Suitability of Stem Latex of *Jatropha Gossypifolia* as a Protein Precipitant for Biochemical Analysis

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Abstract The extract of *Jatropha Gossypifolia* stem was obtained by cutting the stem with a sharp knife and the fluid expressed out. The suitability of the stem latex extract as a precipitant for biochemical analysis was determined. The precipitating efficacy of the extract for creatinine and protein estimation was found to be optimum at 1/4 and 1/5 dilutions respectively aqueous solution. Plasma protein was precipitated with stem extract of *J. Gossypifolia* at the stated dilution. The mean plasma creatinine values obtained from 0.5 % sodium tungstate as a protein precipitant were compared with the values of plasma creatinine obtained when 1/4 dilution of stem extract of *J. Gossypifolia* was used as protein precipitant. Similarly mean cerebrospinal fluid (CSF) and urinary protein values obtained from 3 % Trichloro-acetic acid as protein precipitant were compared with values obtained from 1/5 dilution of stem extract of *J. Gossypifolia* as protein precipitant. The values obtained from the stem latex extract at the stated dilutions were comparable with values obtained from the conventional protein precipitants ($p < 0.05$). The stem latex extract of *J. Gossypifolia* is suitable as a protein precipitant for creatinine, CSF and urinary protein estimations. However further work need to be done to purify the extract and determine the exact concentration at the

stated dilutions as well as the active ingredient in stem latex.

Keywords *Jatropha gossypifolia* · Stem latex · Precipitant · Analysis

Introduction

Precipitation is mostly used for product recovery of biomolecules especially protein and may serve as a reagent in some biochemical analysis. This is usually induced by the addition of salt, organic solvent or by altering the pH of a solution. Binding of water which results in dehydration of biopolymers or molecules is the general mechanism precipitant [1]. *Jatropha gossypifolia*, *Jatropha carcass*, *Jatropha multifida*, *Jatropha podagrica* and *Bridelia ferruginea* all belongs to the family of *eurphorbiaceace* and the order *Geraniale*. Pignut or Fignut is the common name for *J. gossypifolia* while the Yoruba tribe in Nigeria commonly called the plant Lapalapa, the Hausa and Ibo in Nigeria called the plant Binidasugu and Oluluidi respectively [2]. *Jatropha* plants generally is native to Central America but presently cultivated across the world and was brought Africa India by a Portuguese navigator in the 16th century [3].

A *Jatropha* fruit are three-celled with one seed per cell and is the common red species planted around houses with some known therapeutic qualities. A portion of the leaf of *Jatropha gossypifolia* is used for bathing or dressing wounds [4]. The leaf bath is also used for treating sores, sprains, rashes and bewitchment in Latin America and the Carribean [2, 5]. Morton [6] also reported that the poultices of the plant are used for treating sores and pain in Trinidad. The application of the stem sap was reported to stop

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bleeding and itching of the cuts and scratches [6, 7]. In southwest Nigeria the stem latex extract from a crushed fresh plant is routinely used by local people to stop bleeding from fresh wounds. Oduola et al. [5] confirmed the suitability of the leaf extract as anticoagulant for haematological analysis but were unable to establish its coagulant properties.

The precipitating potentials of the stem latex of *Jatropha gossypifolia* was noticed from the coagulum or clot formed when applied to a bleeding sore or wound. This observation formed the basis of this present study aimed at determining the precipitating activity and suitability of the stem latex extract of this plant as a protein precipitant for biochemical analysis.

Materials and Methods

Materials

200 g of fresh stem latex of *Jatropha gossypifolia* was crushed for 5 min and the fresh raw juice was collected by straining the crushed stem latex. The raw juice was centrifuged at 3500 rpm for 10 min and the clear supernatant collected. The minimum concentration that achieved the required optimum analytical precipitation was determined by serial dilution of the extract from neat to 1/10 and each dilution used for precipitating plasma protein for creatinine and urinary protein for creatinine, CSF and urinary protein estimation respectively. The optimum precipitating effect of the stem latex for precipitating plasma protein for creatinine estimation by jaffe's method at 1/4 dilution and at 1/5 dilution for precipitating CSF and urinary protein for their quantitative estimation.

Biochemical Analysis

Creatinine Estimation

This analysis was grouped into 2:

In the first group plasma protein was precipitated by 0.5 ml of 5 % Sodium tungstate ($\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$) and creatinine in the supernatant estimated by conventional Jaffe's method [8].

In the second group, plasma protein was precipitated by 0.5 ml of 1/4 dilution (25 %) of the stem latex extract and creatinine in the supernatant estimated by conventional Jaffe's method [8] except that the stem latex extract was used for plasma protein precipitation.

CSF and Urinary Protein Estimation

The analysis was categorized into 2:

In the first category, 0.3 ml of CSF/urine was added to 3 ml 3 % trichloroacetic acid (TCA) and the protein content determined by method described Baker et al. [9].

In the second category, 3 ml of 3 % TCA in the 1st category was replaced by 3 ml of 1/5 dilution (20 %) of stem latex extract and the protein content in the CSF and urine specimen determined by the methodology described by Baker et al. [9].

Statistics

The mean, standard level of deviation, coefficient of variation and level of significance for the difference between means were computed with SPSS version 15.

Results

See Tables 1, 2 and 3.

Discussion

The mean concentration and coefficient of variation (C.V.) of plasma creatinine estimation obtained by deproteinization with $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ and 25 % of stem latex extract are shown in Table 1. The mean and coefficient of variation of plasma creatinine level obtained by the two

Table 1 Comparison of equality of means and coefficient of variation (C.V.) of plasma creatinine levels between method of deproteinization by $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ and stem latex extract of *Jatropha gossypifolia*

Parameter	$\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$ N = 100	Stem latex extract N = 100	t value	F value	Level of sig.
Creatinine					
Equality of means	164.5 ± 13.5	160.8 ± 14.5	0.259	0.123	$p > 0.05$
Equality of CV	83.2 ± 0.6	87.4 ± 0.8	0.166	0.327	$p > 0.05$

The table shows that there is no statistical significant difference between mean plasma creatinine concentration using the two methods of deproteinization ($p > 0.05$)

Level of significance ($p \leq 0.05$)

Table 2 Comparison of equality of means of urinary and CSF protein concentration determined by 3 % TCA and 20 % (1/5 dilution) of stem latex extract of *Jatropha gossypifolia*

Parameter	3 % TCA <i>n</i> = 100	20 % stem latex extract <i>n</i> = 150	<i>t</i> value	Level of sig.
Urinary protein (mg%)	53.5 ± 5.3	56.7 ± 5.6	0.921	<i>p</i> > 0.05
CSF protein (Mg%)	115.3 ± 14.1	109.3 ± 13.7	0.371	<i>p</i> > 0.05

There is no statistical significant difference in urine and CSF protein values obtained by 3 % TCA and 20 % stem latex extract of *Jatropha gossypifolia* as shown in Table 2 (*p* > 0.05)

Level of significance (*p* ≤ 0.05)

Table 3 Comparison of equality of coefficient of variation (C.V.) of Urinary and CSF protein concentration determined by 3 % TCA and 20 % (1/5 dilution) of stem latex extract of *Jatropha gossypifolia*

Parameter	3 % TCA (<i>n</i> = 100) C.V %	20 % stem latex extract (<i>n</i> = 150) C.V %	<i>F</i> value	Level of sig.
Urine protein	126.2 ± 2.3	125.4 ± 2.1	0.584	<i>p</i> > 0.05
CSF protein	124.2 ± 2.5	123.5 ± 3.5	0.112	<i>p</i> > 0.05

The coefficient of variation of Urinary and CSF protein concentration determined by the two different methods are statistically the same. (*p* > 0.05) as shown in Table 3

Level of significance (*p* ≤ 0.05)

deproteinization agents were comparable (*p* > 0.05). The mean and coefficient of variation of protein in CSF and urine specimen by turbidometry method with 3 % TCA and 20 % stem latex extract as protein precipitant are shown in Tables 2 and 3 respectively. The mean and coefficient of variation of CSF and urine protein level obtained by the two precipitating agents were comparable (*p* > 0.05).

The findings of our study demonstrated the coagulant or precipitating property of stem latex of *Jatropha gossypifolia*. This property could not be demonstrated in the leaf extract of this plant [5]. One of the criteria for selecting a good protein precipitant is that it should quantitatively precipitate [9]. Stem latex of *Jatropha gossypifolia* satisfies this criteria because it quantitatively precipitate protein from plasma used for creatinine estimation. Similarly the protein precipitant obtained when used for CSF and urine analysis was significant enough to be quantified turbidometrically. The precipitating mechanism of stem latex of *Jatropha gossypifolia* appears similar to that of other protein precipitant such as Na₂WO₄·2H₂O and TCA. However the concentration of the active precipitating agent in the stem latex extract of *Jatropha gossypifolia* appears to be very high hence the need for diluting the stem latex extract 1/4(20 %) and 1/5(25 %) for precipitating plasma protein for creatinine estimation and for CSF and urine protein analysis respectively. The study could not explain if the stem latex extract reacted with non creatinine metabolite to produce deeper red tautomer colour (janvoski complex) observed at stage 2 of Jaffe's reaction [8]. To allay this speculation the purified stem latex extract is recommended to be more suitable for protein precipitant for creatinine

estimation by Jaffe's reaction [8]. The active precipitating agent in the stem latex extract has to be extracted and characterized before it can be a better substitute for Na₂WO₄·2H₂O and TCA. We therefore conclude that the stem latex extract of *Jatropha gossypifolia* can be used as a protein precipitant but the active precipitating agent must be isolated, purified and characterized.

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