In-vitro antimicrobial activity of Acacia farnesiana & Rosmarinus officinalis crude extract and isolated compounds against foodborne pathogens

Nabila H Saleem[a], Abeer R Abdelhalim[b], Areej M Alghoul[c], Shaymaa H. Abdel-Rhman[b]

[a] Biology Department, College of Science, Taibah University, Saudi Arabia
[b] Chemistry Department, College of Science, Taibah University, Saudi Arabia
[c] Chemistry Department, Faculty of Science, Yarmouk University, Jordan.
[d] Pharmaceutical Technology Department, college of Pharmacy, Taibah University, Saudi Arabia

DOI: https://doi.org/10.56293/IJMSSSR.2022.4653

IJMSSSR 2023
VOLUME 5
ISSUE 3 MAY – JUNE **ISSN: 2582 - 0265**

Abstract: This study aims at evaluating the antimicrobial activity of the extracts derived from *Acacia farnesiana* and *Rosmarinus officinalis*. The two medicinal plants are used traditionally in Al-Madinah Al-Munawarah for the treatment of diarrhea. The bioassay-guided fractionation of the ethanol extract and further purification of the most antibacterial active fraction led to the isolation and identification of carnosol from *Rosmarinus officinalis*, *O*-galloyl-*D*-glucopyranose, and 5, 6, 3', 5' tetrahydroxy 7,8, 4' trimethoxy flavones from *Acacia farnesiana.* The ethanolic extracts and the three isolated compounds were screened against both Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, and Gram-negative bacteria, *Escherichia coli*. The crude extracts and compounds showed antimicrobial activities against most of the strains with Gram-negative strains being more resistant. The inhibitory effect of carnosol started at 500 µg/ml with an inhibition zone of 7.3 against *S. aureus. Rosmarinus officinalis* extract and *O*-galloyl-*D*-glucopyranose inhibited *B. cereus* growth at a concentration of 250 µg/ml and 125 µg/ml with 10.1 and 11.3 mm inhibition zones, respectively. Carnosol suppressed *S. aureus* and *B. cereus* at concentrations of 500 and 125 µg/ml with 11.1 and 9.1 mm inhibition zones respectively. *Acacia farnesiana* extract inhibited the growth of *B. cereus*, *S. aureus*, and *E. coli* at a concentration of 125 µg/ml with an inhibition zone of 10.5, 7.2 and 12.8 mm, respectively. The presented data show that *Acacia farnesiana* and *Rosmarinus officinalis* could potentially use as an attractive alternative to control food poisoning bacteria and as a natural source of food preservatives.

Keywords: Antimicrobial activity, *Acacia farnesiana* , *Rosmarinus officinalis* and foodborn pathogens

Introduction

Food contamination is one of the most widely recognized reasons for death in the world (Sapkota et al., 2012). Foodborne pathogens are opportunistic organisms that can cause infections and underlining serious damage to food (Pandey and Singh, 2011) and they are one of the major public concerns of causing disease for humans and animals (Mendez et al., 2012). Gram-negative bacteria and gram-positive bacteria are the most associated organisms with food poisoning. Food pathogens are controlled in the food industry by synthetic preservatives and antibiotics to increase the safety and stability of manufactured foods on their whole shelf-life. However, the continuous use of synthetic preservatives resulted in their accumulation in food leading to an increase in microbial resistance (Mostafa et al., 2018a) in addition to their harmful effect on human health. With the increase in antibiotic resistance, there was a need for new effective, safer, and natural antibiotics. Therefore, the new interest for safe food free of any synthetic preservative has been growing rapidly. Plants and their secondary metabolites are natural sources of antimicrobial (Wallace, 2004) and antioxidants agents (Erhabor et al., 2022) making them an attractive alternative to control food poisoning bacteria and a natural source of food preservatives. Various studies have exhibited antimicrobial activity against food poisoning bacteria by plant products (Gonelimali et al., 2018a). For example, the essential oils extracted from thyme, basil, coriander, rosemary, sage, fennel, spearmint, and carraway exhibited considerable inhibitory capacity against all food organisms tested (Lixandru et al., 2010).
In this study, two plant species were selected based on traditional use in Al-Madinah Al-Munawarah for the treatment of foodborne diseases such as diarrhea. *A. farnesiana* is a legume shrub plant and a member of the

Leguminosae family (commonly named Sweet Acacia, Anbar, and futnah) grows throughout tropical and subtropical regions, its flowers characterized by its fragrant scent (Erkovan et al., 2016). Parts of *A. farnesiana* have been used for the treatment of diarrhea (Sánchez et al., 2013). *R. officinalis*, (Lamiaceae), known as rosemary, grow wild in Mediterranean countries and is often cultivated for its aromatic oil (Abdelhalim et al., 2015) and has been used as an antioxidant (Okamura et al., 1994), anti-inflammatory, (Abdelhalim et al., 2017), and antiseptic (Al-Sereiti et al., 1999).

This study aims to evaluate the activity of extracts from *A. farnesiana* and R. *officinalis* against several Gram-positive and Gram-negative bacterial strains *in vitro* and to isolate and identify the compounds responsible for such antibacterial activity.

2. Materials and Methods

2.1 Plant Materials

Fresh *Rosmarinus officinalis* and *Acacia farnesiana* plants were collected from the garden of Taibah University, Al-Madinah Al-Munawarah, Saudi Arabia, in the summer of 2019. Plant voucher specimens prepared, identified and deposited in the herbarium of the biology department, Tiabah University.

2.3 Preparation of the Extracts and Solvent Fractionation

The collected samples were air-dried in shade for three weeks; the plant parts (pods in case of *A. farnesiana* and the whole plants for *R. officinalis*) were then ground to a fine powder using a home grinder, then extracted as follow:

R. officinalis the powdered plant material was extracted with ethanol at room temperature. The crude ethanolic extract was then extracted with ethyl acetate. The ethyl acetate was vaporized under reduced pressure to produce a solid residue, which was then subjected to column chromatography on silica gel (70–230 mesh size). The column was initially eluted with pure n-hexane and then the polarity was increased gradually with ethyl acetate. Seven sub-fractions (F1–F7) were collected. Adding chloroform to sub-fraction F1 leads to the precipitation of impure solid which was then recrystallize by methanol to give white crystals identified as carnosol **(1)** (Figure 1).

A. farnesiana 200 grams of the powdered plant was dissolved in 1L methanol at room temperature. The supernatant was filtered through Whatman filter paper then evaporated under reduced pressure. The yield (12g) was dissolved in water and a brown crystal has been found precipitatedthat which was identified as *O*-Galloyl-*D*-glucopyranose **(2)** (Figure 1). The rest of the methanol extract after dissolving in water was partitioned with chloroform and n-butanol, respectively. While partition with chloroform a yellowish powder has been precipitated which was further purified using column chromatography to afford six sub-fractions (A1–A6). TLC spot under UV showed one spot for fraction number (A6) which was identified as 5, 6, 3', 5' tetrahydroxy 7,8, 4' trimethoxy flavones **(3)** (Figure 1).

2.4 Preparation of Inoculum

Both Gram-positive bacteria [*Bacillus cereus*, *Staphylococcus aureus*] and Gram-negative bacteria [*Escherichia coli*] able to produce food poisoning diseases were used for the assessment of the antimicrobial activity of the plants' extracts. The bacterial cultures were obtained as pure culture from the Pharmaceutics and pharmaceutical technology Department, Taibah University, KSA. They were pre-cultured overnight in Mueller Hinton broth (MHB) at 37°C and their concentrations were adjusted to 0.5 McFarland standard (Bhalodia and Shukla, 2011, Gonelimali et al., 2018b).

2.5 Antimicrobial Screening

Screening for the antibacterial activity of the plants' extracts was performed by agar well diffusion method (Gonelimali et al., 2018b) with minor modifications. Briefly, in a center of a petri dish; 1 ml of fresh adjusted bacterial culture was added and mixed with molten Muller Hinton agar and left for solidification. A sterile cork borer (5 mm in diameter) was used to pinch wells in these agar plates. Afterward, 50 µl of each extract (1000

µg/ml) was added to corresponding wells and the plates were kept in the refrigerator for 30 min to permit the diffusion of the extracts into the agar. Then, the plates were incubated at 37°C for 18 h. The inhibition zone diameters were measured using Caliber in mm. Ampicillin/ clavulanic acid (1000 µg/ml) served as positive control while DMSO and distilled water were used as negative control separately. Three repeats were done for each extract against the tested organisms

2.6 Determination of Minimum Inhibitory Concentration (MIC)

The plant extracts and the isolated purified compounds showed antibacterial activity at a concentration of 1000 µg/ml. So, this concentration was used to determine their MIC by agar well diffusion method (Mostafa et al., 2018b). Two-fold serial dilution was used to prepare various concentrations (1000-15.6 µg/ml) of the effective plants' extracts. In a center of a petri dish, 1 ml of fresh adjusted bacterial culture was added, mixed with molten Muller Hinton agar, and left for solidification. On each plate, four wells were made and 50 µL of the different concentrations of each plant sample and the isolated compound were applied carefully to the corresponding wells. Plates were kept in the refrigerator for 30min and then incubated in an upright position at 37°C for 18 h. The MIC was calculated as the lowest concentration that inhibits the growth of the respective microorganisms. DMSO was used as ethanolic extracts control and distilled water was served as water extracts control. All experiments were done in triplicate.

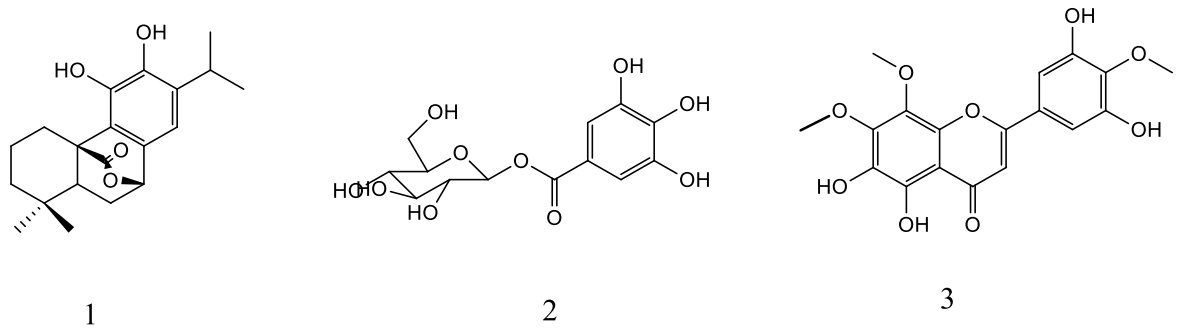
3. Results and discussion

3.1 Physical and Spectroscopic Data of the Isolated Active Compounds

Carnosol (1): white crystals, ESMS *m/z*: 331.0; ^{1}H-NMR (DMSO-d_6) δ ppm: H_{-14} 6.67 (s), H_{-7} 5.54 (dd), H_{-15} 3.22 (sept), $H_{-1\alpha}$ 2.62 (dd), $H_{-1\beta}$ 2.44 (ddd), $H_{-6\alpha}$ 2.11 (ddd), $H_{-6\beta}$ 1.7 (m), H_{-5} 1.69 (dd), $H_{-2\beta}$ 1.52 (m), $H_{-3\alpha}$ 1.41 (dd), $H_{-3\beta}$ 1.22 (ddd), H_{-16} 1.09 (d), H_{-18} 0.78 (s), H_{-19} 0.76 (s); ^{13}C-NMR (DMSO-d_6) δ ppm: C_{-20} 175.9, C_{-11} 142.7, C_{-12} 142.5, C_{-13} 133.9, C_{-8} 131.9, C_{-9} 122.1, C_{-14} 110.7, C_{-7} 77.5, C_{-10} 48.2, C_{-5} 45.3, C_{-3} 41.0, C_{-4} 34.5, C_{-18} 31.6, C_{-6} 29.6, C_{-1} 29.1, C_{-15} 26.5, C_{-17} 22.2, C_{-16} 23.1, C_{-19} 19.7, C_{-2} 19.1.

O-Galloyl-D-glucopyranose (2): brown crystals, HRESI-MS *m/z*: 333.0; ^{1}H NMR (MeOD) δ ppm: $H_{-2,6}$ 7.12 (s), $H_{-1'}$ 5.65 (d), $H_{-6'\alpha}$ 3.86 (dd), $H_{-6'\beta}$ 3.71 (dd), $H_{-2'}$ 3.48 (d), $H_{-3'}$ 3.47 (d), $2H_{-4',5}$ 3.42 (m); ^{13}C NMR (MeOD) δ ppm: C_{-7} 166.0, C_{-4} 146.2, $C_{-3,5}$ 140.1, C_{-1} 122.2, $C_{-2,6}$ 110.3, $C_{-1'}$ 96.1, $C_{-5'}$ 78.1, $C_{-2'}$ 77.1, $C_{-3'}$ 73.1, $C_{-4'}$ 70.1, $C_{-6'}$ 61.1.

5, 6, 3', 5' tetrahydroxy7,8, 4' trimethoxy flavones (3): yellow amorphous solid powder, HRESI-MS *m/z*: 361.0; ^{1}H-NMR (DMSO-d_6) δ ppm: 5_{-OH} 12.53 (s), $5'_{-OH}$ 10.04 (s), 6_{-OH} 9.12 (s), $H_{-2',6'}$ 7.58 (s), H_{-3} 6.98 (d), 7_{-OMe} 3.96 (s), 8_{-OMe} 3.93 (s), $4'_{-OMe}$ 3.90 (s); ^{13}C-NMR (DMSO-d_6) δ ppm: C_{-4} 183.2, C_{-2} 164.4, $C_{-4'}$ 151.4), C_{-7} 148.6, $C_{-3',5'}$ 148.5, C_{-5} 143.7, C_{-9} 142.4, C_{-6} 134.7, C_{-8} 133.4, $C_{-1'}$ 122.2, $C_{-2',6'}$ 120.8, C_{-10} 106.7, C_{-3} 103.3, 8_{-OMe} 62.4, 7_{-OMe} 61.5, $4'_{-OMe}$ 56.4.



1 2 3

Figure 1: Constituents of *A. farnesiana and R. officinalis* used in this study; carnosol **(**1), *O*-galloyl-*D*-glucopyranose (2) and 5, 6, 3', 5' tetrahydroxy 7,8, 4' trimethoxy flavones (3).

3.2 Antibacterial activity of plants extract

A. farnesiana, R. officinalis, and their isolated compounds were examined to estimate their antibacterial activity on two strains of Gram-positive bacteria (*B. cereus* and *S. aureus*) and one strain of Gram-negative bacteria (*E. coli*) using agar well diffusion method. Assessment of antibacterial activity was illustrated in Table 2. The results reveal that the plant extracts and pure compounds were active in inhibiting the food poisoning bacteria growth with variable strength. *A. farnesiana* was the most active extract suppressing the growth of three pathogens (*B. cereus*, *S. aureus*, and *E. coli*) at a concentration of 1000µg/ml. While *R. officinalis* extracts and compound **3** were effective only against *B. cereus*. Carnosol showed antimicrobial activity against both *B. cereus* and *S. aureus*.

Table 2: Screening of antibacterial activity of two plants' extracts, and three pure compounds (1000µg/ml) against some bacterial strains of food poisoning diseases.

Plant extract	Inhibition zones (mm)		
	Gram (+ve) bacteria		Gram (-ve) c bacteria
	B. cereus	*S. aureus*	*E. coli*
Compound 2 (O-Galloyl-D-glucopyranose)	0.0±0.0	14.4 ± 0.5774	0.0±0.0
R. officinalis crude extract	14.3± 0.3333	0.0±0.0	0.0±0.0
Compound 3 (5, 6, 3', 5' tetrahydroxy 7,8, 4' trimethoxy flavones)	15.6±0.3333	0.0±0.0	0.0±0.0
Compound 1 (carnosol)	13.3±0.3333	16±0.3333	0.0±0.0
A. farnesiana crude extract	14.2±0.5774	13±0.5774	18±0.5774

These results propose that *E. coli* was the most resistant strain to plant extracts while *S. aureus* and *B. cereus* were the most sensitive strains to the extracted plants. Furthermore, *A. farnesiana* extracts were the most active and exhibit antibacterial activity against food poisoning bacteria. Accordingly, experiments were administered to determine the plants' extract minimum inhibitory concentration (MIC) against the bacterial strains.

3.3 Minimum inhibitory concentrations (MIC's) of the effective plants' extracts

The MIC of the active plants' extracts and the pure compounds (*R. officinalis*, *A. farnesiana*, compounds 1, 2, and 3) were determined by agar well diffusion method estimates their bacteriostatic activities. The concentration influence of the activity was described in Table 3.

A. farnesiana crude extract inhibited the growth of *B. cereus, S. aureus*, and *E. coli* at concentration 125 µg/ml with an inhibition zone of 10.5, 7.2 and 12.8 mm respectively. While the inhibitory effect of compound **2** started at 500µg/ml with inhibition zone of 7.3 against *S. aureus*. During the fractionation of the pods of *Acacia farnesiana*, it showed a high percentage of hydrolyzable tannins which are mostly responsible for the antimicrobial activity of the plant. Numerous studies have reported the antibacterial activity of tannins against food poisoning pathogens and their mechanism of action. Tannins appear to influence bacterial growth in different ways, for example, restraint of extracellular microbial enzymes, change of the substrates needed for microbial development, or direct activity on microbial metabolism through suppression of oxidative phosphorylation (Sieniawska, 2015). Lipopolysaccharide (LPS) on the external cell membrane of Gram-negative bacteria works as a barrier protecting their cells. The compound's ability to disrupt the cell wall could be the main mechanism of action for their activity since the cell wall is the main distinction between the two groups (Gram-positive and negative) (Dussault et al., 2014).

For example, methyl gallate isolated from *A. farnesiana* was found to affect the membrane activity of *Vibrio cholera*, by decreasing the pH in the cytoplasm and increasing membrane polarity (Sánchez et al., 2013). While Gallic acid caused a rupture in the cell membranes with consequent leakage of essential intracellular constituents (Anabela Borges, 2013). Other studies suggest that they can work in the acidic stomach environment, without affecting the survival of gastric epithelial cells (Wu et al., 1988). The activity of *A. farnesiana* crude extract and compound **2** (O-Galloyl-D-glucopyranose) could be attributed to the tannin content. The results appear to show that *A. farnesiana* crude extracts might be more effective than purified compounds, this loss in activity with isolated compounds

suggests synergistic effects of crude constituents.

Table 3 MIC's of the plants' extracts against tested bacterial strains.

Plant extract	**Conc. µg/ml**	**Inhibition zones (mm)**		
		Gram (+ve) bacteria		**Gram (-ve) bacteria**
		B. cereus	***S. aureus***	***E. coli***
Compound 2 (O-Galloyl-D-glucopyranose)	1000	-	14.4± 0.5774	-
	500	-	7.3±0.333	-
	250	-	0.0±0.0	-
	125	-	0.0±0.0	-
	62.5	-	0.0±0.0	-
Rosmariunus officinalis crude extract	1000	14.3± 0.3333	-	-
	500	12.2±0.228	-	-
	250	10.1±1.1	-	-
	125	0.0±0.0	-	-
	62.5	0.0±0.0	-	-
Compound 3 (5, 6, 3', 5' tetrahydroxy 7,8, 4' trimethoxy flavones)	1000	15.6±0.3333	-	-
	500	14.4±0.43	-	-
	250	13.0±.876	-	-
	125	11.3±0.37	-	-
	62.5	0.0±0.0	-	-
Compound 1 (Carnosol)	1000	13.3±0.3333	16±0.3333	-
	500	11.4±0.37	11.1±0.65	-
	250	10.3±0.65	0.0±0.0	-
	125	9.1±.0.95	0.0±0.0	-
	62.5	0.0±0.0	0.0±0.0	-
Acacia farnesiana *crude extract*	1000	14.2±0.5774	13±0.5774	18±0.5774
	500	13.3±0.333	11.5±0.577	16.4±0.56
	250	11.3±0.37	9.1±0.95	15.2±0.52
	125	10.5±0.65	7.2±0.333	12.8±0.45
	62.5	0.0±0.0	0.0±0.0	0.0±0.0

NMR for the *R. officinalis* showed that the plant contains a wide range of flavonoids and suppressed the growth of *B. cereus* at a concentration of 250 µg/ml with inhibition zones of 10.1mm. Flavones have been found *in vitro* to be active antimicrobial substances against different microorganisms. Their activity is most likely to their ability to complex with extracellular proteins which affect the bacterial cell walls (Cushnie and Lamb, 2005). According to the literature, Flavones with different hydroxy and methoxy substitution patterns showed different ranges of effect on tested microorganisms. Also, position 5 hydroxyl group in flavones is important for their activity against MRSA (Kumar and Pandey, 2013).

According to (Farhadi et al., 2018), Flavones that have at least one hydroxy group in ring A (especially at C-7) are important for antibacterial activity and the activity has been increased in a position such as C -5 and C -6. This could be applied in the case of compound **3** (Fig.3), which has additional OH groups on 6, 3' and 5' positions and inhibit the bacterial growth of *B. cereus* with inhibition zones of 11.3 mm at a concentration of 125 µg/ml. Flavonoids and tannins are reported to have great good antioxidant activities which serve as protection against various diseases, including cancer and cardiovascular diseases. Their free radical inhibition activities are credited to their chemical structures (Lobo et al., 2010).

Carnosol (**1**), isolated from Rosemary (Fig.1) found to suppress the growth of both *B. cereus* and *S. aureus* at concentrations 125 and 500 µg/ml with inhibition zones of 9.1 and 11.1 mm respectively. Carnosol was first isolated from *Salvia carnosa* and showed promising anti-inflammatory and anticancer activity in prostate and breast cancer cells (Johnson, 2011). Rosemary extract is used as a natural antioxidant in food and confirmed as a natural preservative in the food industry. Carnosol has been approved in the European Union (EU) as an antioxidant and food preservative. In addition, in the United States, it has been approved as a Generally Recognized as Safe status as a food additive (Authority, 2008).

Conclusion

The plant extract and isolated compounds have shown different levels of antimicrobial activity against foodborne pathogens the activity was against Gram-positive bacteria than Gram-negative bacteria. This study indicates that the crude extract could be a potential source of antimicrobials, effective against some food-spoiling pathogens, accordingly could be used as a food preservative on itself or in combination with other treatments to increase the effects. Rosemary now is commercially accessible on large scale as food additives. Further exploration is needed to assess the toxicity of isolates in cell lines and/or in animals.

Acknowledgments

This work was supported by the Deanship of Scientific Research (DSR) [grant no. 60339], Taibah University, AlMadinah Al-Munawarrah, Kingdom of Saudi Arabia. The authors appreciate DSR for this technical and financial support.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

1. ABDELHALIM, A., ABURJAI, T., HANRAHAN, J. & ABDEL-HALIM, H. 2017. Medicinal plants used by traditional healers in Jordan, the Tafila region. *Pharmacognosy magazine,* 13**,** S95.
2. ABDELHALIM, A., KARIM, N., CHEBIB, M., ABURJAI, T., KHAN, I., JOHNSTON, G. A. & HANRAHAN, J. 2015. Antidepressant, anxiolytic and antinociceptive activities of constituents from Rosmarinus officinalis. *Journal of Pharmacy & Pharmaceutical Sciences,* 18**,** 448-459.
3. AL-SEREITI, M., ABU-AMER, K. & SENA, P. 1999. Pharmacology of rosemary (Rosmarinus officinalis Linn.) and its therapeutic potentials.
4. ANABELA BORGES, C. F., MARIA J. SAAVEDRA, AND MANUEL SIMÕES 2013. Antibacterial Activity and Mode of Action of Ferulic and Gallic Acids Against Pathogenic Bacteria. *Microbial Drug Resistance,* 19**,** 256-265.
5. AUTHORITY, E. F. S. 2008. Use of rosemary extracts as a food additive - Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food. *EFSA Journal,* 6**,** 721.
6. BHALODIA, N. R. & SHUKLA, V. J. 2011. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *J Adv Pharm Technol Res,* 2**,** 104-9.
7. CUSHNIE, T. P. T. & LAMB, A. J. 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents,* 26**,** 343-356.
8. DUSSAULT, D., VU, K. D. & LACROIX, M. 2014. In vitro evaluation of antimicrobial activities of various commercial essential oils, oleoresin and pure compounds against food pathogens and application in ham. *Meat Science,* 96**,** 514-520.
9. ERHABOR, R. C., ERHABOR, J. O., NKADIMENG, S. M. & MCGAW, L. J. 2022. In vitro antimicrobial, antibiofilm and antioxidant activities of six South African plants with efficacy against selected foodborne pathogens. *South African Journal of Botany,* 146**,** 643-652.
10. ERKOVAN, H. I., CLARKE, P. J. & WHALLEY, R. D. B. 2016. A review on General Description of Vachellia farnesiana (L.) Wight &Arn. *Journal of the Faculty of Agriculture,* 47**,** 71-76.

11. FARHADI, F., KHAMENEH, B., IRANSHAHI, M. & IRANSHAHI, M. 2018. Antibacterial activity of flavonoids and their structure–activity relationship: An update review. *Phytotherapy Research,* 33.
12. GONELIMALI, F. D., LIN, J., MIAO, W., XUAN, J., CHARLES, F., CHEN, M. & HATAB, S. R. 2018a. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. *Frontiers in Microbiology,* 9.
13. GONELIMALI, F. D., LIN, J., MIAO, W., XUAN, J., CHARLES, F., CHEN, M. & HATAB, S. R. 2018b. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. *Front Microbiol,* 9**,** 1639.
14. JOHNSON, J. J. 2011. Carnosol: a promising anti-cancer and anti-inflammatory agent. *Cancer letters,* 305**,** 1-7.
15. KUMAR, S. & PANDEY, A. K. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. *The Scientific World Journal,* 2013**,** 162750.
16. LIXANDRU, B. E., DRĂCEA, N., DRAGOMIRESCU, C., DRAGULESCU, E., COLDEA, I., ANTON, L., DOBRE, E., ROVINARU, C. & CODITA, I. 2010. Antimicrobial activity of plant essential oils against bacterial and fungal species involved in food poisoning and/or food decay. *Roumanian archives of microbiology and immunology,* 69**,** 224-30.
17. LOBO, V., PATIL, A., PHATAK, A. & CHANDRA, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy reviews,* 4**,** 118-126.
18. MENDEZ, M., RODRÍGUEZ, R., RUIZ, J., MORALES-ADAME, D., CASTILLO, F., HERNÁNDEZ-CASTILLO, F. D. & AGUILAR, C. N. 2012. Antibacterial activity of plant extracts obtained with alternative organics solvents against food-borne pathogen bacteria. *Industrial Crops and Products,* 37**,** 445-450.
19. MOSTAFA, A. A., AL-ASKAR, A. A., ALMAARY, K. S., DAWOUD, T. M., SHOLKAMY, E. N. & BAKRI, M. M. 2018a. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi Journal of Biological Sciences,* 25**,** 361-366.
20. MOSTAFA, A. A., AL-ASKAR, A. A., ALMAARY, K. S., DAWOUD, T. M., SHOLKAMY, E. N. & BAKRI, M. M. 2018b. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. *Saudi J Biol Sci,* 25**,** 361-366.
21. OKAMURA, N., FUJIMOTO, Y., KUWABARA, S. & YAGI, A. 1994. High-performance liquid chromatographic determination of carnosic acid and carnosol in Rosmarinus officinalis and Salvia officinalis. *Journal of Chromatography A,* 679**,** 381-386.
22. PANDEY, A. & SINGH, P. 2011. Antibacterial activity of Syzygium aromaticum (clove) with metal ion effect against food borne pathogens. *Asian J Plant Sci Res,* 1**,** 69-80.
23. SÁNCHEZ, E., HEREDIA, N., CAMACHO-CORONA, M. D. R. & GARCÍA, S. 2013. Isolation, characterization and mode of antimicrobial action against Vibrio cholerae of methyl gallate isolated from Acacia farnesiana. *Journal of Applied Microbiology,* 115**,** 1307-1316.
24. SAPKOTA, R., DASGUPTA, R. & RAWAT, D. 2012. Antibacterial effects of plants extracts on human microbial pathogens & microbial limit tests. *International journal of research in pharmacy and chemistry,* 2**,** 926-936.
25. SIENIAWSKA, E. 2015. Activities of Tannins – from in Vitro Studies to Clinical Trials. *Natural Product Communications,* 10**,** 1934578X1501001118.
26. WALLACE, R. J. 2004. Antimicrobial properties of plant secondary metabolites. *Proceedings of the nutrition society,* 63**,** 621-629.
27. WU, Y. C., CHEN, C. & WU, R. 1988. Gallotannins inhibit growth, water soluble glucan synthesis and aggregation of Streptococci mutans. *Journal of Dental Research,* 67**,** 51-5.