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Full Length Research Paper

Effect of light irradiation on the antimicrobial activity of *Zanthoxylum zanthoxyloides* (lam) methanolic extract

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The effect of light irradiation on the antibacterial potentials of methanol root extracts of *Zanthoxylum zanthoxyloides* (Linn) was evaluated against some clinical isolates of gram-positive and gram -negative microorganisms using the agar diffusion methossd. The methanol extracts were exposed to three different sources of radiations: sunlight (28 days), tungsten lamp (72 h) and photoreactor (1 h, 365 nm) after which their antibacterial activity was determined. The colour of the extract monitored by colour visualization and TLC revealed significant colour changes for each treatment; yellow to very light yellow (sunlight and tungsten) while the photoreactor irradiated sample did not show appreciable colour change. The antibacterial activity of the extracts generally reduces significantly ($p < 0.05$) after exposure to the varied radiations. The results in this study indicate a possible compromise of the antimicrobial quality of herbal preparations containing *Z. zanthoxyloides;* this highlights the need for appropriate storage of such herbal products.

Key words: *Zanthoxylum zanthoxyloides*, radiation, antimicrobial activity, herbal products.

INTRODUCTION

Herbal medicines play a major role in the health of thousands of people worldwide (Adewumi and Ojewole, 2004). In the developed countries, such as the United States and Europe, a lot of people are going back to the use of herbal medicines despite great advancement in health care. These medicines are used to treat many illnesses including infectious diseases, allergies and hypertension. Most drugs today are derived from natural products (Gafner and Bergeron, 2005).

Zanthoxylum zanthoxyloides (Rutaceae) commonly called 'toothache bark' or 'candle wood' (English), Orin ata (Yoruba) is widespread in the West Tropical Africa occurring in Savanna and dry forest areas. The plant is also found in the Coastal areas (Iwu, 1999). *Z. zanthoxyloides* contains various secondary metabolites which are known for their diverse biological properties. The plant is known for its antioxidative, anti-inflammatory, antisickling, antibacterial, antiviral, antihepatotoxicity, antiallegic, antitumoral and antihypertensive properties (Sofowora, et al., 1975; Andersson et al., 1996; Adesina, 2005). The methanol extract preparation of the powdered root of *Z. zanthoxyloides* containing flavonoids, chelerythrine, berberine and phenol canthine-6-one have been reported to possess strong antibacterial activity (Odebiyi and Sofowora, 1979; Tsuchiya et al. 1996). They have been used as components of antiseptic, antiparasitic and analgesic preparations for managing small pox, syphilis and related disease conditions (Olatunji, 1983).

The major challenges in herbal medicines are determining the overall quality, safety and efficiency of the herbal product. Virtually all herbal remedies have been reported to cause either allergic sensitization or photo-sensitization (Niggermann and Gruber, 2003). Some members of the Rutaceae family especially *Zanthoxylum* spp have been reported to undergo photodegradation reactions (Pathak et al., 1962).

The aim of this study was therefore to evaluate the effect of light irradiation on the antibacterial fraction (methanol root back extract) of *Z. zanthoxyloides* and the antimicrobial activity of the resulting products with a view to establish the possible clinical implications of the photodegradation process.

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Abbreviations: TLC, Thin layer chromatographic; **UV,** ultraviolet; **DC,** dark cupboard.

EXPERIMENTAL

Collection and preparation of plant extract

Fresh root of *Z. zanthoxyloides* were collected from the wild around Olokemeji Village, Oyo State, Nigeria. The plant was identified and authenticated by Esimekhuia of the Botany Department, University of Ibadan. The roots were sun-dried for about 6 h before the back was removed and further air dried for 2days. The dry root-bark was powdered and extracted with methanol according to an earlier described method (Ogwal-Okeng et al., 2003). The plant extract was screened for phytoconstituents including: anthraquinone glycosides, anthraquinone aglycones, cardiac glycosides, alkaloids, saponins, tannins and flavonoids (Sofowora, 2006).

Test organisms

Three clinical isolates of *Escherichia coli* , *Pseudomonas aeruginosa, Bacillus subtilis* and *Staphylococcus aureus* obtained from Medical Microbiology Department of the University College Hospital, Ibadan were used for microbiological evaluation. In this study, Gentamycin were use also.

Chromatographic analysis of the methanolic extracts

Thin Layer Chromatographic (TLC) analysis of the methanol extracts was carried out using ethylacetate (100%), chloroform: methanol (5:1) and chloroform: methanol: water (65:35:10) as mobile phases. The resulting chromatograms were visualised under ultraviolet (UV) light at 254 and 365 nm. The number of spots and their R_f values were noted.

Evaluation of antimicrobial activity of the plant extract

The agar diffusion method using Mueller Hinton agar seeded with the microorganisms was used (Ndukwe et al., 2005). Stock solution of the plant extract was prepared by dissolving 5 g in 10 ml methanol in a 25 ml volumetric flask, this was made up to volume with methanol. Aliquots of the methanolic plant extract at 200, 100, 50, 25, 12.5 and 6.5 mg/ml were transferred into agar wells.

The agar wells were prepared by boring holes of 8 mm into solidified agar plates which have been earlier seeded with appropriate organisms (Ndukwe et al., 2005). Gentamycin (5 mg/ml) (Sigma Aldrich, USA), was used as a positive control while methanol was used as a negative control. The plates were incubated at 37°C for 24 h. The experiment was carried out in duplicate for all the test bacteria.

Determination of the effect of irradiation on the antibacterial potentials of the methanol plant extracts

Effect of sunlight

The effect of sunlight on the activity of the plant extracts was determined on the powdered, methanol and aqueous (phosphate buffered saline pH 7.4) samples. For the powdered extract, 100 mg of the extract samples were weighed into three sets of test tubes. Two of the test tubes were left under the sun with one tube wrapped completely with aluminium foil (DWS) and the other bare (DES). The third tube was kept in a dark cupboard (DC). All the tubes were exposed to sunlight irradiation for an average of 10 h per day over a period of 28days (Rajendran et al., 2007) . For the methanol solution sample (liquid suspension), 10 ml of the methanol extract (1% w/v) were dispensed into three separate test tubes treated as the powdered samples that is, methanolic solution irradiated with sunlight (MES), methanolic solution wrapped irradiated with sunlight (MWS) and methanolic solution placed in a cupboard (MC). The aqueous samples were also treated as for methanol solution sample that is, aqueous solution irradiated with sunlight (AES), aqueous solution wrapped irradiated with sunlight (AWS) and aqueous solution placed in a cupboard (AC). Unirradiated sample in methanol and water (UMC and UAC) were used as controls.

The samples were monitored for photochemical degradation by observing for colour change and TLC analysis at 1, 3, 7, 14, 21 and 28 days (Adegbolagun et al., 2002). Antibacterial activity of all the samples (irradiated and unirradiated) was evaluated after the period of irradiation.

Effect of white electrical light on activity of the plant extracts

This was carried out by exposing the extracts to irradiation from a 60 W tungsten lamp. In this procedure, 100 mg extract powder, and 10 ml of the methanol and aqueous (phosphate buffered saline pH 7.4) suspensions (1% w/v) each in three sets of test tubes were treated as in solar irradiation. The samples dry powder irradiated with tungsten lamp (DET), dry powder wrapped irradiated with tungsten lamp (DWT), methanolic solution irradiated with tungsten lamp (MET), methanolic solution wrapped irradiated with tungsten lamp (MWT), aqueous solution irradiated with tungsten lamp (AET) and aqueous solution wrapped, irradiated with tungsten lamp (AWT). Unirradiated sample in methanol and water (UMC and UAC) were used as controls.

The samples were kept in an aluminium- lined covered box with the samples at a distance of 30 m from the tungsten lamp for 72 h (Adegbolagun et al., 2002) . The samples were monitored by observing colour change and TLC analysis at 2, 4, 6, 24, 48 and 72 h. Antibacterial activity of all the samples (irradiated and unirradiated) was evaluated after the period of irradiation.

Effect of photoreactor irradiation

Methanol (MPh) and phosphate buffered saline (pH 7.4) (BPh) solutions (0.8% w/v) of the extract were irradiated with mercury arc lamp with radiant energy at wavelength 365 nm for 1 h. The photochemical reaction was monitored by observing colour change and using TLC at 20, 40 min and 1 h. Unirradiated sample in methanol and phosphate buffered saline (pH 7.4) (UMC and UBC) were used as controls.

Antibacterial activities of samples against the microorganisms were evaluated after the period of irradiation of 1 h.

Statistical analysis

Results obtained were expressed as mean± standard deviation and analysed using one way ANOVA and student t-test (Instat 3, Statistical Package) at $p < 0.05$.

RESULTS AND DISCUSSION

Results of extraction of bioactive components of root barks of *Z. zanthoxyloides* using methanol yielded 16.7% w/w of the dried powdered extracts. This is higher than the earlier reported yield of 10.5% w/w by Ogwal- Okeng et al. (2003). Results of phytochemical screening of the root extract also showed the presence of cardiac glycolsides, alkaloids, saponins, tannins and flavonoids. This is in agreement with previous works carried out on the root of *Z. zanthoxyloides* (Adesina, 1986; Adesina, 2005;

Table 1. Zones of inhibition of the methanolic fraction of Z. zanthoxyloides after sunlight irradiation.

Sample	Zones of inhibition of the microorganisms (mm)							
	P. aeruginosa		*E. coli*		*B. subtilis*		*S. aureus*	
	Mean	p value	Mean	p value	Mean	p value	Mean	p value
DES	13.91 ± 5.3	0.38	0.0	0.014*	15.97 ± 6.9	0.27	14.4 ± 5.3	0.081
DWS	17.28 ± 3.2		0.0		15.53 ± 3.9		13.09 ± 3.6	
DC	15.05 ± 6.0		11.9 ± 2.4		15.70 ± 4.6		13.14 ± 1.7	
UMC	20.83 ± 4.5		19.94 ± 2.7		22.60 ± 2.5		20.91 ± 2.6	
MES	14.19 ± 3.3	0.089	0.0	0.03*	13.08 ± 3.0	0.076	9.61 ± 0.1	0.002*
MWS	13.13 ± 3.5		11.71 ± 0.9		15.89 ± 3.6		15.04 ± 1.5	
MC	14.42 ± 1.9		10.38 ± 0.2		16.87 ± 5.4		12.11 ± 2.3	
UMC	20.83 ± 4.5		19.94 ± 2.7		22.60 ± 2.5		20.91 ± 2.6	
AES	0.0		0.0		0.0	0.023*	0.0	0.020^
AWS	0.0		0.0		14.37 ± 2.5		0.0	
AC	0.0		0.0		14.88 ± 4.4		11.85 ± 0.7	
UAC	18.7 ± 2.6		18.66 ± 1.2		18.52 ± 2.3		19.50 ± 4.1	

Values are means of three determinations ±S.D. * - Significant (ANOVA), ^ - Significant (Paired t-test) DES - Dry, irradiated with sunlight, DWS - Dry, wrapped irradiated with sunlight DC - Dry, covered irradiated with sunlight, MES - Methanolic solution irradiated with sunlight

MCS - Methanolic solution wrapped irradiated with sunlight, MC - Methanolic solution placed in a cupboard, AES - Aqueous solution irradiated with sunlight, AWS - Aqueous solution covered irradiated with sunlight, AC - Aqueous solution placed in a cupboard, UMC- Unirradiated sample in methanol, UAC - Unirradiated sample in water.

Chaaib, 2004). Plant constituents have been reported to possess antimicrobial properties (Iwu et al., 1999; Banso and Adeyemo, 2006).

Chromatographic analysis of the methanol extracts using three mobile phases revealed the presence of six components. The best solvent system for the separation of the components was found to be Chloroform: Methanol (5:1).

Microbiological susceptibility determination of the methanol extracts of the plant at the least concentration of 6.25 mg/ml showed activity against the three strains of the “gram-positive” organisms: *S. aureus* (12.33 ± 3.6 mm) and *B. subtilis* (17.64 ± 0.6 mm) as well as those of “gram-negative” organisms: *E. coli* (15.38 ± 2.7 mm) and *P. aeruginosa* (14.42 ± 1.9mm). Adesina (2005) has reported the demonstration of antibacterial activity by many species of *Zanthoxylum* against both “gram-positive and gram-negative” microorganisms, although aqueous extracts of *Zanthoxylum tessmannii* have been shown to be inactive against *P. aeruginosa* and *E. coli* (Ndukwe et al., 2005). Stem and root bark extracts from *Z. zanthoxyloides* have also been reported to have a higher activity against bacteria implicated in periodontal diseases (Taiwo et al., 1999).

Examination of the samples also showed that, there were colour changes after exposure to the radiations. For example, the colour change for the samples before irradiation was bright yellow but this changed to very pale yellow for sunlight and tungsten lamp, while the colour change with the photoreactor was almost insignificant. Though colour change is not a conclusive determinant of decomposition, it is however a sign that, the process of decomposition has started (Greenhill and McLelland, 1990).

The TLC analysis of the sunlight and tungsten lamp irradiated dry samples did not reveal the presence of any additional spot/compound by the end of the study period. However, the sunlight irradiated methanolic solutions showed the presence of two additional compounds, while the aqueous solution showed the presence of one additional spot/compound. The presence of additional compounds indicated possible photochemical degradation of one or more component of the extract. The absence of additional compounds on irradiation with tungsten lamp and photoreactor does not indicate the absence of photodegradation products as the concentration of the photodegradation product(s) may be too low to be detectable by the TLC conditions used.

The evaluation of the antimicrobial activity of the irradiated samples (dry, aqueous and methanolic samples) revealed a total loss of activity for all the sunlight irradiated samples against *E. coli,* as well as wrapped dry and aqueous samples, but a significant ($p < 0.05$) reduction in activity for the wrapped methanol samples (Table 1). The loss in activity of the sunlight irradiated samples proposes that, photochemical degradation of the active compounds has resulted in structural modifications of functional group(s) required by the compounds for the antimicrobial activity. Furthermore, the reduction in activity of the wrapped samples may be as a result of thermal energy from sunlight during irradiation as the sample was shielded from the sunlight irradiation. This

Table 2. Zones of inhibition of the methanolic fraction of *Z. zanthoxyloides* in different mediums against some microorganisms after tungsten lamp irradiation.

Sample	Zones of inhibition of the microorganisms (mm)							
	P. aeruginosa		*E. coli*		*B. subtilis*		*S. aureus*	
	Mean	p value	Mean	p value	Mean	p value	Mean	p value
DET	14.65 ± 5.0		13.71 ± 3.7		15.08 ± 6.0		14.48 ± 1.8	
DWT	17.64 ± 3.9	0.29	14.35 ± 1.9	0.068	15.51 ± 6.1	0.42	18.38 ±6.3	0.41
UMC	20.94 ± 4.4		19.94 ± 2.7		20.77 ±4.1		18.27 ± 0.2	
MET	18.11 ± 8.6		11.18 ± 0.1		16.67 ± 6.4		12.84 ± 0.2	
MWT	16.52 ± 6.1	0.72	12.94 ± 0.7	0.014*	16.76 ± 6.7	0.64	15.55 ± 3.9	0.046^
UMC	20.9 4± 4.4		19.94 ± 2.7		20.77 ± 4.1		18.27 ± 0.2	
AET	0.0		0.0		14.9 ± 4.9		11.41 ± 0.5	
AWT	0.0		0.0		15.3 ± 4.9	0.29	12.36 ± 0.2	0.001*
UAC	16.9 ± 1.0		15.8± 2.4		18.72 ± 1.6		16.21 ± 0.7	

Values are means of three determinations ±S.D.
- Significant (ANOVA), ^ - MET Vs UMC, (Paired t-test)
DET - Dry, irradiated with tungsten lamp, DWT - Dry, wrapped irradiated with tungsten lamp, MET - Methanolic solution irradiated with tungsten lamp, MCT - Methanolic solution wrapped irradiated with tungsten lamp, AET - Aqueous solution irradiated with tungsten lamp, AWT - Aqueous solution wrapped, irradiated with tungsten lamp, UMC- Unirradiated sample in methanol, UAC- Unirradiated sample in water.

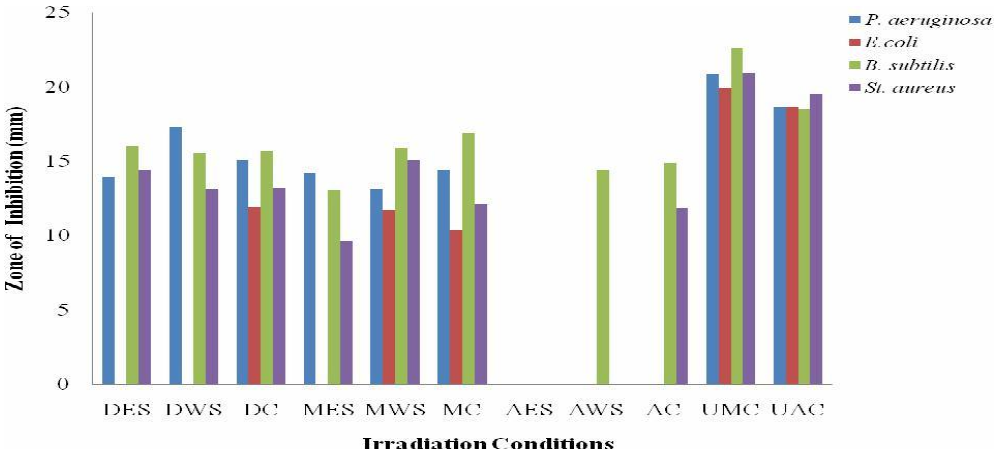

Figure 1. Zones of inhibition produced by sunlight irradiated antimicrobial fraction (methanolic extract) of *Z. zanthoxyloides* against some microorganisms.
DES – Dry, irradiated with sunlight, DWS - Dry, wrapped irradiated with sunlight, MES - Methanolic solution irradiated with sunlight, MWS - Methanolic solution wrapped irradiated with sunlight, AES - Aqueous solution irradiated with sunlight, AWS - Aqueous solution covered, irradiated with sunlight, UMC- Unirradiated sample in methanol, UAC- Unirradiated sample in water.

indicates that, the extract is not stable to heat. A similar trend was observed with aqueous and methanolic samples irradiated under tungsten lamp. The activity of the aqueous samples against *E. coli* and *P. aeruginosa* was totally lost after tungsten lamp irradiation (72 h), while a significant reduction in activity was observed on *S. aureus* ($p < 0.01$) and a non-significant reduction in activity was observed on *B. subtilis* ($p = 0.29$), (Table 2).

Photoreactor irradiation is an accelerated light stability evaluation procedure. A total loss of activity was observed for photoreactor irradiated aqueous solutions against all the microorganisms while, a significant reduction in activity was observed with the irradiated methanolic solution against all the microorganisms ($p < 0.01$, t-test) (Figure 1).

The results obtained in this study indicate that, photo-

chemical degradation has occurred in all the samples with resultant effect of reduction or loss of activity. The significant reduction in the antimicrobial activity of the extracts of *Z. zanthoxyloides*, even in dark cupboard shows the need for appropriate storage conditions. The stability of herbal medicines can be affected by the chemical structure of the component molecules which would result in reactions such as: redox reactions, hydrolysis, isomerizations, condensations and polymerizations. These reactions are induced in the presence of heavy metals, enzymes or the influence of light and oxygen (Olaniyi, 2000; Harnischfeger, 2005). Herbal medicines from *Andrographis paniculata*, widely used in Asian countries, were found to degrade photochemically (Luelak et al., 2003). Chinese medications like *Anthemis nobilis, Rhus toxicodendron* and *Tagetes patula* have also been reported to cause photoinduced contact dermatitis (Niggermann and Gruber, 2003).

Furthermore, light was reported to accelerate the degradation of hypericin and pseudohypericin in the extract solution of *Hypericum perforatum,* resulting in significant reduction in hypericin content of the extract (Wirz et al., 2001). Similarly, the amount of phenolic anthraquinone in Aloe vera sap was reported to be significantly decreased after irradiation of the Aloe vera sap in sunlight or ultraviolet irradiation (Rajendran et al., 2007). Some members of the Rutaceae family to which *Z. zanthoxyloides* belong have been reported to show phototoxic reactions such as dermatitis which is a sign of photodegradation reaction (Zafiropoulo et al., 1968).

Photochemical degradation may arise as a result of improper or inadequate storage and distribution of the products leading to their photo deterioration and chemical decomposition resulting in reduced bioactivity (Kerr, 2002). The colour and characteristics of a product usually changes after photodegradation. This is more common with liquid preparations but for solid products polymeric products are formed instead.

Photodegradation is a major form of instability not only for orthodox medicines but also for herbal products. In the case of herbal preparations the situation is further complicated due to the multi-component nature of the products and the presence of unknown chemical structures (Gafner and Bergeron, 2005; Foster et al., 2005; Adewumi and Ojewole, 2004).

Conclusion

This study revealed the reduction or loss of activity by methanol extracts of Z. *zanthoxyloide* after irradiation with different sources of radiations. This has revealed the photochemical instability nature of the methanol extracts of Z. *zanthoxyloide.* The implication of this is that, herbal products or chemical drugs formulated with components from this plant needs to be adequately stored or a compromise of quality may occur as a result of photo deterioration. There is therefore, the need to educate traditional medicine practitioners on the need to protect their herbal products from direct sunlight as most of them expose their products in open markets in an attempt to attract customers.

Further studies on the implication of the photo irradiation on herbal preparations containing *Z. zanthoxyloides* is in progress.

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