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Evaluation of phytochemical, antioxidant and antibacterial activity of edible fruit extracts of *Ziziphus abyssinica* A. Rich

Nyaberi M. O.[1]; Onyango C. A.[1]; Mathooko F. M.[1]; Maina J. M.[1]; Makobe, M.[2] and Mwaura, F.[3];

[1]*Department of Food Science and Technology of Jomo Kenyatta University of Agriculture and Technology. P. O. Box 62000-00200 Nairobi Kenya;* [2]*Department of Botany Jomo Kenyatta University of Agriculture and Technology;* [3]*School of Human Resource Development Jomo Kenyatta University of Agriculture and Technology.*

Corresponding author email: cakoth2000@yahoo.co.uk

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1 SUMMARY

The conventional chemicals used in preservation of meat are perceived as harmful by health conscious consumers due to their potential toxicity. This has resulted in a general shift of preference to the use of traditional herbal remedies that are proven not to have any known negative effects. The pastoralists from West Pokot district use the fruit paste of *Ziziphus abyssinica* A. Rich for meat processing and preservation. However, the preservative mechanism of *Z. abyssinica* remains unknown. In the present study the aqueous and methanol extracts of the fruit paste of this herb, that is traditionally used by the Kenyan pastoralists of West Pokot for meat preservation, were subjected to qualitative phytochemical analysis using the Trease and Evans (1989) methods; antibacterial properties using agar-diffusion method with the test microorganisms *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Candida albicans*; antioxidant activity using stable radical 2, 2 diphenylpicrylhydrazyl (DPPH), and toxicity using brine shrimps lethality test. From each of the extracts, fractions containing 100 and 200mg/ml were used in all tests. Alkaloids, saponins, flavonoids and polyphenolics, condensed tannins, reducing compounds, sterols and steroids were detected. The diameter of bacterial colony growth inhibition at an extract concentration of between 100 and 200mg/ml ranged between 9 to 15mm. The minimum inhibitory concentration (MIC) of the aqueous extract ranged between 3.13 and 50mg/ml, while the LC_{50} was 270µg/ml. The reducing activity presented as a percentage ranged between 90 - 96%. The fruit extracts of *Z. abyssinica* were found to have highly potent antioxidant activity compared to the control sodium metabisulphite and the results lend scientific credence to justify the use of this plant in the preservation of meat.

2 INTRODUCTION

Meat is one of the most nutritious but also most perishable foods. Unless it is preserved or stored under cool conditions, it rapidly deteriorates and becomes either unfit or unsafe for human consumption. Lack of ready markets has made it necessary for the pastoralists of West Pokot to store excess meat for use during the drought season, which is also vital to their household food security. The pastoralists in West Pokot, Kenya, have applied indigenous knowledge and locally available tree species such as *Ziziphus abyssinica* A. Rich to preserve

meat for hundreds of years (Mureithi, 1996). The pastoral community of West Pokot to treat ailments like diarrhea and various stomach infections also uses this herb for medicinal purposes.

The *Z. abyssinica* or *Angau* (local name) belongs to the family Rhamnaceae that consists of small trees that are indigenous to tropical Africa and India. There is apparently no scientific report on the qualitative phytochemical constituents, antibacterial, antioxidant and toxicity properties of the fruits of this herb. Lack of scientific knowledge has often constituted a major constraint to the use of traditional herbal remedies in conjunction with or as affordable alternatives to conventional preservatives (Krithika & Radhai, 2007). The *Z. abyssinica is a* Tree/shrub or climber of 1.8-8 m. It's bark is grey, deeply furrowed and branches are almost zigzag, with single or paired thorns of up to 12 mm. It is found in wooded grassland, bushed grassland and along rivers. Its Cream pulp and outer skin are eaten. The pulp has a sweet to slightly bitter taste but edible portion is small. It is also used for Construction, as firewood and as fodder. In Eritrea, it is used for charcoal, medicine, bees forage and as live fence (Chikamai et al 2004).

Thus, the extracts of the fruit of *Z. abyssinica* were screened for phytochemical, antimicrobial, antioxidant, and toxicity activity, with the overall objective of determining its potential to preserve meat products.

3 METHODOLOGY

3.1 Plant material: The information on the type of herbs used and the way preservation of meat was undertaken, was obtained by personal observation and focused discussions guided by a questionnaire. Three groups of respondents were involved, elders, women and youth groups. From each group fifteen respondents were interviewed. All the respondents were from Chepararia and Kongelai Divisions of West Pokot District.

The fruits of *Z. abyssinica* were collected from Chepararia and Kongelai Divisions in West Pokot district. This fruits were harvested towards the end of December 2007 and early January 2008. During this time most of the herbs have fruited, the rains have subsided while the concentration of metabolites is at their highest (Freitas and Glories 1999). This fruits were harvested when ripe and had turned red in colour. Taxonomic identification was done at the East African Herbarium of the National Museums of Kenya, Nairobi.

3.2 Extraction of plant materials: A portion of fresh fruits of *Z. abyssinica* weighing 10kg was gently cleaned using running tap water to remove soil. The samples were dried at ambient temperature (25 ± 2 °C) in a room for six days. Fruit samples were ground into moderately coarse powder using an electric grinder (model M10R Japan) and stored until use. A 500g portion of the ground fruit sample was cold extracted with methanol and water, using the method of Regnier and Macheix (1996), with modifications.

In aqueous extraction, distilled water alone was used (Bautista-Banos *et al.*, 2003). In each of the solvents, the fruit sample was completely immersed and the containers shaken for 30 minutes to ensure sufficient contact with the solvents using a Kika Labortechnik Shaker, (Model KS 250 Basic, Staufen, Germany). The mixtures were left to stand for four days in an enclosure at 25 ± 2 °C.

The mixture with distilled water was boiled for one hour, cooled, filtered and centrifuged at 4,000rpm for 10 minutes at a temperature of 4 °C using a Kokusan.

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Figure 1: *Ziziphus abyssinica* A. Rich fruit, collected from West Pokot District in Kenya with some of its leaves (Photo by Nyaberi M. O. 2007).

Centrifuge (Kokusan Corporation, Model 2000C, Tokyo Japan). The supernatant of both solvents were filtered using No. 1 Whatman filter paper. The solvents were evaporated to dryness under vacuum at 80°C using a rotary evaporator (Model RE 100, Staffordshire, England). The extracts obtained were put in stoppered light proof glass containers and stored at 4°C in the dark until used in further tests.

From the extracts, concentrations of 100 and 200mg/ml were prepared. The extracts were subjected to qualitative chemical screening for the presence of various classes of active chemical constituents, that included, alkaloids, saponins, tannins, steroids, flavonoids/polyphenolics, and reducing compounds by the methods of Trease and Evans (1989), El-Olemyl *et al.* (1994) and Wall *et al.* (1954).

3.3 Antimicrobial properties test: Non drug resistant bacterial organisms used included, Gram positive *Staphylococcus aureus* (22923 ATCC), Gram negative *Pseudomonas aeruginosa* (27853 ATCC) and *Escherichia coli* (25922 ATCC), and a fungus, *Candida albicans* (90028 ATCC). Antimicrobial activity was recorded if the zone of inhibition was greater than 9mm (Hassan *et al.,* 2006). Sensitivities of the organisms to the various extracts was evaluated using the cork and bore diffusion method of Bauer *et al.* (1966), Barry *et al.* (1985) and Rojas *et al.* (2003) with slight modifications. The MIC was determined using the standard method of Wariso and Ebong (1996) with modifications. Sodium metabisulphite was used as a standard being a preservative already in the market.

3.4 Antioxidants in fruit extracts: The antioxidant activity of fruit extracts were measured in terms of hydrogen donating radical scavenging ability using the stable radical 2,2 diphenyl picrylhydrazyl (DPPH) (Brand *et al.*, 1995).

3.5 Brine shrimp lethality test: The bioactivity of crude extracts and pure compounds was assessed using brine shrimp (*Artemia salina*) larvae as the test organism to obtain the lethality concentrations (LC_{50} Values) (Meyer *et al.*, 1982).

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3.6 Statistical analysis: One-way analysis of variance (ANOVA) was used and means compared by Duncan's Multiple Range Test (Steel & Torrie, 1980) using SAS program (Version 9.1).

4 RESULTS AND DISCUSSION

Qualitative phytochemical investigations revealed that the aqueous and methanol fruit extracts of *Z. abyssinica* contain saponins, sterols and steroids, alkaloids, tannins, flavonoids and reducing compounds (Table 1). This result, therefore, showed that the phytochemicals in the herb dissolve in both water and methanol. The aqueous fruit extract inhibited the growth of *E. coli* (13.0mm) and *S. aureus* (14.7mm) at 200mg/ml (Table 2), while the methanol extract inhibited *P. aeruginosa* (11.03mm), *E. coli* (12.7mm) and *S. aureus* (15.6mm) at 200mg/ml. The difference in antimicrobial activity between the aqueous and methanol extracts was not significant (P>0.05) indicating that the phytochemicals responsible for antimicrobial activity were equally soluble in both methanol and water (Table 1). The antimicrobial and antioxidant activity was due to the presence of phytochemicals as has variously been reported (Leven *et al.*, 1979; Baratta *et al.,* 1998).

Table 1: Phytochemicals present in the aqueous and organic extracts of *Ziziphus abyssinica*

phytochemicals	**Methanol extract**	**Water extract**
Alkaloids	+	+
Saponins	+	+
Flavonoids and polyphenolics	+	+
Hydrolysable tannins	-	-
Condensed tannins	+	+
Reducing compounds	+	+
Sterols and steroids	+	+
Cardiac glycosides	-	-

- Means absent, + present.

Table 2: The average diameter of inhibition (mm) produced by the methanolic and aqueous extracts of *Ziziphus abyssinica* A. Rich (AZA) on microorganisms grown. Sodium metabisulphite (MS) was used as control.

Microbe	**Methanol AZA**		**Water AZA**		**MS (Control)**	
	200**	100	200	100	200	100
S. aureus *	15.6±0.7^{c}	9.0±0.8^{d}	14.7±0.3^{c}	-	52.7±1.5^{a}	50±1.2^{a}
P. aeruginosa	11.3±0.3^{d}	-	-	-	33.7±1.9^{a}	26.7±0.5^{b}
Candida albicans	-	-	-	-	62.3±1.5^{a}	60±1.5^{a}
E. coli	12.7±0.3^{c}	10.7±0.5^{d}	13.0±0.6^{c}	9.7±0.5^{d}	42±1.2^{a}	33±1.4^{a}

*Gram positive microorganisms; ** Concentration is in mg/ml; MS – Sodium metabisulphite,
- No inhibition or diameter below 9mm. Means followed by the same letter within the same row are not significantly different (P>0.05) by Duncan's Multiple Range test. Figures are mm ± SD.

The aqueous extract of *Z. abyssinica* had a low MIC of 3.1mg/ml for *S. aureus* compared to 0.2mg/ml of sodium metabisulphite for *S. aureus and E. coli* (Table 3). This indicated that *Z. abyssinica* extract was effective compared to sodium metabisulphite which is a synthetic product in the market. *Z. abyssinica* extracts had very effective antioxidant properties that took effect within the first minute (Figure 2). There was no significant difference (P>0.05) in

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antioxidation between the methanol and water extracts (Table 4).

The results of brine shrimp lethality (BSL) obtained in the present study agreed with the findings of Parra et al. (2001) that the in vitro test is highly correlated with in vivo tests, and that it is a useful alternative model for predicting toxicity in plant extracts. According to Meyer et al. (1982) and Parra et al. (2001), LC_{50} values lower than 1000 µg/ml are considered bioactive in toxicity evaluation of plant extracts by BSL bioassay while those values lower than 100µg/ml are considered very highly toxic. Therefore, *Z. abyssinica* is a highly cytotoxic compound, recording LC_{50} value of 270µg/ml (Table 3). The above properties suggest that the pastoralists of West Pokot benefit from both antioxidant and antimicrobial properties of *Z. abyssinica* in preserving their meat and meat products.

Table 3: Minimum inhibitory concentration and toxicity of aqueous extracts of *Ziziphus abyssinica*

Test microorganism	AZA (mg/ml)	Sodium Metabisulphite (mg/ml)	Concentration (µg/ml)	AZA (%) Mean survival
E. coli	25	0.2	781.3	0
S. aureus	3.1	0.2	390.6	22.3±4.0
P. aeruginosa	50	0.4	195.3	68.7±4.5
C. albicans	nd	0.4	97.7	100.0
			LC_{50} (ug/ml)	270

AZA - *Ziziphus abyssinica* A. Rich, nd – Not detected

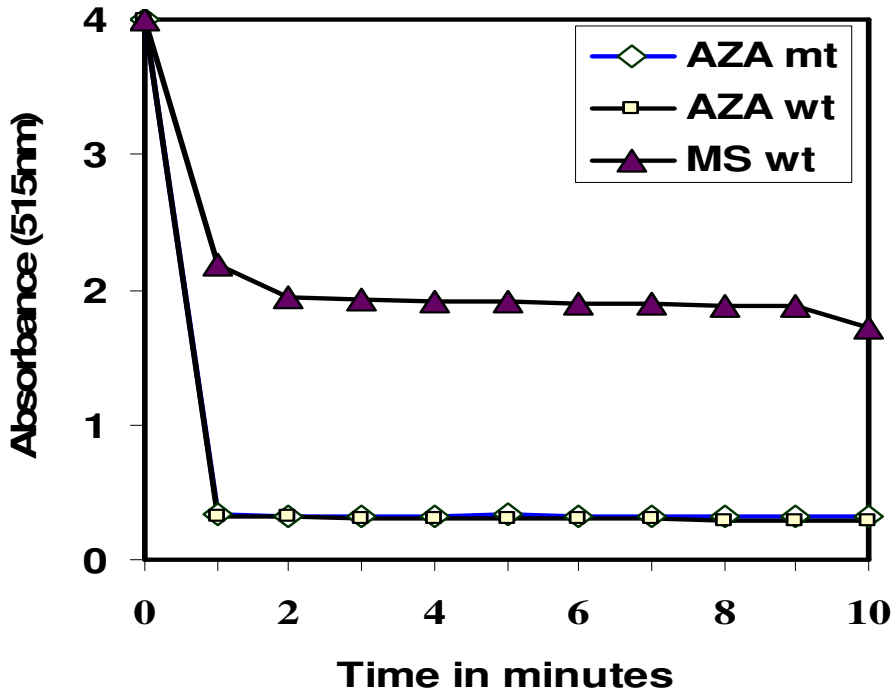

Figure 2: Antioxidant activity of methanol (mt) and water (wt) extracts of *Ziziphus abyssinica* A. Rich (AZA) and control sodium metabisulphite, using the DPPH method.

Table 4: Percentage change in antioxidant capacity of water (wt) and methanol(mt) extracts of *Ziziphus abyssinica* A. Rich (AZA) and the control sodium metabisulphite when the concentration is varied, using the DPPH radical scavenging method

	Concentrations of extracts in mg/ml				
	0.1	0.2	0.3	0.4	0.5
AZA (wt)	96.2±0.1[a]	95.7±0.1[a]	95.3±0.1[a]	92.7±0.1[a]	89.6±0.4[a]
AZA (mt)	93.4±0.1[a]	92.9±0.5[a]	92.3±0.1[a]	91.5±0.2[a]	90.7±0.1[a]
SM	51.3±2.4[b]	59.5±4.6[ab]	57.8±1.7[b]	64.1±1.9[a]	69.4±1.3[a]

Values followed by the same letter within the same row are not significantly different (P>0.05) according to Duncan's multiple range test, MS – Sodium metabisulphate.

5 CONCLUSION

Based on the results of this study we conclude that *Ziziphus abyssinica* A. Rich can be effectively used as a preservative due to its effective phytochemical constituents, antibacterial and antioxidant properties.

Further research is recommended in quantitative phytochemical analysis and the identification of the active principle compounds. A study can also be undertaken to develop an antioxidant product from the fruits of Ziziphus *abyssinica* A. Rich.

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