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Determination of *in-vitro* Antimicrobial Effects of Different Solvent Extracts of *Curcuma zedoaria* (Kunyit putih) rhizome to inhibit the growth of certain types of Gram-negative and Gram-positive bacteria

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ABSTRACT

The importance of medicinal plants remarkably increases. Some of them possess natural products that help them resist antibiotic-resistant bacterial strains. *Curcumazedoaria* is among the most prominent plants of the ginger family highlighted for the presence of antibacterial agents. Methanol, ethanol and isopropanol extracts of *C. zedoaria* rhizome were subjected to phytochemical screening for the presence of total flavonoids content (TFC), antioxidants and phenolic compound. They were also subjected to antimicrobial susceptibility testing using agar-disc diffusion technique at concentrations of 10 mg/mL, 25mg/mL and 50mg/mL against both of Gram positive bacteria including *Bacillus subtilis*(ATCC 6633), *Bacillus cereus*(ATCC 33019), *Staphylococcus aureus*(ATCC 29737), *Propionibacterium acne*(ATCC 6919), *Streptococcus mutans*(ATCC 27351) and *Listeria monocytogenes* and gram-negative including *Escherichia coli*(ATCC 25922), *Klebsiella pneumoniae*(ATCC 13773) and *Acinetobacteranitratus*(A9). Their Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) were also determined. All the extracts had the presence of TFC, Antioxidants and phenolic compounds and exhibited activity against *S. mutans, E. coli* and *B. cereus* at different dilutions used with the inhibition zones range of 7.33 mm to 10.67 mm. They were also active against *B. subtilis* and *A. anitranus* at 25 and 50 mg/mL concentrations. However, there was no activity of the extracts at all concentrations against the isolates of *K. pneumoniae, S. aureus, L. monocytogenes* and *P. aeruginosa*. The MIC and MBC values for all the extracts were equal against *S. mutans* and *B. cereus*. Isopropanol extract had the lowest MIC value of 1.25 mg/mL against *E. coli* while ethanol extract had the lowest MIC value of 0.632 mg/mL against *B. subtilis*. Meanwhile, both the two extracts exhibited the lowest MICs of 2.5 mg/mL against *A. anitranus*. Other values remain the same. The refined extracts of *C. zedoaria* rhizome could be alternative source of cure of diseases caused by the test organisms.

تحديد التأثيرات المضادة للميكروبات لمستخلصات مختلفة من ريزومات نبات الكركم زيدواريا معمليا على انواع بعض السلالات المنتخبة من البكتيريا السالبة والموجبة لصبغة جرام

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الملخص

الكلمات المفتاحية

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تزداد أهمية النباتات الطبية بشكل ملحوظ ، حيث تمتلك بعضها مركبات طبيعية تساعدها على احداث تأثيرعلى نمو السلالات البكتيرية المقاومة للمضادات الحيوية، و يعد الكركم زيدواريا من أبرز نباتات عائلة الزنجبيلية المميزة لوجود عوامل مضادة للبكتيريا، لذلك تم إجراء تقديرا كميا لمحتوى مستخلصات رايزومات الكركم من المركبات الفلافونويدية ،مضادات الاكسدة و المركبات الفينولية، و تم إخضاعهم أيضًا لاختبار الحساسية المضادة للميكروبات باستخدام أقراص ترشيح مشبعة بتركيزات 10 ملجم / مل، 25 ملجم / مل و 50 ملجم / مل على سطح الاجار المغذي ضد كل من البكتيريا الموجبة لصبغة جرام وشملت العصوية الرقيقة ، العصوية الشمعية، المكورات العنقودية الذهبية ، البروبيونية العدية ،السبحية الطافرة والليستيريا المستوحدة، وأيضا البكتيريا سالبة لصبغة جرام وشملت العصيات القولونية ، الكلبسيلة الرئوية والمكورة الراكدة ، كما تم تحديد الحد الأدنى المثبط والحد الأدنى القاتل من التركيزات ،وأوضحت النتائج احتواء جميع المستخلصات على المركبات الفلافونيدية ،مضادات الأكسدة والمركبات الفينولية وأظهرت نشاطًا ضد العقدية الطافرة ، العصية القولونية و العصوية الشمعية في التخفيفات المختلفة المستخدمة مع اقطار مناطق تثبيط تراوحت من 7.33 - 10.67 ملم ، وكانت نشطة أيضًا ضد العصوية الرقيقة و والمكورة الراكدة بتركيزات 25 و50 ملغم/مل، خلاف ذلك، لم يكن هناك أي نشاط للمستخلصات في جميع التراكيز ضد عزلات الكليبسلة الرئوية ، المكورة العنقودية ، والليستيريا المستوحدة، و كانت قيم الحد الأدنى المثبط والحد الأدنى القاتل لجميع المستخلصات متساوية ضدالبكتيريا السبحية الطافرة والعصوية الشمعية وكان لمستخلص الأيزوبروبانول أقل قيمة تأثير مثبط تبلغ 1.25 مجم / مل ضد العصية القولونية بينما كان لمستخلص الإيثانول أقل قيمة تأثير مثبط تبلغ 0.632 مجم / مل ضد العصويات الرقيقة ، وفي الوقت نفسه أظهر كلا المستخلصين أدنى مستوى مثبط بلغ 2.5 ملغم/مل ضد المكورة الراكدة ، وبذلك يمكن أن نستنتج إمكانية استخدام كركم زيدواريا مصدرًا بديلاً لعلاج الأمراض التي تسببها الكائنات الحية التي تم اختبارها.

الكركم الأبيض.
النشاط المختبري.
مستخلصات المذيبات.
سالبة الجرام.
موجبة الجرام

Introduction

Several plants and plant products have been significantly employed as antimicrobial agents due to the presence of secondary metabolites [1].Their emergence as source of natural products has been in existence for many centuries [2]. These plants have been explored and tested severally for their antimicrobial efficacies [3]. It has been reported that *Curcumazedoaria* (white turmeric) rhizomes possess various compounds that are potential antibacterial agents. For many centuries, *C. zedoaria* has been employed in various countries particularly the South-East Asia in which the dried rhizomes had been used in making drinks and also the extracts as traditional medicine in the treatment of stomach infections, stagnation of blood and in the treatment of diarrhoea. This plant contains substances with high antimicrobial effect such as curcumin, gums, arabin, essential oils etc [1-4].

The plant *Curcuma zedoaria* has greatly been utilizedas the agent of coloring and as a spice with medicinal potentials [5]. It is a member of the rhizomatous family, the Zingiberaceae also known as ginger [6]. Several plant parts are used to cure various illnesses like coryza,stomachdiseases,diarrhea,liver protection,skin disorders,rheumatism and facilitation of menstruation [7].

Microorganisms persistently weaken various antimicrobial agents in the medical field despite their abundance. This is achieved via the evolution of various mechanisms by the organisms against such drugs [8]. This may equally be as a result of the production of enzymes such as beta lactamase and other enzymes [9]. Microbial resistance to antibiotics is an issue of concern worldwide and as the resistance occurs, the possibility of treatment due to pathogenic microorganisms becomes greatly reduced [10-11].

Various solvents have successfully been employed in the extraction of bioactive principless from plants [12-13]. Water being a universal solvent, has been employed in the extraction of products from plants proved to have had antimicrobial potential. However, plant products extracted using organic solvents reported higher antimicrobial activity than those extracted using water although water had been traditionally used by local healers [3]. In addition, water soluble flavonoids especially anthocyanins indicate no antimicrobial activity; while, water soluble phenolics showed antioxidants activity. It has also been investigated that many phytochemical contents such as phenolic compounds, saponins, flavonoids, anthraquinones, alkaloids etc, were obtained from the organic solvents extracts of plants such as C. zedoaeia [14].

The uses of plant products in the production of medicinal metabolites have been established recently [15]. Researchers have taken the advantage of the biosynthetic ability of plant in producing valuable products and also to study metabolism [16].This study therefore aimed to determine the potential for the ethanol, methanol and isopropanol extracts of *C. zedoaria* rhizome as antibacterial agents with the objective of investigating their phytochemical contents using the organic solvents methanol, methanol and isopropanol for extraction and their activity against *Streptococcus mutans*, *Bacillus cereus*, *Escherichia coli*, *Bacillus subtilis*, *Acinetobacter anitratus*, *Klebsiellapneumoniae*, *Staphylococcusaureus*, *Listeria monocytogenes* and *Propionibacterium acnes* so as that they can serve as alternative to commonly used antibiotics to which these organisms proved resistant.

Materials and methods

Sample collection and preparation of plant extracts

The C. zedoaria rhizome was obtained from a farm in Temerloh, Pahang. The procedure was carried out in accordance with the procedure of Wilson with some modifications [17]. The rhizome was washed three times thoroughly with clean water to remove dust, cut into pieces, placed in perforated paper bags air-dried at room temperature for 24 hours in an oven gradually at varying temperatures of 40 oC for three days, 50 oC for three days, and 60 oC for 24 hours. With the aid of laboratory grinder, the dried rhizome was ground to powder using a laboratory grinder. After drying, 15 g of the powder was soaked in 250 mL of each of the solvents; ethanol 90%, methanol 90% and isopropanol 30% (which were chosen based on the results of the phytochemical trial) in a 500 mL volumetric flask plugged and covered with aluminium foil and shaken using ultrasonic path sonicator (Branson, model 8510E-MTH, Danbury,USA). The mixture was left to stand for 1 hour in a shaking water bath maintained at 40 °C, then filtered and the filtrate was then evaporated under reduced pressure at 40 oC to obtain the extracts of each solvent using rotary evaporator.

The extracts obtained were weighed to determine the total extractable compounds and then stored in desiccators away from light. Moreover, 100 mg of the extracts of the solvents was dissolved in 1 mL dimethylsulfoxide (DMSO) (Fisher Scientific, Leicestershire, United Kingdom) to obtain stock solution with a concentration of 100 mg/mL. Further dilutions were made with the DMSO to obtain solutions with concentrations of 50, 25, and10mg/mL (50,000, 25,000 and10, 000 µg/mL). The DMSO served as a negative control.

2.2 Extraction and PhytochemicalScreeningof Solvents Extracts of C. zedoaria Rhizome

The three phytochemicals namely, flavonoid, antioxidant and phenolic compounds were determine by weighing 0.1 g of either the kunyit putih (C. zedoaria) leaves or rhizomes powders, dissolved in 10 mL of various concentrations (0, 10, 30, 50, 70, 90 and 100%) of each solvent and shook at 90 rpm for an overnight. The solutions were filtered at room temperature using Whatman's filter papers (0.2 µm) and subjected to secondary metabolites analysis [18-19], were methanol (90%) gave the best results for phenols, while isopropanol (30%) gave the best results for flavonoids and antioxidant, whereas ethanol (90%) was chosen as an additional solvent because it gave very close results to what was achieved with methanol (optimization trail).

2.3 Microorganisms

The bacterial strains used in the study were the members of both Gram positive and Gram-negative bacteria (GeneBank, NCBI). The Gram-positive ones include Bacillus subtilis (ATCC 6633), Bacillus cereus (ATCC 33019), Staphylococcus aureus (ATCC 29737), Propionibacterium acne (ATCC 6919), Streptococcus mutans (ATCC 27351) and Listeria monocytogenes. The gram-negative ones include Escherichia coli (ATCC 25922), Klebsiella pneumoniae (ATCC 13773) and Acinetobacter anitratus(A9).

2.4 Inoculum Standardization

All the microorganisms were sub-cultured in Mueller Hinton broth (MHB, Difco, Sparks, Maryland, UnitedStates) and incubated at 30 °C for 24 hours prior to the susceptibility testing. The resultant suspension was standardized using fresh MHB as described by Rukayadi et al. [20].

2.5 Antimicrobial Susceptibility Testing

The crude extracts of C. zedoaria rhizome were tested for antimicrobial activity against the testorganismsbased on the Clinical and Laboratory Standard Institute guideline [21]. A swab of overnight culture was aseptically spread onto Mueller Hinton agar (MHA) for all the bacterial tested strains using a sterile cotton swab. Sterile paper disc of 6 mm diameter was placed onto the agar and about 10 µl of each prepared concentration of 10mg/mL (10,000 µg/mL), 25mg/mL (25,000 µg/mL), and 50mg/mL (50,000 µg/mL) of the crude extract was pipetted onto the disc. Chlorhexidine with a concentration of 0.1 mg/mL (100 µg/mL) was used as positive control. The negative control was 10 µl of the DMSO. The plates were incubated at 30 °C for 24 hours. Zones of inhibition as clear zones around the disc were observed and their diameter measured in mm. All bioassays were conducted intriplicates and the results recorded.

2.6 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

This was conducted according to Rukayadi et al. [22] and in accordance with the guideline of Clinical and Laboratory Standard Institute M07-A9 (2012). This was carried out in a 96-well U-shaped microtitre plate using two-fold standard broth micro-dilution method with an inoculum density of approximately 105 – 107CFU/mL. The stock solution (10mg/mL) of the crude extract of C. zedoaria rhizome was mixed in two-fold dilution in the medium containing the standardized inoculum. The column 12 of the microtiter plate contained the highest concentration of extract (5mg/mL) while column 3 contained the lowest concentration of extract (0.0097 mg/mL). Column 1 served as negative control (only medium, no inoculum, and no antimicrobial agent). Meanwhile, column 2 served as positive control for all samples (only medium and inoculum or antimicrobial agent-free well). Microtiter plates were incubated at 30 °C for 24 hours. The MIC was defined as the lowest concentration of antimicrobial agent that resulted in the completed inhibition of visible growth [23].

Results

Phytochemical Screening of C. zedoaria methanol, ethanol and isopropanol extracts

Following the phytochemical tests of themethanol, ethanol and isopropanolextracts of the C. zedoaria rhizome, Table 1 shows that both the extracts indicated the presence of total flavonoids contents (TFC), antioxidant and phenolic compounds although they were extracted in various concentrations (not to mention that they are distinguished from other solvents that were subjected to the same experimental conditions in previous trail). Based on the various concentrations used in the extraction of the rhizome, all the extracts were able to produce the phytochemicals in various quantities, but the 90% of Methanol and Ethanol with 30% of Isopropanol considered the best solvents and concentrations among the others.

Table 1: Phytochemical contents of C. zedoaria solvents extracts

Phytochemical/Solvent	Methanol (90%)	Ethanol (90%)	Isopropanol (30%)
TFC	+	+	+
Antioxidant compound	+	+	+
Phenolic compound	+	+	+

+ = present

TFC= Total flavonoids contents

Antimicrobial activity of the methanol, ethanol and isopropanol extracts C. zedoaria

The in-vitro antimicrobial activity of the methanol, ethanol and isopropanol extracts of C. zedoaria rhizomes is displayed in Table 2 All the extracts exhibited activity against S. mutans in which at a concentration of 10 mg/mL the methanol, ethanol and isopropanol extracts recorded zones of inhibition of 8.5±0.55, 8.67±0.52 and 8.33±0.82 mm respectively; at 25 mg/mL concentration with inhibition zones of 9.83±1.17, 9.5±1.05 and 9.5±0.84mm respectively; finally at 50 mg/mL with inhibition zones of10.67±0.82, 10.33±0.82 and 10.5±0.84 mm respectively. However, there was no activity by all various solventsextracts of C. zedoaria rhizomes at 10 mg/mL concentration against B. cereus but inhibition zones of 8.83±0.55, 9.17±0.75 and 9.33±0.52 mm of methanol, ethanol and isopropanolextracts, respectively at 25 mg/mL concentration as well as 9.5±0.75, 9.67±0.82 and 9.83±0.75 mm respectively at 50 mg/mL. Moreover, against E. colithe methanol, ethanol and isopropanol extract of C. zedoaria rhizomes at 10 mg/mL with inhibition zones of 7.5±0.55, and 8±0.63 mm respectively. At 25 mg/mL concentration the zones of inhibition recorded by the extracts were 9.17±0.75, 9.33±0.82 and 9.5±0.55 mm respectively

while at 50 mg/mL the zones of inhibition were 9.5±0.55, 9.67±0.52 and 9.83±0.75 respectively. All the extracts showed activity against B. subtilis in which at 10 mg/mL concentration the methanol,ethanol and isopropanol extracts had inhibition zones of 7.33±0.52, 7.5±0.55 and 7.67±0.52 mm respectively while at 25 mg/mL the inhibition zones were 8.83±0.75, 9.33±0.82 and 9.17±0.75 mm respectively and 10.33±0.82, 10.17±0.75 and 10.5±0.55 mm respectively at 50 mg/mL concentration. The entire C. zedoaria methanol, ethanol and isopropanol extracts exhibited no antimicrobial activity against A. anitranus at 10 mg/mL concentration but recorded inhibition zones of 7.67±0.82, 8.17±0.75 and 7.83±0.75 respectively at 25 mg/mL while at 50 mg/mL the inhibition zones were 8.83±1.17, 9.5±1.05 and 9.33±0.82 mm respectively. However, there was no activity of the extracts at all concentrations against the isolates of K. pneumoniae, S. aureus, L. monocytogenes and P. aeruginosa.

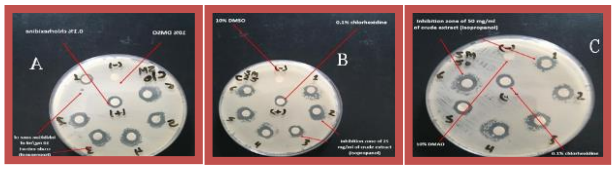

Fig.1: Inhibition zones of C. zedoaria rhizome Isopropanol crude extracts against Streptococcus mutans. Plates A, B and C indicate the 10, 25 and 50 mg/mL concentrations of the extracts

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Solvent Extracts of C. zedoaria

The MICs of the methanol extracts of C. zedoaria rhizome were found to be 0.312, 1.25, 2.5, 0.625 and 5.0 against S. mutans, B. cereus, E. coli, B. subtilis andA. anitranus respectively. However, those of the ethanol extract were 0.312, 1.25, 2.5, 0.312 and 2.5 against the organisms respectively. The MICs for the isopropanaol extract against S. mutans, B. cereus, E. coli, B. subtilis andA. anitranus were 0.312, 1.25, 1.25, 0.625 and 2.5 respectively. Meanwhile the MBCs of the methanol, ethanol and isopropanol extracts against S. mutans, B. cereus, E. coli, B. subtilis and A. anitranus remain the same for all as 0.625, 2.5, 2.5, 0.625 and 5.0 respectively, as shown in Table 3.

Discussion

The activity of these solvent extracts has been dependent on the phytochemicals presence. The fact that all the solvent extracts contain flavonoids, antioxidant and phenolic compounds is a function of their antimicrobial efficacy. Flavonoids and phenolic compounds have been investigated to have antimicrobial efficacy against different types of bacterial isolatesas well as antioxidant activity [23-24].

There were no clear differences in the recorded readings of all the solvents used, or in other sense the effects were very convergent , as there was no clear pattern in which we could be certain that there was a preference for any of them over the others, which reveals clear indications that they have given the best results in phytochemical optimization testing, which was conducted to show which was the best solvent and the best concentration of a group of solvents tested with different concentrations. However, there is a tendency to have a slight effect of ethanol compared to methanol, in most cases it showed an inhibitory effect, while other dilutions of isopropanol reported a relatively greater effect in some cases, compared to extracts of other solvents, with the general closeness that is evident between them.

Table 2: In-vitro antimicrobial activity of the methanol, ethanol, and isopropanol extracts of C. zedoaria rhizome

Bacteria	Concentration	Methanol (90%)	Ethanol (90%)	Isopropanol (30%)
Streptococcus mutans	10mg/mL	8.5±0.55	8.67±0.52	8.33±0.82
	25mg/mL	9.83±1.17	9.5±1.05	9.5±0.84
	50mg/mL	10.67±0.82	10.33±0.82	10.5±0.84
Bacillus cereus	10mg/mL	-	-	-
	25mg/mL	8.83±0.55	9.17±0.75	9.33±0.52
	50mg/mL	9.5±0.75	9.67±0.82	9.83±0.75
Escherichia coli	10mg/mL	7.5±0.55	7.83±0.75	8±0.63
	25mg/mL	9.17±0.75	9.33±0.82	9.5±0.55
	50mg/mL	9.5±0.55	9.67±0.52	9.83±0.75
Bacillus subtilis	10mg/mL	7.33±0.52	7.5±0.55	7.67±0.52
	25mg/mL	8.83±0.75	9.33±0.82	9.17±0.75
	50mg/mL	10.33±0.82	10.17±0.75	10.5±0.55
Acinetobacter anitratus	10mg/mL	-	-	-
	25mg/mL	7.67±0.82	8.17±0.75	7.83±0.75
	50mg/mL	8.83±1.17	9.5±1.05	9.33±0.82
Klebsiella pneumoniae	10mg/mL-50mg/mL	-	-	-
Staphylococcus aureus	10mg/mL-50mg/mL	-	-	-
Listeria monocytogenes	10mg/mL-50mg/mL	-	-	-
Pseudomonas aeruginosa	10mg/mL-50mg/mL	-	-	-

Table 3: Minimum Inhibition Concentration (MIC; mg/mL) and Minimum Bacterial Concentration (MBC; mg/mL) of different solvents extracts

	Solvent Used	Methanol		Ethanol		Isopropanol	
	Concentrations (mg/mL)	MIC	MBC	MIC	MBC	MIC	MBC
Bacteria	*Streptococcus Mutants*	0.312	0.625	0.312	0.625	0.312	0.625
	Bacillus Cereus	1.25	2.5	1.25	2.5	1.25	2.5
	Escherichia Coli	2.5	2.5	2.5	2.5	1.25	2.5
	Bacillus Subtilis	0.625	0.625	0.312	0.625	0.625	0.625
	Acinobacter	5.0	5.0	2.5	5.0	2.5	5.0

The antimicrobial activity of C. zedoaria rhizome against S. mutans showed that the ethanol extract had slightly highest activity followed by methanol extract and finally the isopropanol extracts. However, isopropanol extract had the highest activity against B. cereus at the concentrations, and then follows the ethanol and methanol extracts. This activity was similarly recorded against E. coli. Meanwhile, the ethanol extract recorded the highest activity against A. anitranus followed by isopropanol extract and the least was the methanol extract at both the 50 mg/mL and 25 mg/mL concentrations although there was no activity at the lowest concentration of 10 mg/mL.

The activity of ethanol extract of C. zedoaria against these organisms was similar to a study conducted by Wilson et al. [17]. This result was found to be higher than obtained in another study in Malaysia [23]. However, the result of the ethanol extract in this study was slightly lower than obtained

in another study [25] which has also reported the activity of both methanol and ethanol extracts of C. zedoaria against various Gram positive bacteria (B. cereus, B. megaterium, B. subtilis, S. aureus and Sarcina lutea), various Gram negative bacteria (Vibrio parahaemolyticus, V. minicus, Salmonella paratyhi, S. typhi, Shigella dysenteriae, S. boydii, E. coli and P. aeruginosa) The highest activity of the ethanol extract of C. zedoaria was against B. subtilis at the highest concentration. Activity against other organisms such as S. aureus, B. cereus as Gram positive bacteria as well as the Gram negative E. coli and P. aeruginosa was also reported [26] which contradict the findings in this study.

There was no antimicrobial activity recorded by the extracts at all concentrationsagainst K. pneumoniae,S. aureus, L. monocytogenes and P. aeruginosa.These findings do not agree with aforementioned study as nearly all the resistant organisms were found to be susceptible to C. zedoaria rhizome extracts at various concentrations using a variety of extracts. This shows that more concentrations of these extracts could serve batter as antimicrobials against the resistant organisms tested.

Meanwhile, the lowestMIC was observed in ethanol extract against B. subtiliswhile all other MICs of all the extracts were similar against all the organisms tested herein. This signifies the higher efficacy of the antimicrobial activity of ethanol extract than the methanol and isopropanol extract as the lower the MIC concentration the higher the activity [27]. However, the MBC results for all the extracts remain the same at all concentrations tested against all the isolates. The concept of MIC and MBC is indicative of bacteriostatic and bactericidal activity of antimicrobials. Wherein the both the MIC of an antimicrobial remains the same, it indicates that such concentration is able to kill all bacterial isolate in question. However, when the concentration differs it signifies that at the MIC is bacteriostatic; able to inhibit the growth and the MBC indicates the bactericidal action which indicates complete elimination of the isolate at such concentration [28].

Conclusion

Overall, isopropanol extract of the C. zedoariarhizome had relatively the highest activity against all the sensitive organisms viz; S. mutans, B. cereus, E. coli, B. subtilis and A. anitranus. This was followed byethanol and finally the methanol extracts,with minor exceptions considering that we use different dilutions of each solvent.Whereas the ability of ethanol is evident in the case of (A. anitranus) bacteria.High dilutions of methanol (25 and 50mg/mL) were distinct in their effect in the case of S. mutans bacteria. There was no activity againstK. pneumoniae, S. aureus, L. monocytogenes and P. aeruginosa. However, this does not indicate inactivity against these isolates. Higher concentrations may prove effective. It is noteworthy that these are crude extracts of the C. zedoaria rhizome using the three different solvents. Refined extracts are hereby recommended so that the antimicrobial property of the extracts will manifest again the resistant isolates. Further study should be conducted using other solvent extracts and with other plant parts such as leaves, flowers and stem of C. zedoaria to ascertain the highest antimicrobial activity of the plant for recommendation in both traditional and orthodox medicine.

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