INVESTIGATING THE CHEMICAL COMPOSITIONS AND THE MEDICINAL VALUES OF Zanthoxylum zanthoxyloides STEM

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ABSTRACT

Zanthoxylum *zanthoxyloides also known as fagara* is a useful traditional medicinal plant growing in Africa for treating of array pathological condition. The stem bark of *Zanthoxylum zanthoxyloides* was analyzed for proximate compositions in (%); moisture (4.65), ash (5.95), protein (19.69), crude fat (2.1) and carbohydrate (56.51).Elemental composition in (ppm) includes; Iron (0.132), m agnesium (1.400), zinc (0.260), copper (0.084), manganese (2.105), phosphorus (0.144), calcium (2.111), potassium (0.099), sodium (0.144), copper (0.084), manganese (2.105), phosphorus 0.144), calcium (2.111), potassium (0.099), sodium (0.144). The quantity phytochemical revealed saponins (3.77mg/l), tannins (0.215mg/l), alkaloids (0.96g), flavonoids (40.10 mg/l) and glycosides (21.88g/ml).The infrared analysis of Zanthoxylum *zanthoxyloides* of stem exhibited a characteristic absorption band at 3300-3500cm^{-1} and 2920-2950 cm^{-1} indicating presence of O-H alcohol, phenol , hydrogen bonded OH and C-H stretch alkyl, methylene respectively, at 130-1700cm^{-1} and 1422 cm^{-1} for C=O carbonyl and CH_2 bending aromatic ring respectively. More so at 1313.5, 1023.2 and 700-800cm-1 there is C-O stretch, C-O and aromatic C-H in that order. The vitamins composition revealed that Zanthoxylum *z.* contained vitamin C (90mg/100g), vitamin B_1 (21.02 µg/L), vitamin B_2 (22.10g/L) and vitamin B_{12}.(0.124g/l). The antimicrobial activity of the plant extract in both methanol and ethanol solvents was tested against six microorganisms*: Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albican, and Candida tropicalis.*

Key words: *Zanthoxylum zanthoxyloides, Klebsiella pneumoniae, Staphylococcus aureus*

INTRODUCTION

Medicinal plants which are known as herbal medicine were brought to the limelight and used from the inception as medicine practices since prehistoric times. Among is Zanthoxylum *zanthoxyloides* stem *also called as fagara* is a useful traditional medicinal plant growing in Africa for treating of pathological condition. Zanthoxylum *zanthoxyloides* commonly known as wild lime, is a flowering plant that, despite its common name, does not belong to the genus Citrus. Instead, it is a member of the Rutaceae family, which also includes species such as Sichuan pepper. Zanthoxylum *zanthoxyloides* Lam is a much-branched tree with dense, dark-green foliage and abundant prickles. These prickles or thorns extend to the leaf stalks and the leaflets midribs. The leaves are elliptic, slightly obovate. (Okagu, *et al.*, 2021; Ozigis *et al.*, 2023 The flowers are greenish-white in narrow axillary and terminal panicles usually without thorns. It is always in fruit between July and September every year. A major characteristic of the plant is that the trunks, branches, branchlets, leaf stalks and inflorescence axes are covered by prickles or what others describe as spines. This makes it a big nuisance to farmers who always mark them for destruction when cited on farms and vegetation's the leaflets and inflorescences contain volatile oils which bring forth sweet smells and fragrances. (König *et al.*, 2021; Ozigis *et al.*, 2023). The spines are thrown into fire to give off a scented smoke. The leaves, which smell like citronella and the seeds, which taste strongly like cinnamon or pepper are commonly used to season foods from the seeds, necklaces are made.

Aim and objective of this research work is to evaluate the nutritional composition, antioxidant capacity, antimicrob ial activity and infrared spectral characteristic of Zanthoxylum *zanthoxyloides* in order to establish its medicinal potential and nutritional values.

Materials and Method

Material and sample collection

The *Zanthoxylum zanthoxyloides* samples used for this study were bought at Ojaoba market at Iree, Ifelodun Local Government area of Osun State, Nigeria. The stems were cut into pieces and Grounded using a grinder until a smooth texture was achieved. The resulting smooth substance Were stored in an airtight container for further analysis.

Method

Proximate analysis:

The grounded stem was analyzed for its moisture, crude fat, crude fiber, crude protein, carbohydrate and ash content. The moisture content was taken using an A&D MS-70 Moisture Analyzer at 105 °C. The proximate parameters (crude fat, crude protein, crude fiber and ash value) of the grounded *Zanthoxylum zanthoxyloides* stem was determined using the Association of Official Analytical Chemists, 2016 method (AOAC, 2016). While the carbohydrate content was calculated by difference using the formulae;

Total Carbohydrate (%) = [100 - % (Moisture + Fat + Protein + Fiber + Ash)]

Elemental analysis

The method described by (AOAC 2016 and Abiola *et al.*, 2020) was adopted for the digestion and elemental analysis of the sample. The prepared sample solution was analysis with the AA-7000 Atomic absorption spectrophotometer (Schimmadza Japan) for macro and micro elements.

Phytochemical analysis

Preliminary phytochemical analysis was carried out on the 70% ethanolic extract of *Zanthoxylum zanthoxyloides* stem using standard qualitative methods with slight modification. Quatitative screening of the aqueous leaf extract of *Zanthoxylum zanthoxyloides* stem were carried out using appropriate methods as previously documented (Oyeleke *et al.*, 2021). The FTIR of the sample was carried out by employing the standard kb pellet technique.

The vitamins analyses were carried out using the method of Okwu and Josiah (2006).

Antimicrobial analysis

The method of Oyewale (2012) was employed. The *Zanthoxylum zanthoxyloides* stem was concentrated to 25 mg/ml. After incubations at 37^0C for 24 hours, zones of inhibitions were measured for each microorganism.

RESULT AND DISCUSSION

Table 1: Proximate analysis of *Zanthoxylum zanthoxyloides* stem (%)

Parameters	Constituents
Moisture	4.65
Ash content	5.95
Crude fibre	11.10
Crude protein	3.15
Crude fat	2.1
Carbohydrate by difference	56.51

Table 2: Elemental analysis of *Zanthoxylum zanthoxyloides* stem (mg/g)

Parameters	Constituents
Iron (Fe)	0.132
Magnesium (Mg)	1.400
Zinc (Zn)	0.260
Copper (Cu)	0.084
Manganese (Mn)	2.105
Phosphorus (P)	0.144
Calcium (Ca)	2.111
Potassium (K)	0.099
Sodium (Na)	0.144

Table 3: Qualitative phytochemical analysis of *Zanthoxylum zanthoxyloides* stem

Parameters	Constituents
Tannins	+
Glycosides	+++
Alkaloids	+
Saponins	++
Flavonoids	+++

Key: +++ = Highly presence, ++= moderately present, + =lightly present

Table 4: Quantitative phytochemical analysis of *Zanthoxylum zanthoxyloide* stem

Parameters	Constituents
Saponins (mg/L)	3.77
Tannins (mg/L)	0.215
Alkaloids (g)	L 0.96
Flavonoids (mg/L)	40.10
Glycosides (mg/ml)	21.88

Table 5: FTIR spectra table of *Zanthoxylum zanthoxyloide* stem

Maximum Peak (cm^{-1})	Group function	Class of compounds	Significance in medicine
~3400–3200	O–H stretching	Tannins, flavonoids and Phenols	Antimicrobial and antioxidant
~2920–2850	C–H stretch (aliphatic)	The fatty acids	Antibacterial and anti-inflammatory
1730 cm^{-1}	C=O stretch	Aldehydes, flavonoid glycosides and Esters	Antimicrobial and anti-inflammatory
1630–1600 cm^{-1}	C=C /Amide	alkaloids and Aromatics	Suppression of enzyme
1400–1200 cm^{-1}	C-N/C-O	glycosides and Alkaloids	Antimicrobial and analgesic,
1050–1020 cm^{-1}	C–O-C	glycosides and Alkaloids	Strengthen the immune system and healing wound

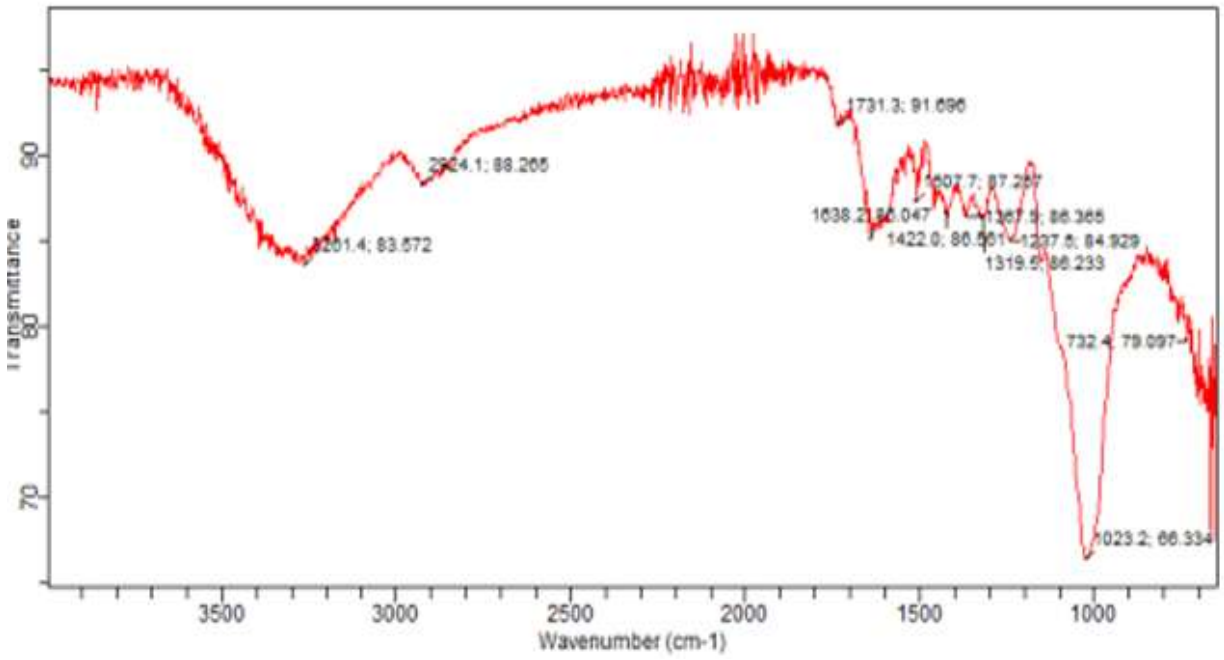

Fig 1: FTIR spectra of *Zanthoxylum zanthoxyloide* stem

Table 6: Vitamin analysis of *Zanthoxylum zanthoxyloides* stem

Parameters	Constituents
Vitamin C (mg/100g Ascorbic acid)	90
Vitamin B_1 (µg/L Thiamine)	21.02
Vitamin B_2 ((µg/L Riboflovin)	22.10
Vitamins B_{12} (Cyanocobalamin)	0.124

Table 7 Antimicrobial sensitivity of *Zanthoxylum. zanthoxyloides* stem extracts

Test organism	Methanol extract (mm)	Ethanol extract (mm)	Control (AUG, mm)	Control (GEN, mm)
K. pneumoniae	7.5	10	10	13
Staphylococcus aureus	17	8	20	11
Pseudomonas aeruginosa	11	5	10	6
E. coli	22	9	23	15
Candida albicans	13	2	8	13
Candida tropicalis	9	11	13	–

Key: = (–) = No inhibition, 6 - 9mm = low inhibition, 10 - 15mm = moderate inhibition, ≥ 15mm = high inhibition
AUG = Augmentin, GEN = Gentamycin

Zanthoxylum zanthoxyloides stem has a low moisture content (4.65%), according to the proximate analysis (Table 1), indicating acceptable storage quality and decreased microbial deterioration. According to recent research by Okagu *et al.*, (2023), which found similar low moisture content in West African medicinal species, medicinal plants with moisture levels below 10% are thought to be more shelf-stable. A moderate mineral load is suggested by the ash percentage (5.95%), which indicates the existence of significant inorganic elements. This figure is similar to the ash level (5–7%) found in other Zanthoxylum species that are utilized in traditional medicine (Adesina *et al.*, 2022).The percentage of crude fiber (11.10%) is comparatively high. Stems high in fiber are linked to better gastrointestinal health and cleansing. Similar fiber levels exceeding 10% were found in Zanthoxylum leprieurii stems by Koussou *et al.*, (2021), confirming the plant's nutritional significance. As would be expected for woody plant tissues, the stem has low crude fat (2.10%) and low crude protein (3.15%). According to Onoja *et al.*, (2020), low protein concentration suggests a limited contribution to dietary protein, whereas low fat content improves oxidative stability. The greatest component, at 56.51%, is carbohydrates, suggesting that the stem is high in energy. Medicinal plant stems typically have high carbohydrate fractions (Nwankwo *et al.*, 2022), which may also aid in the plant's phytochemical synthesis and metabolic functions.

The stem includes significant macro and trace minerals that are necessary for physiological and metabolic functions, according to the mineral analysis (Table 2). The highest concentrations of calcium (2.111 mg/g), magnesium (1.400 mg/g), and manganese (2.105 mg/g) were found. Magnesium is needed for energy metabolism and enzyme activation, while calcium is essential for healthy bones and muscles. Manganese-superoxide dismutase is one of the antioxidant enzyme systems that manganese supports. These results align with the findings of Ekor *et al.*, (2021), who reported mineral-rich profiles in a number of medicinal plants. The production of hemoglobin, cellular metabolism, and immunology all depend on trace minerals like iron (0.132 mg/g), zinc (0.260 mg/g), and copper (0.084 mg/g). Similar trace mineral values in Zanthoxylum zanthoxyloides roots and bark extracts were reported by Yakubu *et al.*, (2022). Lower levels of sodium (0.144 mg/g) and potassium (0.099 mg/g) were found, which support neuromuscular function and electrolyte homeostasis.

Significant amounts of bioactive chemicals that are known to contribute to the therapeutic value of the plant are shown in the qualitative and quantitative phytochemical composition in Table 3 and 4 respectively. The amount of flavonoids in the stem is extremely high (40.10 mg/L). Strong antioxidants, flavonoids have been connected to anti-inflammatory, antibacterial, and anti-diabetic properties. According to Mensah *et al.*, (2022), Zanthoxylum extracts rich in flavonoids have strong antibacterial action, which supports the plant's traditional use. There were also a lot of glycosides (21.88 mg/mL), suggesting possible detoxifying, antiinflammatory, and cardioprotective actions. Their existence is consistent with research by Akinmoladun *et al.*, (2023), who found notable glycosides in medicinal plants used to treat malaria and fever. Tannins (0.215 mg/L) and saponins (3.77 mg/L) support the antibacterial, antihelminthic, and astringent qualities. These substances frequently aid in detoxification and wound healing (Osei-Djarbeng and Essuman, 2021). Alkaloids, one of the most pharmacologically active classes of phytochemicals, make up 0.96 g of the stem. Alkaloids have antibacterial, analgesic, and antimalarial properties. Alkaloids were also found to be important bioactive components in Zanthoxylum species by Ndam *et al.*, (2024).

O–H stretching vibrations of hydroxyl groups, which are commonly found in phenols, flavonoids, and tannins, are represented by a broad and strong band seen at about 3400–3300 cm^{-1}. These substances are widely known for their potent antibacterial and antioxidant properties because they can destroy microbial membranes and stabilize free radicals by donating hydrogen ions. Akinyemi *et al.*, (2021) found a high correlation between the antibacterial effectiveness of plant extracts and the presence of phenolic O–H groups. The presence of fatty acids and substances generated from lipids was indicated by absorptions around 2920–2850 cm^{-1}, which were attributed to C–H stretching of aliphatic chains. As previously shown by Musa *et al.*, (2023), who found that long-chain fatty acids in medicinal plants improve antimicrobial performance, these compounds are linked to anti-inflammatory and membrane-disrupting antibacterial actions. The C=O stretching of carbonyl groups, which is typical of esters, aldehydes, flavonoid glycosides, and organic acids, was shown by a strong peak at 1730 cm^{-1}. It is well recognized that phytochemicals containing carbonyl have antibacterial, antifungal, and anti-inflammatory properties. Carbonyl functional groups are crucial structural elements of many antibacterial secondary metabolites, according to Zhang *et al.*, (2020).

C=C stretching in aromatic rings or amide I vibrations in alkaloids or protein-like structures were identified as the causes of the absorption band between 1630 and 1600 cm^{-1}. Flavonoids and tannins are examples of aromatic chemicals that have anti-inflammatory, enzyme-inhibitory, and antioxidant properties. This conclusion is consistent

with the findings of Ali and Mohamed (2023), who connected polyphenol-rich extracts with strong therapeutic benefits to aromatic ring vibrations in FTIR spectra.

The stem has a significant amount of vitamin C (90 mg/100 g), which is exceptionally high for plant stem materials, as Table 6 demonstrates. As a strong antioxidant, vitamin C helps give medicinal plants their anti-inflammatory and immune-boosting qualities. Antioxidant-rich plants utilized in West African ethnomedicine have been shown to have similar high vitamin C levels (Nwachukwu *et al.*, 2022).These B vitamins aid in erythropoiesis, nervous system function, and energy metabolism. Additionally, small but noteworthy B-vitamin concentrations were found in medicinal herbs that are traditionally utilized for metabolic support and vitality (Habila *et al.*, 2023).
Z. zanthoxyloides' methanol extract showed the strongest inhibitory efficacy against E. coli (22 mm) and Staphylococcus aureus (17 mm). K. pneumoniae exhibited poor inhibition (7.5 mm), but Pseudomonas aeruginosa and Candida albicans showed moderate action. Although the ethanol extract's efficacy against bacterial strains was generally weaker, it did demonstrate moderate suppression against Candida tropicalis (11 mm) and K. pneumoniae (10 mm).
The difference in inhibitory zones across solvents indicates that ethanol may selectively extract chemicals more efficient against specific fungus, whereas methanol is more successful in extracting bioactive compounds with antibacterial action. This is consistent with other research showing that phytochemical extraction and antibacterial effectiveness are strongly impacted by solvent polarity (Olajuyigbe and Afolayan, 2012) .Notably, Candida albicans exhibited varying susceptibility depending on the solvent, suggesting preferential antifungal action of specific phytochemicals in the extracts, but E. coli was the most susceptible bacterial strain. The traditional usage of Z. zanthoxyloides stem to treat bacterial and fungal illnesses is supported by the found antibacterial activity as revealed in table 7

Conclusion

The stem of *Z. zanthoxyloides* may be a possible source of natural antibacterial and antifungal drugs due to the combination of antimicrobial activity and bioactive functional groups. The overall findings show that the stem of *Zanthoxylum zanthoxyloides* has a rich profile of vitamins, minerals, phytochemicals, and nutrients. Strong antioxidant and anti-inflammatory properties are suggested by the high flavonoid and vitamin C content. The plant has long been used to cure infections, pain, fever, and inflammatory disorders due to the presence of alkaloids, glycosides, and saponins. Additionally, by supporting physiological and metabolic processes, the mineral content especially calcium, magnesium, and manganese improves the stem's therapeutic significance. These results corroborate previous findings that *Zanthoxylum* species are widely used medicinal plants in African ethnomedicine that are both pharmacologically effective and nutritionally significant (Mensah *et al.*, 2022; Ndam *et al.*, 2024). The corresponding author, similarly used the *Zanthoxylum zanthoxyloides stem to ease tooth ache*

REFERENCES

Adesina, S. K., Ogunleye, J. A., & Kuye, O. (2022). Mineral and proximate composition of selected medicinal shrubs in Nigeria. *Scientific African*, 18, e01502.

Akinmoladun, A. C., Olaleye, M. T., & Farombi, E. O. (2023). Phytochemical and pharmacological significance of glycoside-rich medicinal plants used in sub-Saharan Africa. *Journal of Ethnopharmacology*, 305, 116232.

Akinyemi, O. O., Ogunleye, A. O., and Popoola, A. R. (2021). Phenolic-rich plant extracts as antimicrobial agents. *Journal of Ethnopharmacology*

Ali, H., and Mohamed, S. (2023). FTIR analysis of medicinal plant compounds and their therapeutic relevance. *Phytomedicine Research*

Abisola, A.M, Olushola, S., Ukachi E. I, Godfrey A., Ibilola A. D. and Chima C. I. (2020). Elemental analysis and phytochemical characterization of *Zanthoxylum zanthoxyloides (Lam.) Zepern*. and Timler stem bark . *Journal of Pharmacognosy and Phytochemistry*. 9(5):41-46

AOAC (2016).Association of Official Analytical Chemists. Official Methods of Analysis, 20th ed. Association of Official Analytical Chemists International, Rockville MD,

Ekor, M., Odewabi, A. O., & Olayemi, F. O. (2021). Mineral characterization of traditional medicinal trees from West Africa. *BMC Complementary Medicine and Therapies*, 21(1), 112.

Habila, N., Suleiman, M., and Ahmed, A. (2023). Vitamin profile of commonly used medicinal plants in northern Nigeria. *Heliyon*, 9(4), e15142.

Koussou, M. O., Kouadio, K. J., and Goué, L. (2021). Nutritional evaluation of *Zanthoxylum leprieurii* stem bark used in traditional medicine. *African Journal of Plant Science*, 15(7), 215–223.

Mensah, J. K., Osei-Tutu, P., & Darko, M. (2022). Antimicrobial and antioxidant activities of flavonoid-rich extracts of *Zanthoxylum* species. *Journal of Medicinal Plants Research*, 16(4), 112–121.

Musa, M. B., Sulaiman, I.A., and Abdullahi, F I. (2023). Role of fatty acids in the antimicrobial potential of plant extracts. *BMC Complementary Medicine*

Ndam, T. S., Mbah, J. A., & Chia, V. (2024). Phytochemical investigation and therapeutic relevance of *Zanthoxylum* species in African ethnomedicine. *Phytomedicine Plus*, 4(1), 100360.

Nwankwo, F. N., Eze, C. N., & Ugwuoke, E. I. (2022). Proximate and nutrient evaluation of plant stems used in ethnomedicine. *Frontiers in Nutrition*, 9, 934812.

Nwachukwu, C. A., Uba, A., and Okoro, C. (2022). Antioxidant composition of selected Nigerian medicinal plants. *South African Journal of Botany*, 151, 543–551.

Okagu, I. U., Ndefo, J. C., Aham, E. C., and Udenigwe, C. C. (2021). Zanthoxylum Species: A Comprehensive Review of Traditional Uses, Pharmacological and Phytochemistry, Nutraceutical Applications. Molecules, 26(13), 4023.

Okagu, I. U., Ndefo, J. C., Alagwu, E. A., Okpallah, I. C., Uzochukwu, I. C., Ugwuoke, C. C., Nweke, K. O., & Agu, K. C. (2023). *Phytochemical composition, antioxidant and anti-inflammatory properties of selected medicinal plant extracts*. Journal of Medicinal Plants Research, 17(4), 112–124.

Osei-Djarbeng, S., and Essuman, E. K. (2021). Quantitative analysis of tannins and saponins in medicinal plants used for wound healing. *Journal of Herbal Medicine*, 28, 100434.

Onoja, S. O., Onukwube, I. E., & Okafor, A. E. (2020). Nutritional and biochemical characterization of traditional medicinal stems from southeastern Nigeria. *Journal of Applied Sciences and Environmental Management*, 24(3), 531–537.

Olajuyigbe, O. O and Afolayan, A. J. (2012). Antimicrobial potency of the ethanolic crude bark extract of *Ziziphus mucronata Willd*. subsp. mucronata Willd. *African Journal of Pharmacy and Pharmacology* . 6(10) : 724-730.

Oyeleke OM, Bamigboye MO, Olowookere BD, Alabi KE, Oladele SK, Oladele JO (2021). Comparative studies on phytochemical constituents and in vitro antioxidant activities of methanol and ethylacetate extracts of Talinum triangulare. Journal of Basic and Applied Research in Biomedicine 7(1):18-23.

Oyewale, M.O. (2012). Antibacterial properties of Fagara xanthoxyloides compared with commercial toothpastes readily in Nigeria. Journal of Agriculture and Rural Development, 10(2): 1-11.

Ozigis, H.O., Olaifa, K.A., Agbeja, A.O., Asabia, L.O., Akindolu , D.R , Majolagbe, M.O. (2023). Qualitative phytochemical analysis of leave and stem bark of *Zanthoxylum zanthoxyloides* and *Zanthoxylum gilletti.* *Journal Chemical Society of Nigeria*, 48 (3): 578 - 584

Yakubu, M. T., Musa, I. G., & Adeleke, A. A. (2022). Mineral distribution in *Zanthoxylum zanthoxyloides* roots and bark extracts. *Journal of Trace Elements in Medicine and Biology*, 72, 126982.

Zhang, L., Han, X. and Chen, W. (2020). Carbonyl phytochemicals and their antimicrobial mechanisms. *Food Chemistry*. , 329, 127092.