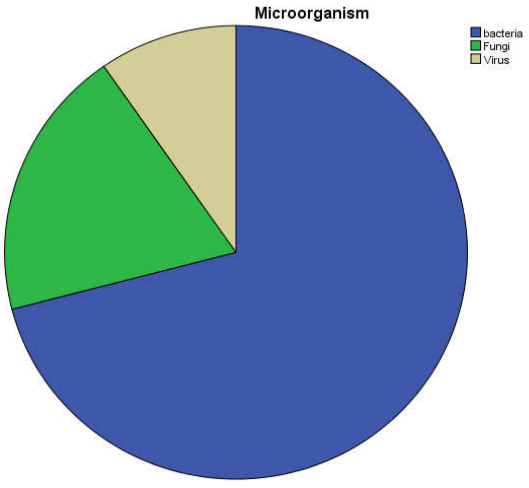

Figure 2. Type of microorganisms was used in antimicrobial assessment of OLE

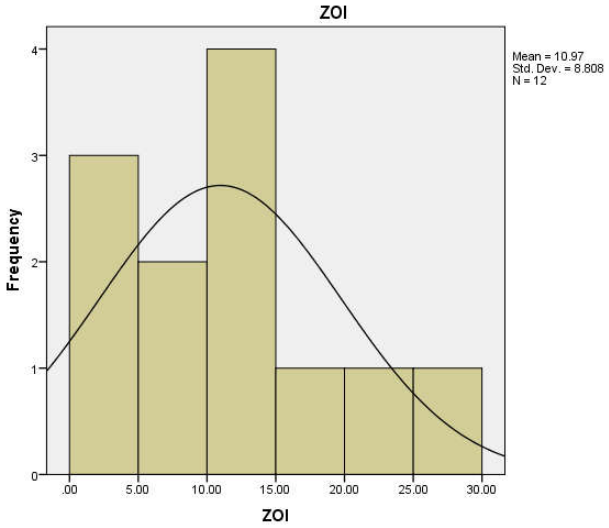

Figure 3 .Zone of inhibition

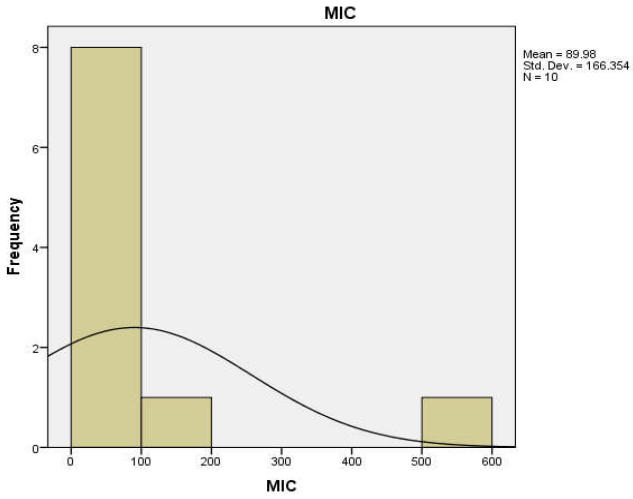

Figure 4.Minimum of inhibitory concentration

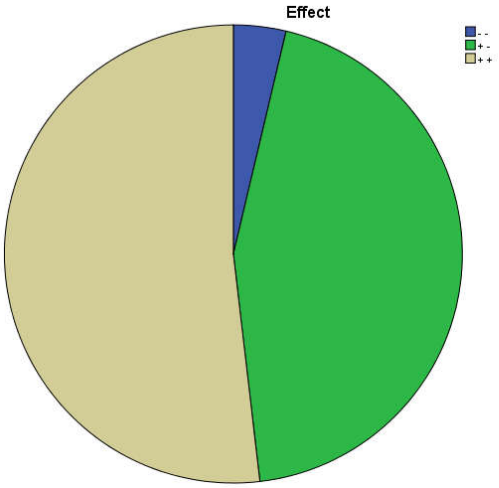

Figure 5. Type of antimicrobial effect of OLE . ++: studies which are effective against all tested n microorganisms.+-:studies which are effective against some strains of tested microorganism .--: studies which are had no effect on tested microorganism

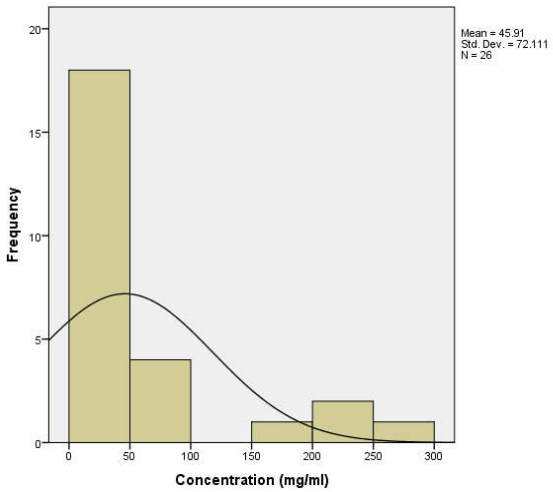

Figure 6 . Different concentration of OLE used against microorganism.

DISCUSSION

Nowadays increased pathogen resistance and adverse side effects of Antibiotics is a concerning challenge in treatment of infectious disease [10]. In addition incidence of treatment failure and opportunistic infection was increased by commen antifungal agents [11] , because of this problems people prefer to use natural antimicrobial alternative instead of synthetic chemical antimicrobial agents.

Olive leaf extract has been used for medicinal purposes for decades. It possesses a variety of bioactive ingredients play a role for their biological properties. No one main active compounds responsible for therapeutic effect alone and it may be due to the combined action of different constituent present in the plant [12] . the biological effects of OLE are mainly derived from phenolic components [13].

Many studies confirm positive role of OLE in inhibitory pathogenic microorganisms .Olive leaf extract present activities against some of both gram positive and gram negative bacterial strains [1-20] and also has been shown to inhibit or delay the rate of growth of a range of fungi in varying degree thus they might be useful as alternative for antifungal agents [11,21,22,23].

OLE has antiviral effect due to the prevention of viruses entry in to the cells [6,24,25].

Different studies demonstrated different solvent and concentration was used to provide OLE and thereby different antimicrobial effect was obtained .Varying degree of susceptibility in different extracts revealed that some of solvent are more effective in extracting the active ingredient than the others [26-30]. The use of methanol ,ethanol and water as solvent proved to be more efficient .the maximum activities were found for the methanolic extract against tested bacterial strains in Altaf Husseins study [29]. In paudels study extracts obtaine by water is not effective agent against the tested pathogen(31) but Korukluoglu reported that the aqueous extract showed the high activity [27] and markin also report that water extract of OLE with a concentration of 0.6% are effective but it would be more efficient when the concentration was increased to 20 % [26] .Ethyle acetate and acetone extracts demonstrate a wide range of antimicrobial activity ().these result may be due to phenolic distribution and concentration in extract ,and antimicrobial activity against tested bacteria.[27]

CONCLUSION

Olive leaf extract was not broad spectrum in action against all microorganism but could be a useful source of antibacterial agent against special bacterias. .antimicrobial effect of the extract varied in response to the solvent used .OLE has antiviral activity due to the prevention of virus entry in to the cells also demonstrated inhibitory activity against fungi .these results indicate that OLE can be considered as a promising natural antimicrobial alternative limited studies is available on pharmacokinetics of OLE ingredients and it is not possible to hypothesize if this substance retains its antimicrobial properties *in Vivo* . Further research is necessary to approve this point.

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