



ANTIMICROBIAL SCREENING AND STABILITY STUDIES OF THE CRUDE EXTRACT OF *JATROPHA CURCAS* LINN LATEX (EUPHORBIACEAE)

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ABSTRACT

A preliminary antimicrobial screening of the latex of *Jatropha curcas* was carried out. The stability of the liquid, dried (powdered) latex and ethyl acetate extract (EAE) was also studied. The latex showed a broad spectrum of antimicrobial activity against *Bacillus subtilis*. NCTC 8326, *Escherichia coli* NCTC 10418, *Pseudomonas aeruginosa* NCTC 6750, *Staphylococcus aureus* NCTC 6571, *Streptococcus pyogenes* NCTC 8198, *Candida albicans*. NCTC 3151A and clinical isolates of *Trichophyton* sp., using agar and broth dilution methods. The diameter of zones of inhibition ranged between 20 to 26 mm, when the activity of the latex was compared with that of established antibiotics, it was shown that the latex seemed to be more active. The stability studies were carried out over 14 weeks under different storage conditions. The I.R. spectra of all the samples were taken at two weekly intervals for four weeks to elucidate the effect of light and moisture. It was also found that exposure to light decrease the antimicrobial activity with decrease in the diameter of zone of inhibition. The decrease was as high as 10 mm over the period of 14 weeks of storage. The liquid latex completely lost its activity over 48 hours. The pH value of latex and EAE also increased with time from 3.1 to 5.0 with accompanying decrease in aqueous solubility. The implications of these findings in the use of *Jatropha curcas* in traditional medicine are discussed.

Key words – Latex, dermatophyte, preservative, photolabile, antimicrobial

INTRODUCTION

Jatropha curcas Linn is commonly grown as hedges and fences around gardens and households in Northern Nigeria. It has been documented to have medicinal uses for human and veterinary purposes (Irvine, 1961). The latex combined with the powdered leaves is applied to sluggish wounds while when formulated as enema it is used for the treatment of gonorrhoea (Irvine, 1961). It is also used as an antiseptic against cuts and wounds. The healing effect of curcain a proteolytic enzyme from the latex on wound has been demonstrated (Nath and Dutta, 1992).

However, one of the major problems of natural products is instability. Stability of an active substance is the capacity of that substance in a specific container/closure system to remain within its physical, chemical, microbiological and toxicological specification (Linter, 1975). Instability of active ingredients could result from interaction between active and inactive components, enzymatic degradation, environmental condition which include air (O₂ and CO₂), light, heat, water (hydrolysis) and duration of time between storage and usage. Degradation of products due to any of the above could result in decrease in therapeutic

activity of product and appearance of toxic substances as by-products of degradative reaction.

An active product can be affected chemically by radiation of a particular wavelength only if it absorbs radiation of that wavelength and the energy exceeds a threshold. Ultraviolet radiation is said to be the cause of many degradative reactions (Linter, 1975).

An increase in temperature can also cause an increase in the rate of chemical reaction. This is true for all active products and can lead to deterioration of some thermolabile products.

In natural products, enzymatic degradation also plays a significant role in the stability of such products (Ferdinand, 1976). Changes in the physico-chemical nature of active molecules normally accompany degradative changes e.g. pH, colour e.t.c

Jatropha curcas latex has been reported to have strong antimicrobial activities (Oyi *et al.* 2002), but is rather unstable leading to loss of activity with time. So this work was designed to determine the spectrum of antimicrobial activity, compare with established agents, look into the source of the instability of the latex and proffer solutions to it.

MATERIALS AND METHODS

Extraction

The liquid exudates from the cut stalk of leaves and young stem were collected into amber-coloured bottles from April to October and stored at 4⁰C until needed for use. To obtain the powdered latex, it was spread in thin layers over clean glass sheets and kept in a dark cupboard to dry overnight (Irvine, 1961). The dried latex was subsequently scrapped off the glass sheet with a sharp razor blade. This was pulverized and packed in amber coloured bottles.

A known quantity of the fresh latex (50 mls) was successively extracted with 100mls each of petroleum ether, chloroform, ethyl acetate, methanol and water. The solvents of the filtrates were distilled off with a rotary evaporator.

Protection with Antioxidant and Preservative

The freshly collected samples of latex were protected separately with 0.1%w/v sodium benzoate and 0.1%w/v sodium metabisulphite and then stored at 4⁰C. The antibacterial activities of both were compared to that of the unprotected latex stored at 4⁰C and at room temperature of 28⁰C to 32⁰ C.

Susceptibility Testing

Sterile nutrient agar plates were inoculated with the diluted cultures of the test organisms (10³ cfu/ml) by flooding and excess discarded (Anderson, 1970). The plates were dried for 10 mins in the incubator. Using No. 4 (8 mm) sterile cork borer, holes were bored on the plates and different concentrations of the latex or extract (0.1 ml) for the liquid latex, 20 mg for dried latex and Ethyl acetate extract (EAE) were applied to different holes. One hour pre-diffusion time was allowed after which the plates were incubated at 37⁰C for 18-24 hours. After the incubation, the diameter of zones of inhibition was measured to the nearest millimeter. For fungi incubation was at 25⁰ C for 5-7 days. For comparative purpose the activities of some antibiotics were also tested. The mean value of three experiments was taken as the reading.

Determination of some physical changes of the extract on storage

The pH of the aqueous solution of the latex and EAE were taken at different time intervals. A set of 10mg/ml solutions were prepared and used for pH determinations. The pH meter was zeroed using standard buffer solution before analyzing the latex and the

extract solutions. To determine the solubility, 250 mg each of latex and EAE was added to 5 ml of distilled water in a 25 ml conical flask. Dissolution was effected by turning the flask continuously for 15 min. shaking was avoided to prevent excessive foaming of the products. The mixture was filtered through a pre-weighed whatman No. I filter paper. The residue and filter paper were dried at room temperature (to constant weight). The difference in weight between the filter paper with residue and without residue was taken as the weight of material that did not dissolve.

Infra-red (IR) Spectrophotometric Studies

The IR spectra of the samples of EAE were taken for four weeks to elucidate the effects of light and moisture.

The study was carried out at the National Research Institute of Chemical Technology (NARICT) Bassawa, Zaria.

RESULT

The latex has a broad spectrum of antimicrobial activity, because it shows inhibitory activity against Gram positive, Gram negative bacteria and fungi (Table 1), but the activity is higher on bacteria compared to the fungi.

Table 1: Activity of fixed inhibitory concentrations of Latex of *Jatropha curcas* Linn against 10³ cells or spores/ml of Test organisms

Test organisms	Zone of inhibition (mm)
<i>Ps. aeruginosa</i>	25
<i>B. subtilis</i>	26
<i>C. albicans</i>	24
<i>E. coli</i>	24
<i>Strept. pyogenes</i>	25
<i>Staph. aureus</i>	21
<i>Trichopyton sp.</i>	20

A comparative study of the inhibitory activity of the latex with some commonly prescribed

antibiotics also showed a comparative activity (Table 2).

Table 2: Comparative Inhibitory activities of the fixed concentrations of Latex with commonly prescribed antibiotics

Agents	Zone of Inhibition (mm)						
	<i>E. coli</i>	<i>Ps. aeruginosa</i>	<i>Strept. pyogenes</i>	<i>B. subtilis</i>	<i>Staph. aureus</i>	<i>C. albicans</i>	<i>Tricophyton sp.</i>
Latex	24	25	25	26	21	24	20
Tetracycline 30ug	8	20	16	8	16	8	NT
Gentamicin 10ug	20	19	15	18	23	19	NT
Pen.G 10 I.U	8	10	8	8	16	13	NT
Procaine Pen 10 I.U	NT	10	8	8	16	13	NT
Fluconazole 10 mg	NT	NT	NT	NT	NT	NT	40
Griseofulvin 10 m	NT	NT	NT	NT	NT	NT	19

*Note: Diameter of cork borer is 8 mm
All readings are mean of three experiments
NT – Not tested*

There was slight difference in the diameter of zones of inhibition produced by the protected

and unprotected latex (Table 3). These differences were found to be statistically insignificant at 99.05% confidence level.

Table 3: Effect of protection with 0.1% w/v sodium metabisulphite and 0.1% w/v sodium benzoate on the antibacterial activity of latex

Period of Storage (weeks)	Organism with zone of inhibition (mm)														
	E.coli			Ps .aeruginosa			Stept. pyogenes			S.aureus			B. subtilis		
	SM	C	SB	SM	C	SB	SM	C	SB	SM	C	SB	SM	C	SB
1	23	21	22	24	24	25	24	22	24	24	22	23	24	21	25
2	23	20	20	24	21	23	24	20	24	24	22	22	24	19	22
3	23	19	19	23	18	23	23	18	23	21	19	20	23	16	21
4	19	16	16	20	16	20	21	NI	20	21	18	20	21	15	18
5	17	14	14	19	13	19	20	NI	17	19	17	17	18	10	17

Control: 0.2ml of 0.1%^{w/v} of sodium benzoate had no activity on all the organisms.

0.2ml of 0.1%^{w/v} of sodium metabisulphite had no activity on all the organisms

Key:

- a. SM = Latex with sodium metabisulphite (0.1%^{w/v})
- b. SB = Latex with sodium benzoate (0.1%^{w/v})
- c. C = Unpreserved Latex
- d. NI = No Inhibition

There was general reduction in the activity of latex and EAE with time, however, this reduction is more pronounced within the first two weeks of storage. The liquid latex lost all its activity within a week of storage while the dried latex retained its activity for up to 14 weeks period of study.

The aqueous solubilities of latex and EAE were found to be affected on storage. The solubilities decreased while the pH value increased. Table 4 gives details of the loss of activity with time under varying storage conditions.

Table 4: Susceptibility of *E. coli* and *Staph aureus* to 30mg of the latex and EAE under different storage conditions and time

Duration (weeks)	Diameter of zone of Inhibition (mm)											
	<i>E. coli</i>						<i>Staph. Aureus</i>					
	A1	A2	B1	B2	C1	C2	A1	A2	B1	B2	C1	C2
0	38	38	36	36	38	36	32	32	24	24	32	34
1	30	33	30	29	34	32	26	28	30	29	28	32
2	23	30	21	26	33	30	24	28	27	28	28	28
4	22	26	20	25	33	28	24	26	25	27	28	27
6	21	26	20	24	28	27	24	26	23	25	28	27
10	21	25	20	24	28	27	24	26	23	25	28	25
12	20	25	18	23	28	26	23	26	22	24	28	25
14	20	25	18	23	28	26	23	26	22	24	28	25

Key:

- AI = EAE in colourless bottle;
- BI = Whole latex in colourless bottle;
- CI = Desiccated EAE in coloured bottle;
- A2 = EAE in coloured bottle
- B2 = Whole latex in coloured bottle.
- C2 = Desiccated whole latex in coloured bottle

However latex is more soluble than EAE (Table 5). The latex dried at room temperature (28 – 32° C), 50° C and 100° C were found to

have different pH values. With increase in temperature, the pH values increased (Table 6) and solubilities decreased.

Table 5: Solubilities and pH Values of Latex and EAE with Time

Substance and colour	Period of Storage (weeks)			14 weeks		
	pH	Colour	Solubility (mg/ml)	pH	Colour	Solubility (mg/ml)
A1	4.1	off-white	22	5.1	brownish-red	11
A2	4.1	off-white	22	4.6	brownish-red	15
B1	3.1	off-white	30	4.9	brownish-red	20
B2	3.1	off-white	30	4.3	brownish-red	25
C1	4.1	off-white	22	4.4	brownish-red	18
C2	3.1	off-white	30	4.0	brownish-red	27

KEY:

- A1 - EAE in colourless bottle
- A2 - EAE in coloured bottle
- B1 - Whole latex in colourless bottle
- B2 - Whole latex in coloured bottle
- C1 - Desiccated EAE in coloured bottle
- C2 - Desiccated whole latex in coloured bottle

Table 6: Effect of temperature on pH and solubility of latex

Temperature (°C)	pH	Solubility (mg/ml)
28 – 32	3.4	28
50	4.0	25
100	4.7	13

Table 7: IR peaks of samples A1, A2 and C_I after two and four weeks of storage

Sample	Peak at 2 weeks (cm-1)	Peak at 4 weeks (cm-1)	Tentative assignments
A1	3282	3265	-C-H stretch
	1461	1455	-C-H stretch
	1377	1376	-C-H stretch
	1174	1170	-C-H stretch
		1612	-C=C stretch
A2	3323	3257	-O-H stretch
	1455	1461	-C-H stretch
	1167	1377	-C-H stretch
		1612	-C=C stretch
C1	3323	3257	-O-H stretch
	1459	1464	-C-H stretch
	1158	1111	-C-H stretch

- A1 - EAE in colourless bottle
- A2 - EAE in coloured bottle
- C1 - Desiccated EAE in coloured bottle

DISCUSSION

The latex showed a good antimicrobial activity against all the tested organisms. The activity of the latex against both Gram negative and Gram positive bacteria is comparable; showing its broad spectrum of activity. The latex also showed a very good activity against *Candida albicans* and *Tricophyton* sp. The antimicrobial activities of the latex could be due to the presence of secondary metabolites such as tannins, flavonoids and saponins which have been confirmed to be present (Levens *et al.*, 1979).

Tannins coagulate the cell wall proteins resulting in bactericidal activity in high concentrations, while saponins are surface – active agents; they alter the permeability of the cell wall thus facilitating the entry of toxic materials or leakages of vital constituents from the cell. Flavonoids are phenolic in nature, they act as cytoplasmic poisons. They inhibit the activity of enzymes (Iwu *et al.*, 1990; Pathak *et al.*, 1991). The antibacterial activity of the latex seems to be as a result of the combined effects of the metabolites. The addition of sodium metabisulphite and sodium benzoate was intended to take care of oxidation and microbial contamination while storage at low temperature was to retard the rate of deterioration. The activity of the latex reduced with time in both protected and unprotected cases (Table 3), a statistical analysis of the data revealed that these differences in activity were not significant at $P < 0.05\%$. indicating that oxidation and heat may not be the only degradative pathways involved in the degradation of the latex, other mechanisms are involved.

The liquid latex lost all its activity within a week, while the dried latex maintained its activity for up to fourteen weeks period of study. This indicates that hydrolysis plays a major role in the degradation of the latex. The main target classes of hydrolytic reactions are the amides,

esters and lactams (Florence and Attwood, 1981), suggesting that these compounds may be the main components of the latex.

The dried latex lost its activity gradually on exposure to light (Table 4) suggesting also that the active components of the extract are also susceptible to photochemical degradation. The aqueous solubility of samples AI, A2 and C2 were also found to be affected by deterioration. The solubility decreased with increase in pH values with the development of colour changes (Table 4). The application of heat to dry the latex also produced physicochemical changes in the nature of the latex (Table 6). Changes in the chemical nature of substances are usually accompanied by physical changes as evidenced by the changes in pH, colour and solubility.

The antibacterial activity reduces with time of storage (Table 4) confirming that the initial compounds with acidic pH are more active than those with high pH value (pH changed from 3.1 to 5.1)

The IR spectrum of EAE indicated the presence of aromatic phenolic compounds and phenolic compounds are known to generally behave as acids (Gisvold, 1977) with high antimicrobial activity. The biological activities of phenolic compounds are greatly affected by pH. At low pH the compound remains in the unionized form, which is more lipid soluble than the ionized form and can gain more entry through cell membranes.

Phenolics are susceptible to attack by oxygen of the air and by oxidizing agents, which converts them to the corresponding quinones (Gisvold, 1977). The degradation of the latex by hydrolysis and heat may suggest that the main active components of the latex are phenolic in nature, undergoing hydrolysis and oxidation in the presence of unsuitable conditions.

A close examination of the IR spectra of the samples indicates that new peaks which were not present in the initial samples appeared on storage of the samples e.g new

peaks at Cm-1 1461 and 1377 were observed after two weeks and at Cm-1 1455 and 1376 after four weeks of storage. While for sample A2 new peak at Cm-1455 after two weeks and Cm-1 1461 and 1377 after four weeks (Table 7). For sample C₁ at 1459 and 1464 after 2 weeks and four weeks respectively. Although spectral changes were observed in all the samples, C₁ retained the closest features to the original EAE spectra. This suggests that all the samples are undergoing degradative changes but at different rates. Between four and fourteen weeks of storage the active compounds in the sample seem to have stabilized. Therefore, giving more uniform antibacterial activities (Table 4). Enzymatic activities may also have been taking place in the powdered sample as the latex has been confirmed to contain some enzymes (Nath and Dutta, 1992, Auvin *et al.*, 1977). Enzymes are often present in natural products and active even in solid state and the presence of moisture accelerates their activity. It can be concluded that the latex and ethyl acetate extract of *Jatropha curcas* are highly unstable under ambient storage condition and that the antimicrobial activity of these products decrease with time. The instability of these products is catalyzed or accelerated by factors like moisture, temperature and light. For a stable product to be obtained, these factors must be excluded. Traditional healers using this product must come to term with the fact that this product is very unstable, particularly those in aqueous solution and efforts must be made to find solutions to the instability of this product which inspite of its unstatic potency still find a good use in traditional medicine.

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