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STUDY THE ANTIBACTERIAL IMPROVEMENT OF ANTIBIOTIC ACTIVITY BY USING A NEW POLYMER PREPARED FROM SUMAC EXTRACT

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ABSTRACT : The purpose of this study is natural products such as plants and herbal extracts have been used as antimicrobial agents. Sumac has been known as an antimicrobial effective natural product. In this study, we examined the antimicrobial effect of the polymeric sumac derivatives. Sumac extracted has been polymerized with citric acid and folic acid separately and then its antibacterial activities have been tested against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method. Results shows that the diameter of inhibition zone (DIZ) of polymer I, polymer II and $FeSO_4$ against *Staphylococcus aureus* was 18mm, 15mm, and 16mm respectively while the MICs was 0.1%, 0.2% and 0.2%, respectively. Polymer II and $FeSO_4$ also displayed a significant activity against the *Escherichia coli* with DIZ ranging from 17–22 mm. However, polymer I did not show any effect. *Escherichia coli* appears to be more sensitive to $FeSO_4$ compared to polymer I. In which the MICs was 0.2% and 0.3%, respectively. Polymer I, II and $FeSO_4$ exhibited a synergistic effect on the gentamicin antimicrobial activity compared with the free drug and polymer or $FeSO_4$ alone. A similar trend was observed for II and $FeSO_4$ against *Escherichia coli.* In conclusion, the study's results revealed the antibacterial activities of using the polymer I, polymer II and $FeSO_4$. Combinations of gentamicin with polymer I, polymer II and $FeSO_4$ demonstrated a remarkable synergistic effect on *Staphylococcus aureus* and *Escherichia coli.*

Key words : Gallic acid, sumac, antibacterial, natural products.

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INTRODUCTION

Since ancient times, medicinal plants have been extensively conducted to treat a wide spectrum of medical conditions (Mahady *et al*, 2008). It has been reported that aromatic and medicinal plants naturally contain a wide range of bioactive phytochemicals such as essential oils and phenolic compounds (Ghrairi *et al*, 2019). Phenolic compounds generally divided into simple and complex compounds based on the number of aromatic rings in the chemical structure. Phenolic acid is perhaps one of the most important classes of plant phenolic compounds. Gallic acid, caffeic acid, and salicylic acid are the most common phenolic acids (Kahkeshani *et al*, 2019). Gallic acid is one of the natural hydroxylated phenol that exposed to be toxic to microorganisms. Its molecule has three -OH groups and one carboxylic acid (Cowan, 1999) (Fig. 1). Gallic acid naturally found in a wide range of natural products such as sumac, oak bark and gallnuts. Iron chelators compounds have been investigated and shown to possess antibacterial properties. Gallic acid has been reported to form a chelating complex when it reacts with ferrous (Fe^{2+}) sulphate (1:1) (Thompson *et al*, 2012; Haslam *et al*, 1994; Shidfar *et al*, 2014) (Fig. 2). Moon and colleagues, carried out research to exam into the ability of iron chelators deferoxamine (DFO) and deferasirox (DFRA) as antibacterial and antibiofilm. They reported that DFRA displayed strong antimicrobial activity against planktonic *P. intermedia*, but the bacterial growth was partially inhibited. They also found that DFO was incapable to inhibit the bacterial growth completely in the concentration range tested (Moon *et al*, 2013). In this study, we aim first to isolate gallic acid from sumac and then polymerized it with citric acid and folic acid separately. The second step is to study their ability to improve the biological properties including inhibition of the growth of harmful microorganisms in present/without iron.

MATERIALS AND METHODS

Sumac was purchased from a local market and citric acid from Thomas Baker, Mumbai, India. Folic acid was purchased from Sigma Aldrich, UK.

Extract of sumac

Sumac fruits were ground into small particles firstly with household (silver crest) grinder, then, 10 g of sumac powder was soaked in 200 ml of warm distilled water with occasional stirring until the water colure became bright maroon this process takes around half-hour. The mixture was filtered to obtained the extract followed by concentrated and dried it.

Polymers preparation

Polymers were synthesized by esterification of hydroxyl groups of the sumac polyphenols extract with carboxylic acid groups of citric acid and folic acid separately. Polymerization was carried out at 2:1 wt/wt feed ratio sumac extract: carboxylic acid.

Microorganisms

Staphylococcus aureus and *Escherichia coli* were used in this study. The tested bacteria were obtained from the microbiology laboratory of Al SADR teaching hospital in Basrah city.

Culture media

Mannitol salt agar was used for confirmation of *Staphylococcus aureus* (Kateete *et al*, 2010). Furthermore, MacConkey agar and Eosin methylene blue was used for confirmation of *Escherichia coli* (Leininger *et al*, 2001; Lüscher and Probes, 1994).

Mueller-Hinton agar was used to study the susceptibility of the examined bacteria to the new polymers (Stubbings *et al*, 2004).

Isolation and identification of *Staphylococcus aureus*

Microbiological confirmation methods were used for isolation and identification of *Staphylococcus aureus*, such as the growth of isolates on Mannitol Salt Agar, colony morphology, Gram stain and detection of catalase activity (Forbes *et al*, 2007). The isolates were further investigated and confirmed as *Staphylococcus aureus* by detection of coagulase activity, the susceptibility to polymyxin B and novobiocin (Tille, 2015). Further conformation bacterial identification was performed by using the Vatic2 system (Biomerieux direct).

Isolation and identification of *Escherichia coli*

Bacterial samples were first streaked onto the Eosin Methylene Blue Agar (EMB) agar, incubated at 37°C for 24-48 hrs. The obtained bacteria were streaked on MacConkey agar (Merck, Germany) and incubated for 24h at 37°C. From plates, the colony displed bacterial growth of *Escherichia coli* features (Gill *et al*, 2014) were further analyzed by using Gram staining and biochemical API 20E identification system (BioMerieux, Inc, Durham, NC).

Antimicrobial sensitivity test

Disk diffusion method was used to assess the antimicrobial activities of polymer I, polymer II and $FeSO_4$ against *Staphylococcus aureus* and *Escherichia coli*. The agar plates were streaked with bacterial suspension that adjusted to 0.5 Mac Farland standard (1.5×10^8 CFU/ml).

Fix amount of different concentrations of each tested material was loaded on the centre of the sterilized filter paper (about 6 mm in diameter), which applied on the bacterial culture agar. Blank disk and Gentamicin loaded disk were used as negative and positive control, respectively. The plates were left for 1h at the refrigerator (4^0C) to ensure diffusion of the tested materials in the agar. The plates were then incubated at 37^0C for 24 h. The zone of inhibition around each filter paper disk was calculated (Stengel and Connan, 2015).

Determination of the minimum inhibitory concentration (MIC) and bactericidal concentration (MBC)

The minimum inhibitory concentration of the tested polymers and $FeSO_4$ was determined by the broth dilution method. Different concentrations of each polymer were prepared using sterile Muller Hinton broth. These concentrations range from 0.03, 0.003 and 0.0003. Moreover, $FeSO_4$ concentrations ranged from 0.02,0.002 and 0.0002.

The bacterial stock of 10^8/ml was prepared from fresh bacterial cultures after diluted in Muller Hinton broth. The bacterial stock was added to each individual tube of extract to reach the final concentration of 5×10^5 CFU/ml. One of the tubes was left without extract and used as a control tube. All the tubes were incubated at 37^0C for 18h and examined for occurring bacterial growth.

The minimum inhibitory concentration was determined, in which the lower concentration of extract had no growth of bacteria. The minimum bactericidal concentrations (MbCs) were determined by streaked the samples from the tube above the (MICs) on the Mueller-Hinton agar. The MBC was determined as the highest dilution in which no single bacterial colony appear on the agar plates.

RESULTS AND DISCUSSION

Synthesis and Characterization of polymers

Sumac extract was obtained as a dark burgundy solid after dried with a yield 60%. The results revealed that polymer I displayed bright burgundy color with a yield 90%. The results also showed that polymer II displayed light brawn color with a yield 95% (Fig. 1).

FTIR spectrum

The FTIR spectrum of sumac extract (Fig. 2) indicate the presence of a broad band of O-H at 3458 cm^{-1}, C=O group at 1743-1714 cm^{-1} and C-C aromatic 1530 cm^{-1}.

Synthesis of polymer I was verified by FTIR analysis; however, visually this can be easily verified by the formation of more rigid material with polymer-like appearance. FTIR spectra of polymer I (citric acid and sumac extract) is shown in the Fig. 3. From FTIR spectra can be identified broad band at3435 cm^{-1} related to the non-reacted OH groups. The FTIR spectrum also indicates two peaks appeared at 1728 cm^{-1} and 1635 cm^{-1} due to the presence of C=O groups. The sharp intense peak at 1226 cm^{-1} was attributed to the C-C-O peaks stretching confirming the combination of the sumac extract with citric acid.

The esterification reaction of folic acid and sumac extract is expected to occur easily and short time. Folic acid conjugation was confirmed using FTIR spectroscopy. The FT-IR spectrum (Fig. 4) indicate the presence of a broad band of 0-H at 3431 cm^{-1}, assigned to the O-H Stretch this absorption overlaps the N-H (from folic acid) stretching peaks. C=O group at 1743 cm^{-1} and 1639cm^{-1}, C-C aromatic 1375 cm^{-1} peaks confirming the combination of the folic acid with sumac extract.

The diameters of inhibition zones around the disk were measured to assess the antimicrobial activity of tested materials.

Table 1 : Antimicrobial activity of polymer I and $FeSO_4$ against *Staphylococcus aureus*.

Material	Amount (µl)	Low concentration	Medium concentration	High concentration
PI	10	-	-	8
PI	20	-	6	18
PI + Disk	10:30	19	19	19
PI + Disk	20	19	19	20
Fe	10			
Fe	20		9	16
Fe + Disk	10	19	19	14.3
Fe + Disk	20	19	19	22
PI +Fe	10:10	-	-	8
PI +Fe	10:20	-	6	16
PI +Fe	20:10		6	18
PI +Fe + Disk	10:20:30	19	19	21
PI +Fe + Disk	20:10:30	19	19	22

Low concentration of polymer I (PI) = 0.0003, Medium concentration of polymer I (PI) = 0.003, High concentration of polymer I (PI) = 0.03: Low concentration $FeSO_4$ (Fe) = 0.0002, Medium concentration of $FeSO_4$ (Fe) = 0.002, High concentration of $FeSO_4$ (Fe) = 0.02: Antibiotic disk of gentamicin (Disk) = 30 mg

Table 2 : Antimicrobial activity of polymer II and $FeSO_4$ against *Staphylococcus aureus*.

Material	Amount (µl)	Low concentration	Medium concentration	High concentration
PII	10		-	
PII	20			15
PII + Disk	10:30	19	19	19
PII + Disk	20:30	19	19	20
PII +Fe	10:10	-	-	
PII +Fe	10:20	-	9	16
PII +Fe	20:10		6	18
PII +Fe + Disk	10:20:30	19	19	20
PII +Fe + Disk	20:10:30	19	19	21

Low concentration of polymer II (PII) = 0.0003, Medium concentration of polymer II (PII) = 0.003, High concentration of polymer II (PII) = 0.03: Low concentration $FeSO_4$ (Fe) = 0.0002, Medium concentration of $FeSO_4$ (Fe) = 0.002, High concentration of $FeSO_4$ (Fe) = 0.02: Antibiotic disk of Gentamicin (Disk) = 30mg

Fig. 1 : Colure change according to the polymerization with I) citric acid II) folic acid.

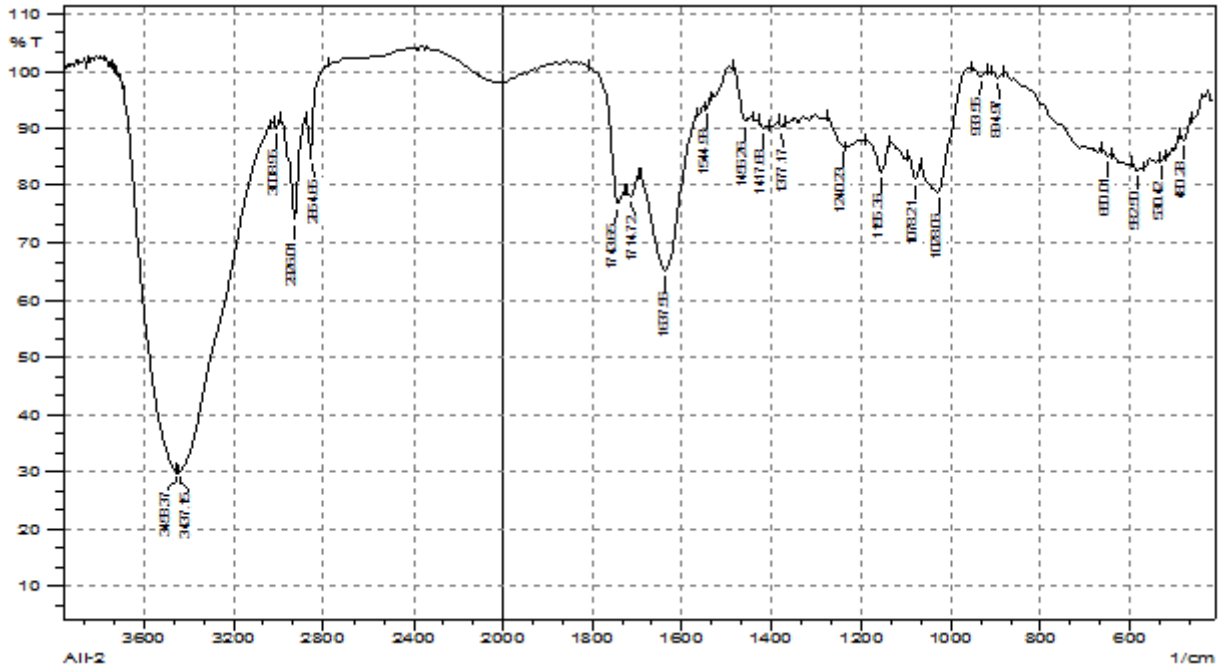

Fig. 2 : FTIR spectra of sumac extract.

The antimicrobial activity of Polymer I, Polymer II and $FeSO_4$ against *Staphylococcus aureus* and *Escherichia coli* were determined by the formation of an inhibition zone.

Regarding *Staphylococcus aureus*, the antimicrobial activity of each of Polymer I and Polymer II was increased when 20 µl of high concentration was used. In which the diameters of the inhibition zone were 18 mm and 15 mm, respectively. It was found that the mean inhibition zone of sumac extract increased by increase the concentration of the extract from 0.1% to 0.5% (w/v) in which the inhibition zone ranges from 10 to 24.7 mm (Nasar-Abbas and Halkman, 2004). Moreover, by the use of 20 µl of high concentration of $FeSO_4$ the diameter of the inhibition zone was 16mm (Tables 1 and 2).

The synergistic effect of polymer I, $FeSO_4$ and gentamicin were evaluated. In which the antimicrobial activity against staph was increased when used each of polymer I with gentamicin and $FeSO_4$ with gentamicin. In which the diameters of the inhibition zone were 20 mm and 22 mm, respectively. However, no synergistic effect was obtained when polymer I and $FeSO_4$ were used (Table 1).

Similarly, the synergistic effect of polymer II, $FeSO_4$ and gentamicin were evaluated. In which the antimicrobial activity against staph was increased when used each of polymer II with $FeSO_4$ and gentamicin. The inhibition zone was 20 mm when the amount of polymer II and $FeSO_4$ was 10 and 20, respectively. Moreover, the inhibition zone was 21 mm when the amount of polymer II, $FeSO_4$ and antibiotic was 20 µl, 10 µl and 30 mg, respectively (Table 2). However, no synergistic effect was obtained when polymer II and $FeSO_4$ were used (Table 2).

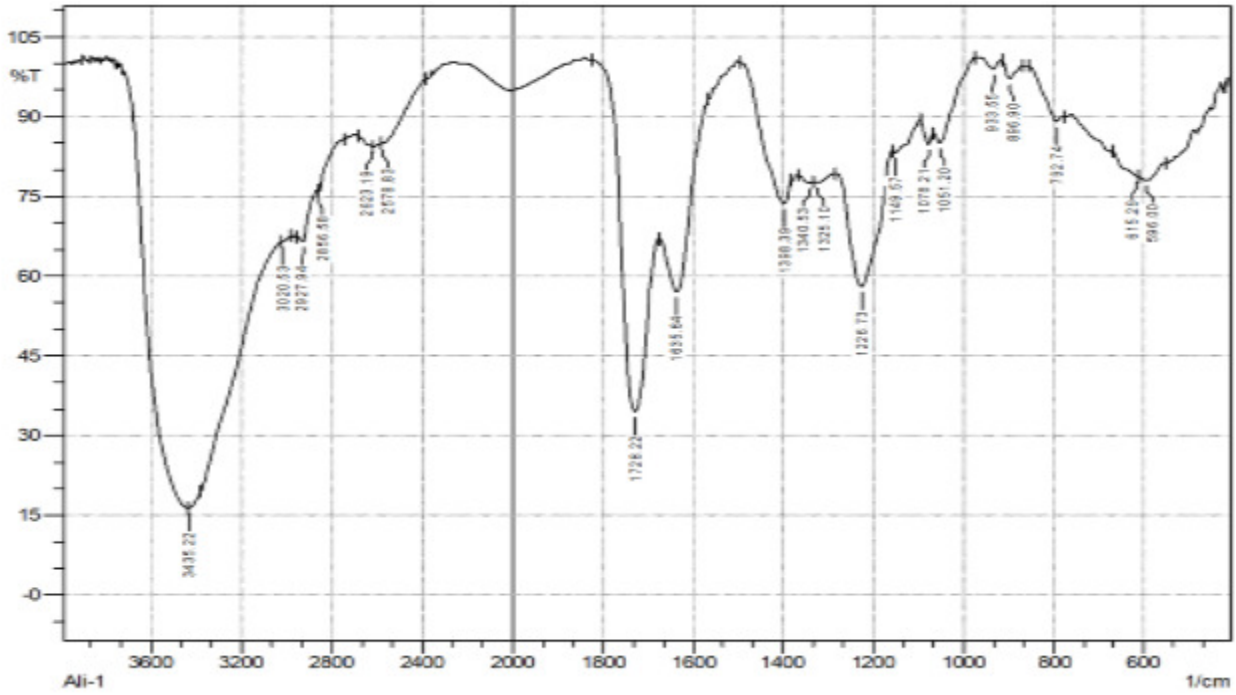

Fig. 3 : FTIR spectra of polymer I.

Table 3 : Antimicrobial activity of polymer I and $FeSO_4$ against *Escherichia coli.*

Material	Amount (µl)	Low concentration	Medium concentration	High concentration
PI	10	-	-	6
PI	20	-	-	17
PI + Disk	10:30	20	20	20
PI + Disk	20	20	20	20
Fe	10		5	20
Fe	20		12	22
Fe + Disk	10	20	20	20
Fe + Disk	20	20	20	23
PI +Fe	10:10	-	-	20
PI +Fe	10:20	-	6	24
PI +Fe	20:10		6	23
PI +Fe + Disk	10:20:30	20	20	21
PI +Fe + Disk	20:10:30	20	20	22

Low concentration of polymer I (PI) = 0.0003, Medium concentration of polymer I (PI) =0.003, High concentration of polymer I (PI) = 0.03: Low concentration $FeSO_4$ (Fe) = 0.0002, Medium concentration of $FeSO_4$ (Fe) = 0.002, High concentration of $FeSO_4$ (Fe) = 0.02: Antibiotic disk of Gentamicin (Disk) = 30 mg

The antimicrobial activity of polymer I, polymer II and $FeSO_4$ against *Escherichia coli* was also evaluated according to the formation of the inhibition zone.

The antimicrobial activity of polymer I and $FeSO_4$ was increased when used a high concentration of 20 µl of each one individually, in which the zone of inhibition was 17 mm and 22 mm, respectively (Table 3). However, the zone of inhibition was 6 mm when a high concentration (20 µl) of polymer II was used (Table 3).

The synergistic effect of Polymer I, $FeSO_4$ and gentamicin were evaluated.

The antimicrobial activity against *Escherichia coli* was increased when used $FeSO_4$ with gentamicin. In which the inhibition zone was 20 mm and 23 mm when 10 µl and 20 µl of $FeSO_4$ was used, respectively (Table 3).

Polymer I, polymer II and $FeSO_4$ were further investigated to evaluate the potential of antimicrobial activities by determined the minimum inhibitory concentrations and minimum bactericidal concentrations (Table 4).

It was found that *Staphylococcus aureus* was more

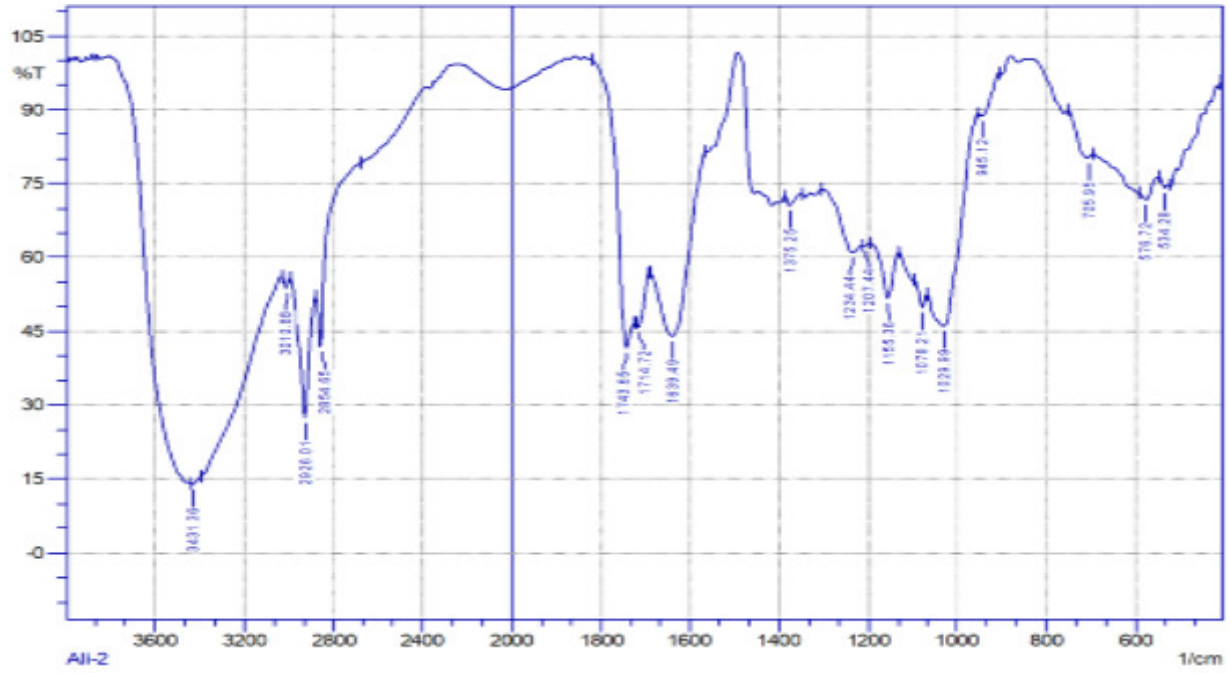

Fig. 4 : FTIR spectra of polymer II.

Table 4 : The minimum inhibitory concentration (MICs) and bactericidal concentration (MBCs) of Polymer I, Polymer II and $FeSO_4$.

Name of bacteria	MICs			MBCs		
	Polymer I	Polymer II	Iron (II) sulfate	Polymer I	Polymer II	Iron (II) sulfate
Staphylococcus aureus	0.15	0.2	0.2	20	20	20
Escherichia coli	0.3	Not detected	0.2	60	Not detected	40

sensitive to polymer I compared to polymer II and $FeSO_4$. In which the MICs was 0.1%, 0.2% and 0.2%, respectively (Table 4). It was found that sumac has MICs on a different type of bacteria including *Staphylococcus aureus* in which the MICs was 0.10% (Fazeli *et al*, 2007). Antibacterial action of polymer I may relate to the gallic acid which is found in the sumac composition which prevents biofilm formation (Liu *et al*, 2017). The MBCs of Polymer I, Polymer II and $FeSO_4$ against staphylococcus was 20% for each (Table 4).

However, *Escherichia coli* appears to be more sensitive to $FeSO_4$ compared to polymer I. In which the MICs was 0.2% and 0.3%, respectively. This result in line with Yadav *et al* (2011) that shows $FeSO_4$ is significantly inhibited the growth of *Escherichia coli.* Furthermore, the inhibitory effect of polymer I may be related to the present of gallic acid which is one component of sumac (Kang *et al*, 2018). However, no result was detected for polymer II (Table 4). The MBCs of polymer I, polymer II and $FeSO_4$ against *Escherichia coli* was 60, 40 and 40, respectively (Table 4).

CONCLUSION

In this research, we have successfully developed a new polymeric antibacterial system from sumac. The synthesis procedure involves two steps. Firstly, phenolic compounds were extracted from sumac. Polymers were then prepared by RAFT polymerization with citric acid and folic acid. The prepared polymers were characterized successfully using FTIR analysis and then its antibacterial activities have been tested against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion method. Polymeric sumac extracts have showing a promising antibacterial activity individually and in combination with $FeSO_4$ and gentamicin against *Staphylococcus aureus* and *Escherichia coli*.

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Author contributions

AA carried out the chemistry laboratory work part while RA and NA carried out the microbiology laboratory work part. All authors have contributed to the writing and approved the manuscript before submission.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that

could have appeared to influence the work reported in this paper.

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