Journal of Pharmacy and Bioresources
Vol. 2 no. 1, pp. 5-12 (March 2005)

Journal of
PHARMACY AND
BIORESOURCES

Evaluation of *Jatropha curcas* latex ointment formulations *in-vitro* and *in-vivo*

Rukayat A. Oyi[1*], Josiah A. Onaolapo[1] and Kaita A. Haruna[2]

[1]*Department of Pharmaceutics and Pharmaceutical Microbiology,*
[2]*Deparment of Pharmaceutical and Medicinal chemistry, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Nigeria.*

Received 12th December 2004; Revised; Accepted 17th March 2005

Abstract

The dried latex of *Jatropha curcas* was formulated into ointments of 2.5, 5 and 10% $^{w}/_{w}$ concentrations in hydrocarbon base B.P.C, using the fusion and levigation methods. The efficacy of the ointments was evaluated *in-vitro* using the agar diffusion method and cup-plate technique with the following organisms: *Escherichia coli* (N.C.T.C. 10418), *Staphylococcus aureus* (N.C.T.C. 6571), *Microsporum sp., Epidermophyton sp. and Trichophyton sp.*, which were clinical isolates. For the *in-vivo* studies, volunteers with *Tinea* capitis from a primary school were used. The results indicated that the ointment formulations were active both *in-vitro* and *in-vivo*. The activities of the test formulations compared favorably with those from the standard formulations, i.e. Whitfield's ointment B.P. and penicillin ointment B.P.C. The results also indicated that the stability of latex was maintained within the ointment base over the nine months period of storage.

Keywords: *Jatropha curcas*; Latex; Ointment; Formulations; *Tinea capitis*.

Introduction

Jatropha curcas latex has been reported to be useful in the treatment of whitlow, ringworm, eczema, sores and wounds (Williams, 1949; Elewude, 1986). It has also been reported that the fresh latex is caustic on the skin (Irvine, 1961). This may be due to the acidic nature of latex with pH of 3.1 (Oyi *et al.*, 2001). One of the ways by which standard characteristics such as quality, safety, efficacy and reproducibility can be guaranteed with the use of phytomedicines is by formulating them into dosage forms (Elujoba, 1996).

Creams and ointments are the most popular dosage forms for treating skin or external disease conditions. Creams are more elegant than ointments and also possess faster release characteristics. However ointments give greater protection to the skin and active ingredients, which are susceptible to degradation by moisture and air. The use of hydrocarbon bases does limit water loss from the skin (Aulton, 1988).

The aim of this work is to formulate different concentrations of *J. curcas* latex and its ethyl acetate extract (EAE) into ointments and to evaluate these formulations *in-vitro* and *in-vivo*. Ointment formulation was embarked upon to protect the latex and EAE from the effect of moisture which has been

* Corresponding author.
ISSN 0189-8442

found to have deleterious effects on the stability of latex (Oyi *et al.*, 2001).

Experimental

Materials and Methods. Latex of *J. curcas*, EAE of latex, nutrient agar (Oxoid), nutrient broth (Biotec), Sabouraud Dextrose agar (SDA) (Lab. M), Sabouraud Dextrose broth (Biotec), white soft paraffin, liquid paraffin, beeswax, cetostearyl alcohol, hard paraffin and emulsifying ointment were all from BDH Poole-England but purchased from Lanaks Co. Ltd Zaria-Nigeria. Benzoic and salicylic acids were from Hopkins and Williams-Essex. Daily Needs Nig. Limited-Lagos manufactured penicillin ointment B.P.C. The organisms used were: *Escherichia coli* (N.C.T.C 10418); *Staphylococcus aureus* (N.C.T.C 6571); *Microsporum sp.*; *Epidermophyton* and *Trichophyton sp.* (the last three were clinical isolates)

Authentication of plant The plant used for this study was collected from area 2 junior staff quarters of Ahmadu Bello University, Zaria-Nigeria. Samples of the leaves, stem and roots were collected and taken to the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria where the plant was authenticated (herbarium no. 1911).

Collection and Drying of Latex. After authentication, the latex was collected by plucking the stalks of the leaves, or cutting the young stems, and the liquid exudate, which came out in drops were collected into an amber coloured bottle and carried in an ice pack to the laboratory. To obtain the powdered latex, the liquid latex was spread in thin layers on clean glass sheets and kept in a dark cupboard to dry overnight (Irvine, 1961). The dried latex was subsequently scrapped off the glass sheets with a razor blade, pulverized and packed into an amber coloured bottle.

Extraction with ethyl acetate. Different organic solvents were passed through a sample of latex in order of increasing polarity to extract varying components using a separatory funnel (Fig. 1).The extracts were concentrated using a rotary evaporator.

Isolation of dermatophytes. Pupils with ringworm of the scalp (*Tinea capitis*) at Saidu primary school, Samaru-Zaria, Nigeria, were used as the source of the organisms. Twenty children aged 8-11 years (male and female) were identified from different classes. The affected area was first swabbed with 70% $^{v}/_{v}$ ethanol using cotton wool. The swabbed area was scrapped with razor blade and sterile cotton was used to swab the area into sterile normal saline in McCartney bottles. These were properly labelled and taken to the laboratory in an ice pack. The cotton swabs were subsequently used to swab the surfaces of SDA plates prepared as follows:-

The SDA used for the isolation of dermatophytes was prepared by incorporating 40,000 units of streptomycin, 20,000 units of procaine penicillin and 0.5g of cycloheximide per litre. This is to prevent the growth of bacteria and non-pathogenic fungi (British Pharmacopoeia, 1988).The plates were subsequently incubated at 25^0C for 5-7 days.The organisms isolated were identified under the microscope using their morphologies (Cruickshank *et al.*, 1973; Thomas, 1988).They were stored on agar slants at 4^0C till needed for use.

Bacterial cultures and inoculum preparation. All the standard cultures obtained were purified by the streak-plate method of culture purification (Hugo and Russell, 1988). The pure cultures were subsequently stored on slants. From the organisms stored on slants, overnight cultures in nutrient broth were prepared when needed. The dilution of culture from broth was 1:1000 for Gram positive bacteria and fungi while 1:5000 dilution were made for Gram negative organisms in normal saline to obtain about 10^6 c.f.u./ ml for bacteria and 10^6 c.f.u/ml for fungi (Anderson, 1970).

Formulation of ointments. The calculated quantities of ingredients (Table 1) for preparing the ointments were weighed and melted together in an evaporating dish. The molten base was cooled down to 40^{0}C before the incorporation of latex or EAE powder (75um fraction). This was stirred into the molten base until ointment solidified. The Whitfield's ointment was prepared by levigating the salicylic and benzoic acids into the emulsifying base (Table 2).

Test for activity of ointment formulations

In-vitro testing. The release of incorporated medicaments was tested in-vitro by the agar diffusion method using the cup plate technique (Rawlin, 1977). Twenty milliliters of molten agar was poured into sterile plates and allowed to solidify. A diluted solution of overnight culture in normal saline was used to flood the surface of the plates ,dried in the incubator before boring holes using sterile No. 4 cork borer .One gram of the ointment was melted and mixed with one gram of liquid paraffin to reduce viscosity and enhance diffusibility. The 1:1 mixture of ointment and liquid paraffin was poured into the holes (0.2ml) and left at room temperature for two hours pre-diffusion, after which the plates were incubated at 37°C for bacteria and 25°C for fungi. The zones of inhibition were measured after 48hours for bacteria and 5-7 days for fungi. A control using only the ointment base was set up alongside the main experiments. These tests were repeated at intervals of three months for nine months.

In-vivo testing -/ clinico-pathological studies. Children with ringworm of the scalp from whom the dermatophytes used for susceptibility testing were obtained, were selected for this study. They had their heads shaved before the application of ointment by massaging mornings and evenings everyday for a period of sixteen weeks. Five pupils were placed on latex ointment treatment, while five others were placed on Whitfield's ointment as it is the commonest drug used for ringworm in this area. Inspections of pupils were carried out at weekly intervals. Four out of these ten cases are presented in figures 2-5. After the study, scalps of the volunteers were re-sampled for fungal isolates.

Results and Discussion

Ointment formulation. The formulated ointments retained their physical properties (colour and consistency) throughout the period of study. The 5% $^{w}/_{w}$ latex ointment was used for antimicrobial screenings because it produced zones of inhibition which were similar to those produced by Whitfield's and penicillin ointment (Table 3).

In-vitro, testing of ointment formulation. The results of these tests indicated that the ointments maintained their activity throughout the nine months period of study. The zones of inhibition obtained with the organisms indicated no loss in the activity of latex and EAE ointments (Table 4). No significant differences were obtained on statistical analysis ($p<0.05$).

In-vivo evaluation

P_1 - This is an eight-year-old pupil infected with *Microsporum* and *Trichophyton* species. He was placed on Latex ointment treatment. The initial bald circular patches on his head improved gradually as evidenced by the increase in hair density within the affected areas with time (Fig. 2).

P_2 - This is also an eight-year-old boy with infection of *Microsporum, Trichrophyton* and *Epidermophyton* species. He responded well to the latex ointment (Fig. 3)

P3 - This is a nine-year-old boy who had *Microsprum, Trichophyton* and *Epidermophyton* isolated from his scalp. He responded slowly to Whitfield's ointment. (Fig. 4).

P4 - This is an eleven-year-old boy with *Microsporum* and *Epidermophyton*

infections. He also responded slowly to Whitfield's ointment (Fig.5).

In all cases (P1 -P4) complete recrudescence was obtained after sixteen weeks of treatment. Subsequent scrapping of their scalps revealed the absence of these organisms.

Skin diseases are treated with drugs dispersed or dissolved in ointment or cream bases. A hydrocarbon ointment base was chosen for formulating the latex EAE in order to protect these products from the effects of moisture and light. These were previously found to have deleterious effects on the stability of latex and its extracts (Oyi *et al.*, 2001).

The results of the antimicrobial screening of the ointments confirms the release of the active products from the ointment base used; but the activity obtained with the 2.5%$^{w}/_{w}$ ointments were less than that of 5%$^{w}/_{w}$ which was similar to the 10%$^{w}/_{w}$ formulations. An increase in the concentration of active agents did not give an expected corresponding higher activity. This could be due to low diffusibility of active agents from ointment base.

Table-1 : Quantities of ingredients for preparing 200g of ointment.

Ingredients	Formulation/ Quantities (g)	
	1	2
White soft paraffin	171	171
Beeswax	3.8	3.8
Cetostearyl alcohol	9.5	9.5
Hard paraffin	5.7	5.7
EAE	-	10.0
Whole latex(WL)	10.0	-

1-WL ointment 5% $^{w}/_{w}$; 2-EAE ointment 5% $^{w}/_{w}$

Table 2-Formula for Whitfield's ointment B.P.C

Ingredients	B.P.C Quantities(g)	Quantities used(g)
Benzoic acid	60	12
Salicylic acid	30	6
Emulsifying ointment	910	182
TOTAL	1000	200

Table 3- Results of antimicrobial screening of latex, EAE, penicillin and Whitfield's ointments.

Ointment type	Organisms with zones of inhibition(mm)				
	EC	SA	MS	TS	ES
Latex 2.5% $^{w}/_{w}$	11	12	10	11	13
5.0%	19	22	18	16	16
10.0%	20	21	19	15	16
EAE 2.5%	14	15	0	0	0
5.0%	17	21	0	0	0
10.0%	16	22	0	0	0
Penicillin	20	18	0	0	0
Whitfield's	NT	NT	22	18	18
Ointment base	0	0	0	0	0

EC- *Escherichia coli;* SA- *Staphylococcus aureus;* MS- *Microsporum* species ; TS- *Trichophyton* species; ES- *Epidermophyton* species

Table 4 - Assessment of the antimicrobial activity of ointment formulation on storage

Time (months)	Ointment type	Organisms with zones of inhibition(mm)				
		EC	SA	MS	TS	ES
0	Base	0	0	0	0	0
	WL	19	22	18	16	16
	Whitfield's	NT	NT	22	18	18
	Penicillin	20	18	0	0	0
	EAE	17	21	0	0	0
3	Base	-	-	-	-	-
	WL	20	21	19	16	17
	Whitfield's	NT	NT	20	18	17
	Penicillin	20	18	NT	NT	NT
	EAE	18	19	NT	NT	NT
6	Base	-	-	-	-	-
	WL	21	22	19	18	16
	Whitfield's	NT	NT	21	17	16
	Penicillin	20	18	NT	NT	NT
	EAE	19	19	NT	NT	NT
9	Base					
	WL	20	21	19	18	16
	Whitfield's	NT	NT	21	17	16
	Penicillin	20	19	NT	NT	NT
	EAE	18	20	NT	NT	NT

NT - Not tested; - = zero inhibition

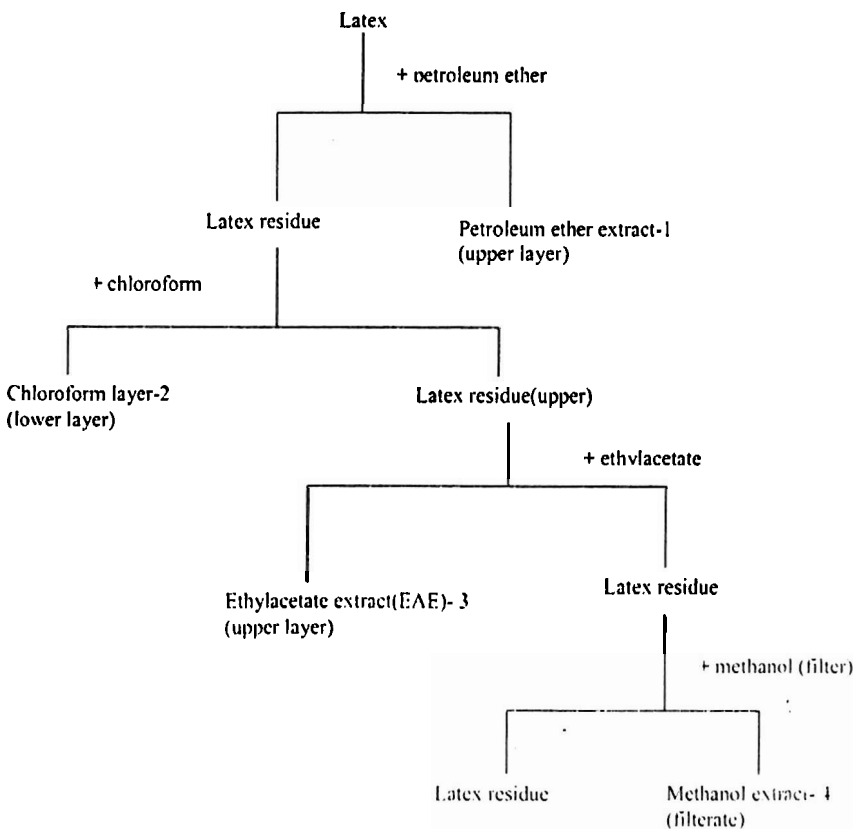
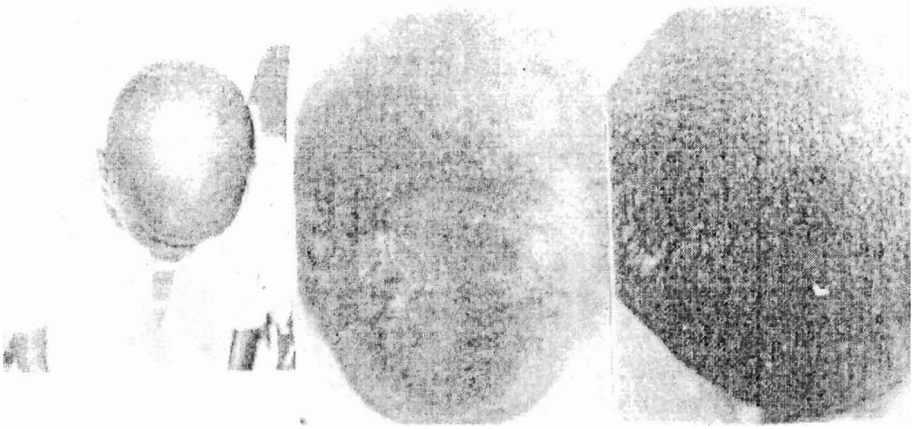
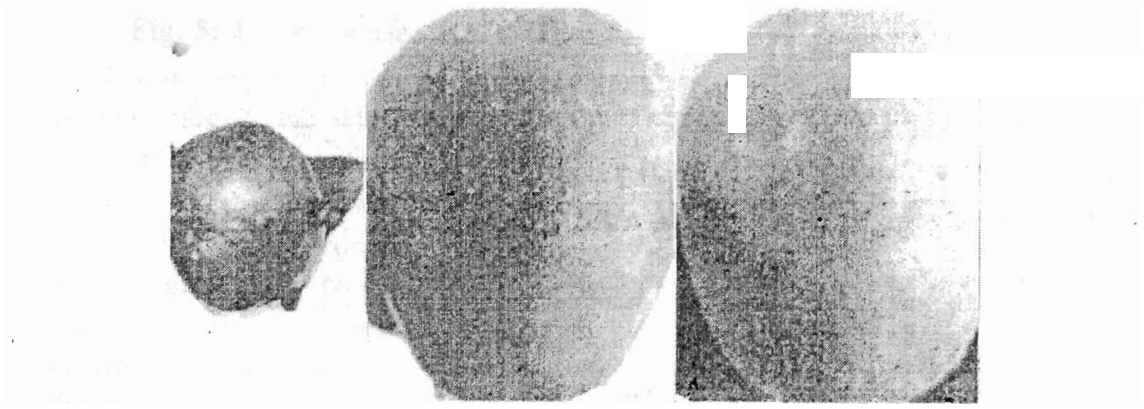


Figure 1- Sequence of organic solvent separation



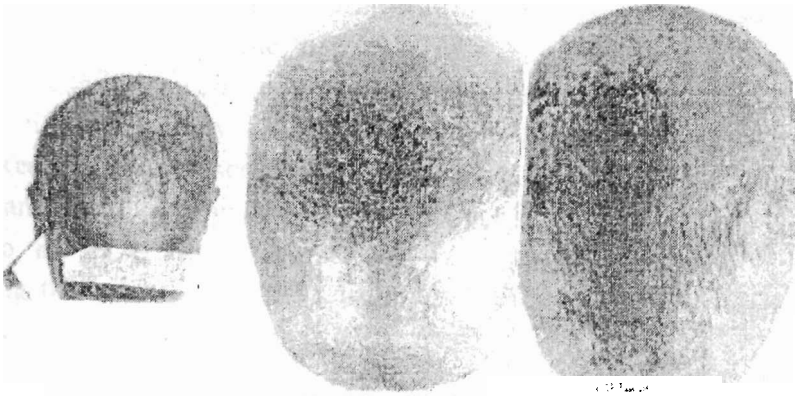
At Zero Week After Five Weeks At Ten Weeks

Fig. 2: The response of P_1 to Treatment



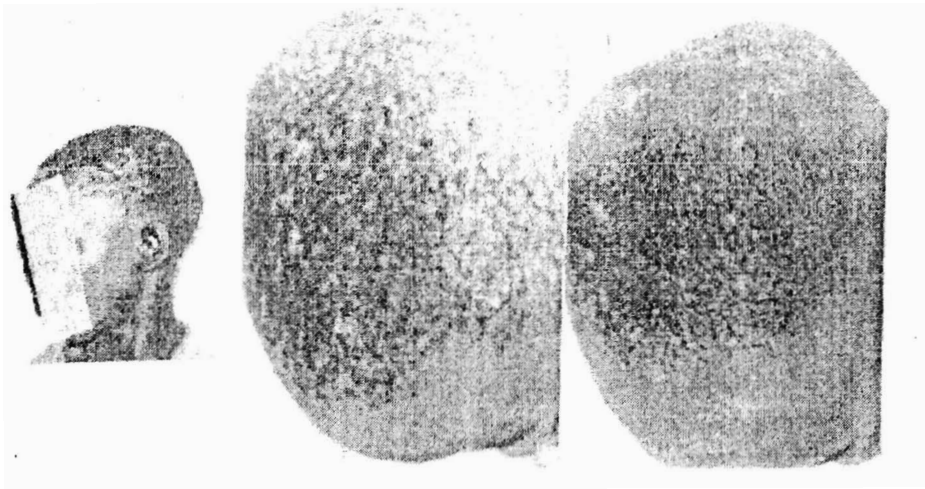
At Zero Week After Five Weeks At Ten Weeks

Fig. 2: The response of P_2 to Treatment



At Zero Week After Five Weeks At Ten Weeks

Fig. 4: The response of P_3 to Treatment



At Zero Week After Five Weeks At Ten Weeks

Fig. 5: The response of P_4 to Treatment

The *in-vitro* antimicrobial activity of the formulations (Table 4) indicates that the ointment is active for up to nine months. This activity is corroborated by the *in-vivo* assessments, where a complete recrudescence was obtained after 16 weeks of ointment treatment. (Fig 1-5). Due to the occlusive nature of hydrocarbon bases water loss from the skin surface is prevented and thus water accumulating underneath the ointment could aid in concentrating the active compounds (mainly phenolic in nature) in which they are soluble. This further aids the penetration of active ingredients through the hair roots to carry out their antifungal effects.

The antifungal activity of the latex had been linked to the presence of tannins, saponins and flavonoids present in the latex (Onaolapo *et al.*, 1998). Tannins act by coagulating the cell wall proteins, saponins are surface active agents which alter the permeability of the cell wall thus facilitating the entry of toxic materials or leakages of vital cell constituents (Adesina *et al.*, 1988).Flavonoids are phenolic in nature and act as cytoplasmic poisons, inhibiting the activity of enzymes (Shebanovaskii, 1971; Iwu *et al.*, 1990). Flavonoids have also been reported to have fungicidal and virucidal activities (Hugo and Russell, 1983; Pathak *et al.*, 1991).

Conclusion

This formulation and evaluation studies of *J. curcas* latex ointments have confirmed the stability of latex in a hydrocarbon base with maintenance of activity. It has also established that 5% $^{w}/_{w}$ latex ointment formulation in a hydrocarbon base elicited a better activity than that obtained from Whitfield's ointment B.P.C.

References

Adesina, S. K., Iranloye, T. A., Olugbade, T. A., Agbedahunsi, J. M., Sonemekun, R. O. and Akinwusi, J.O (1988). Chemical antimicrobial and formulation studies on *Acalypha wilkensiana* Muell Arg (Euphobiaceae). Drug Production for Natural Products. Proceeding of the International Symposium 9-12 October 1988. Book of Abstracts Pp 125.

Anderson. G.T. (1970). "Testing of susceptibility of antimicrobial agents and antimicrobial agents in body fluids". Manual of clinical microbiology by American society for microbiology. First edition. p 299-310.

Aulton M. E (1988) Topical Preparations. In-Pharmaceutics: The science of dosage form design. Edited by M. E. Aulton. Churchill-Livingstone.p318-429.

British Pharmacopoeia (1973), University printing house, Cambridge

Cruickshank R, Dugid J.P, Marmion B.P and Swain R.H. (1973). Medical microbiology. Twelfth edition. ELBS Edinburgh, London. p 10-17 and P549-560.

Elewude J.A.(1986). *Jatropha curcas*-lapalapa funfun: In the state of medicinal plant research in Nigeria. Edited by Abayomi Sofowora. Ibadan University Press-Nigeria. P 47-51.

Elujoba A.A (1996). "Standardization of phytomedicines." Proceedings of an international workshop on commercial production of indigenous plants as phytomedicines and cosmetics. Lagos - Nigeria. p 19.

Hugo W.D and Russell A.D(1983). Pharmaceutical Microbiology, third edition. Blackwell scientific publications. Oxford -London. p 262-266.

Irvine F.R. (1961). Woody plants of Ghana. Published by Oxford University press, London. First edition. p 233-236.

Iwu M.M, Igboko O.A, Okunji C.D and Tempesta M.S. (1990). Inhibition of enzyme activity of aldosoreductase by some flavonoids. *J. Pharm. Pharmacol*:42: p 290-292.

Leven M., Vandan-Berghe D.A, Marten I., Vilienttsrice A. and Lomiveas E.C.(1979).Screening of higher plants for biological activity. *Planta Medica* 36: p 311-312.

Onaolapo J.A., Oyi A.R., Aremu O.K and Bayode E.S. (1998). Preliminary studies on the antimicrobial activities of the latex of Jatropha curcas Linn (family Euphorbiaceae). Paper presented at a conference on: Natural products in drug development, O.A.U, Ile-Ife, Nigeria, 18-21st Oct.1998.Book of abstract p44.

Oyi A.R., Onaolapo J.A. , Haruna A.K. and Morah C.O.(2001). Stability studies of the latex and an extract of *J. curcas*. Journal of medicinal plants (In the press).

Pathak D, Pathak K. and Singla A.K(1991). Inhibition of xanthine-oxidase activity . *Fitoterapia*, 62:371-385.

Rawlin E.A (1977) Bently's textbook of Pharmaceutics, eight edition, Baillere Tindal , London p433-447.

Shebanovaskii L. (1971) "Rast Resus" Gos *Nikitskii Bot. Sad alta*. U.S.S.R.,7,No.1: p133-141.

Thomas C.G.A (1988). Medical Microbiology, sixth edition, ELB. Baillere Tindal, London p80-120.

Williams R. O. (1949). *Jatropha curcas* Linn- In: The useful and ornamental plants of Zanzibar and Pemba p316-319.