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Bioactive fractions in the stem charcoal of *Ozoroa insignis* used by the pastoral communities in West Pokot to preserve milk

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ABSTRACT

Objective: To determine the potential of *Ozoroa insignis* Del to preserve milk as practiced by the pastoralists of West Pokot, Kenya.

Methodology and results: The aqueous and organic solvents extracts of the peeled stem charcoal of *Ozoroa insignis* were screened for qualitative phytochemical composition by the Trease and Evans (1989) method. Antimicrobial activity was determined using the cork and bore diffusion method against test organisms *Staphylococcus aureus* (ATCC 22923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 90028). Antioxidant activities measured as hydrogen donating radical scavenging ability was determined using the stable radical 2, 2 diphenyl picrylhydrazyl (DPPH) (Brand *et al.* 1995). Toxicity test was carried out using brine shrimp larvae (*Artemia salina*, Aqua farm, USA) as the test organism (Meyer *et al*., 1982). Flavonoids, saponins, sterols and steroids were detected in the three extracts. Alkaloids were detected in the chloroform and methanol extracts, while hydrolysable tannins were detected in the methanol extract. The chloroform and methanol extract fractions were significantly ($p<0.05$) active against *P. aeruginosa, E. coli* and *S. aureus* compared to the aqueous extract that had no activity against any of the test organisms. This implies that alkaloids were directly responsible for the inhibition and growth of micro-organisms and also that the effective phytochemicals were not able to dissolve in water. The antioxidant activity in all the extracts was very high and reduced 2, 2 diphenyl picrylhydrazyl (DPPH) in the first minute by about 84.5%.

Conclusions and application of findings: These results show that there is scientific justification for the use of *Ozoroa insignis* Del in the preservation of milk by the pastoralists of West Pokot. This herb can therefore be considered as viable substitute for the chemical preservatives in the markets where more consumers show greater preference for products preserved with non-artificial compounds.

Key words: *Ozoroa insignis,* antibacterial activity, *in vitro* screening.

INTRODUCTION

Ozoroa insignis Del. (Verbenaceae) or *kromwa* as known locally, is a shrub or tree that grows to a height of 6.5 m. It is found in the Sudanian savannah, Senegal, Niger, Nigeria, Ethiopia, Zaire and E. Africa (Burkill, 1985). The pastoralists of West Pokot peel the bark, dry the stem and burn it

into charcoal. They use this charcoal to preserve milk. The root, bark, leaves and stem of this herb have been found to have a wide range of healing properties and are used in the treatment of diarrhoea and venereal diseases (Rea *et al*. 2003), tapeworms and hookworms (Gelfand *et al*. 1985), schistosomiasis (Ndamba *et al.* 1994; Mølgaard *et al*. 2001) and kidney ailments (He *et al*. 2002).

Although O. insignis is used to preserve and flavour milk for use in the dry season, the leaves, flowers, twigs, bark, roots and stems have not been submitted to any significant phytochemical investigation (Wall et al. 1996; Yonghong et al. 2006). This lack of scientific knowledge on its phytochemical constituents, antibacterial antioxidant and toxicological properties limits the use of O. insignis in traditional herbal remedy or an affordable alternative to conventional preservatives.

Thus, in this study the aqueous and organic extracts of the stem charcoal of O. insignis were screened for their phytochemical composition, activity against selected microorganisms, antioxidant potential and toxicity against brine shrimp larvae with the objective of establishing its preservative potential in foods.

MATERIALS AND METHODS

Plant material: The *O. insignis* herb (Figure 1) was identified and collected from the field by the use of a structured questionnaire administered to focus groups and key informants in selected areas of West Pokot District. The taxonomic identification was done at the East African Herbarium of the National Museums of Kenya, Nairobi.

Extraction of plant materials: Fresh peeled stems of *O. insignis* weighing 10kg were gently cleaned using running tap water to remove soil before drying in an air oven at 25°C for 6 days. The stems were burned for charcoal and the charcoal ground into moderately coarse powder using an electric grinder (model M10R Japan) and stored until use. A 500g portion each of sample was cold extracted with methanol, chloroform and water. The organic solvents were used to extract the sample using the method of Regnier and Macheix (1996), with modifications. For each extraction, the herb sample was completely immersed in the solvent, and the container shaken for 30 minutes for sufficient contact using a Kika Labortechnik Shaker (Model KS 250 Basic, Staufen, Germany). For aqueous extraction, distilled water alone was used (Bautista-Banos et al., 2003)

Figure 1: Leaves, stems and bark of *Ozoroa insignis* Del. collected from West Pokot District in Kenya (Photo by Nyaberi M. O. 2007)

The mixture was left to stand for four days at 25 ± 2°C in a stoppered flask, and then brought to boiling and held for one hour, left to cool, filtered and centrifuged at 4,000rpm for 10 minutes at a temperature of 4°C using a Kokusan Centrifuge (Kokusan Corp., Model 2000C, Tokyo, Japan). The supernatant was filtered using No. 1 Whatman filter paper. All the solvents were evaporated to dryness under vacuum at 80°C using a rotary evaporator (Model RE 100, Staffordshire, England

The extract obtained was put in a light proof glass container and stored at 4°C until use. From the extracts, concentrations of 0.1, 0.2 and 0.3mg/ml were prepared. The extracts obtained were subjected to qualitative chemical screening for the presence of various chemical constituents, including alkaloids, saponins, tannins, steroids, flavonoids/polyphenolics, and reducing compounds. Methods used were as described by Trease and Evans (1989), El-Olemyl *et al*. (1994) and Wall *et al*. (1954).

Test microorganisms: Non drug resistant bacterial organisms were obtained from Kenya Agricultural Research Institute (KARI). They included Gram positive *S. aureus* (22923 ATCC), Gram negative *P. aeruginosa* (27853 ATCC) and *E. coli* (25922 ATCC), and a fungus, *C. albicans* (90028 ATCC). Antimicrobial activities were recorded if the zone of inhibition was greater than 9mm (Hassan *et al.* 2006). Sensitivity of the organisms to the various extracts was done using the cork and bore diffusion method of Bauer *et al*. (1966), Barry *et al*. (1985) and Rojas *et al*. (2003) with some modifications. The MIC assay was undertaken using the standard method of Wariso and Ebong (1996) with modifications.

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

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

Statistical analysis: One-way analysis of variance (ANOVA) was used. Mean comparisons were performed by Duncan's Multiple Range Test (Steel & Torrie, 1980). Statistical analysis was carried out using SAS program (Version 9.1).

RESULTS AND DISCUSSION

Preliminary qualitative phytochemical investigations revealed the presence of saponins, sterols and steroids in methanol, chloroform and aqueous extracts. Alkaloids were detected in both chloroform and methanol extracts and tannins were detected only in the methanol extract (Table 1). The results showed that methanol was a better extracting solvent than chloroform while water had the least extraction ability. Higher yields of phytochemicals from the methanol and chloroform extracts compared to the aqueous extracts suggest higher proportion of water-insoluble plant components (Nkere & Iroegbu 2005). The phytochemical compounds detected such as saponins, tannins, flavonoids and alkaloids, have previously been reported to have antimicrobial and antioxidant activity (Leven *et al.*1979).

The aqueous extract of the stem charcoal of *O. insignis* did not show significant activity against any of the test microorganisms. The chloroform extract showed significant activity against *E. coli* and *P. aeruginosa* (both 15.4mm inhibition zone at 0.2 mg/ml). The methanol extract showed significant antibacterial activity in the order of *E. coli* (12.3mm at 0.2 mg/ml) >*P. aeruginosa* (9.6mm at 0.2 mg/ml). However, both methanol and chloroform extracts failed to show any antifungal activity against *C. albicans* (Table 2).

These findings suggest that antimicrobial activity of *O. insignis* may be primarily due to the presence of alkaloids (Table 1). Furthermore, organic solvent extracts of the plant were very effective against Gram negative organisms. Therefore, milk being an emulsion of oil dispersed in water, could have dissolved the phytochemicals from the herb into its oil fraction from where they were able to impart their antimicrobial effect.

Antioxidant analysis (Figure 2) showed that all the extracts reduced DPPH in the first minute by about 84.5% due to scavenging effect of the phytochemicals in the herbs. However, here was no significant difference ($P<0.05$) between the three extracts. This

indicates that all the extracts had factors that promote antioxidant activity (Table 3). The results showed that the factors promoting antioxidation were soluble in both aqueous and organic solvents. This implies that the herbs can prevent the production of off flavours that are caused by fat oxidation (Namki 1990).

Table 1: Phytochemicals present in the aqueous and organic extracts of *O. insignis*.

Phytochemical	Water extract	Chloroform extract	Methanol extract
Condensed Tannins	-	-	-
Hydrolysable Tannins	-	-	+
Flavonoids	+	+	+
Saponins	+++	+	++
Sterol and steroids	+	+	+
Reducing compounds	-	-	-
Alkaloids	-	+	+

- Absent, + present, ++ present in high *O. insignis* proportion (from the colour intensity).

Table 2: Average inhibition zone diameter (mm) of extracts of *O. insignis* against test organisms.

	Chloroform		Methanol
Organism	0.2mg/ml	0.1mg/ml	0.2mg/ml
E. coli	15.4±0.6a	12.9±0.6b	12.3±0.6c
C. albicans	-	-	-
P. aeruginosa	15.4±0.9a	13.0±0.9b	9.6±0.5c

Values followed by the same letter within the same row are not significantly different (P>0.05). All the figures are in millimetres (mm). Values are means ± SD of n = 3. Values for *C. albicans* and methanol at 0.1mg/ml were below 9.0mm thus not considered.

Table 3: Percent antioxidant activities at different concentrations of the water, chloroform and methanol extracts of *O. insignis*

	Concentration of extract in mg/ml				
	0.1	0.2	0.3	0.4	0.5
Water	84.9±1.4ab	85.0±0.06a	85.0±0.08a	85.0±0.03a	85.0±0.05a
Chloroform	87.9±0.5a	87.9±0.05a	88.5±0.03a	88.5±0.3a	88.5±0.5a
Methanol	83.4±0.3ab	80.2±0.08a	74.1±3.2b	79.2±2.6ab	88.3±0.04a

Values followed by the same small letter within the same row are not significantly different (P>0.05) values are means ± SE and n=3, water extract, chloroform extract and methanol extract

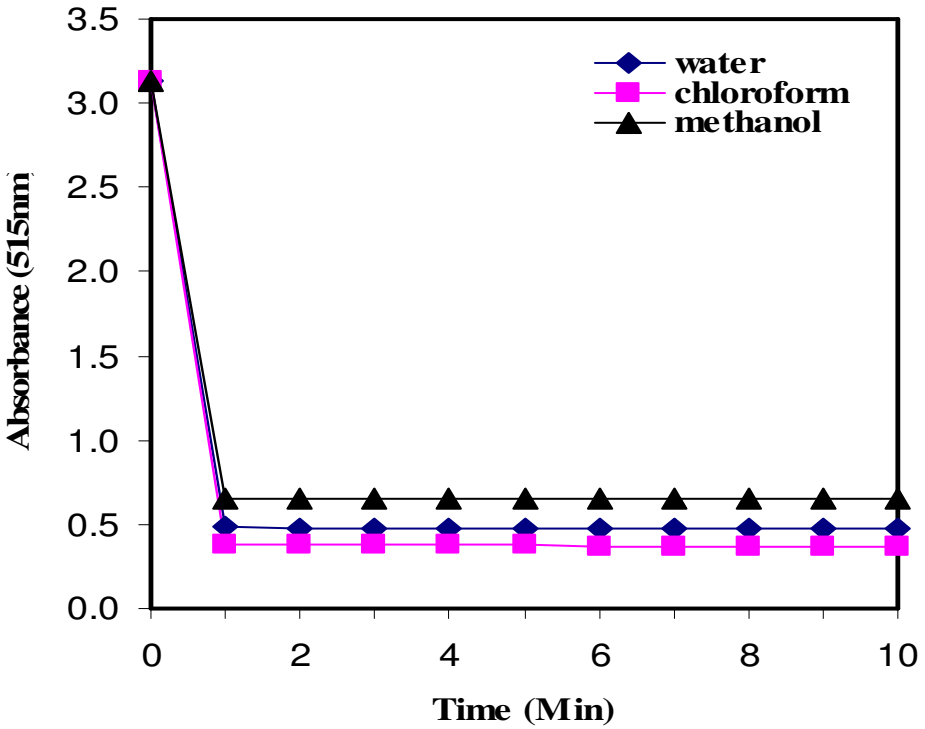

Figure 2: Antioxidant activity of the water, chloroform and methanol extracts of *O. insignis* at 515nm using the DPPH method n=3

CONCLUSION

Since the pastoral communities of West Pokot use water as their extracting solvent, and in the study showed that the aqueous extract had no significant antimicrobial activity, the property that may be largely contributing to the preservation of milk is the antioxidant capacity. More properties of the herbs would be harvested if the Pastoralists used methanol as their solvent other than water. Based on these findings and the practice of preserving milk by the pastoralists of West Pokot using this herb, the application of the extract of the stem charcoal of *O. insignis* in the preservation of milk is justified. Further research is recommended to further determine the specific compounds responsible, the synergism between them and the optimum portions required to exert maximum stability.

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