

Thin Layer Chromatographic Examination of *Croton zambesicus* Muell Arg. Stem Bark.

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Abstract: The stem bark of *Croton zambesicus* Muell Arg. was collected, gabled and pulverized. This was air dried and subjected to gradient extraction with the soxhlet apparatus using petroleum ether, ethylacetate, methanol and distilled water respectively. Finally, the extract fractions were subjected to thin layer chromatographic examination. The gradient extraction of *C.zambesicus* yielded 17.197g of a yellowish oily paste for petroleum ether, 50.56g of a dark brown mass for ethylacetate, 52.023g of brown mass for methanol and 26.4g of brown mass for distilled water extract respectively, while the thin layer chromatographic examination of the extracts yielded 8 discrete spots for petroleum ether extract, 9 spots for ethylacetate extract, 5 spots for methanol extract and 3 spots for the distilled water extract.

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Key words: Thin layer chromatography, *Croton zambesicus*, stem bark.

1. Introduction

Croton zambesicus Muell Arg. (Keay, 1989., Arbonnier, 2004), commonly known as *Koriba* or *Icen maser* in Hausa, *Ajekofole* in Yoruba, *Mfam* in Ekoi languages (Agishi and Shehu, 2004) and *Moramora* in Kilba language (Reuben, *et al*, 2008., 2009abc) belongs to the *Euphorbiaceae* family. It is a shrub of 10-16m high, often branching low down with a spreading crown and characteristic hanging leaves, silvery beneath. The bark is whitish to pale gray, slash thin and yellowish with a strong pharmaceutical smell. Flowering usually at the beginning of the dry season. It is found in Sudan and Guinea savannah zones and distributed from Cameroon to tropical Africa (Arbonnier, 2004).

The leaf decoction of *C. zambesicus* is used in the treatment of urinary tract infection, as antihypertensive and to treat fever associated with malaria. The leaf alkaloidal fraction has also been reported to have broad spectrum antibacterial property (Arbonnier, 2004 and Okokon, *et al*, 2005). Reuben, *et al*. 2008., 2009abc have also reported broad spectrum activity of the methanolic extract, petroleum ether extract, ethylacetate extract and the partition fractions of the crude methanolic extracts, all of the stem bark of *C. zambesicus*. These reports have therefore motivated this research with the aim of carrying out preliminary investigation by thin layer chromatography (TLC) method, for the possible number of compounds present in the stem bark of *C. zambesicus*, since it has these promising reports and the wide application in folk- low medicine.

Thin layer chromatography (TLC) is a technique of separation and identification of chemical substances by taking advantage of their different rates of movement in a suitable moving solvent (mobile

phase) on a thin layer of suitable adsorbent medium (stationary phase) (Smith and Feinberg, 1962, Trease and Evans, 2002).

2. Materials and methods.

Plant material: the plant parts of *Croton zambesicus* muell arg. Was collected in December 2006 at longitude 13°15'E, latitude 10°15' Mubi, Adamawa state Nigeria and was authenticated by a taxonomist, Prof. S. S. Sanusi, of the department of Biological sciences, University of Maiduguri, Nigeria. A voucher specimen with No. 190520081/2 were deposited in the department of biological sciences, university of Maiduguri, Maiduguri Nigeria.

3. Preparation and Extraction

The stem bark of *C. zambesicus* was gabled for removal of adulterants and then pulverized. It was air dried at room temperature and four hundred grams(400) of the pulverized part was exhaustively and sequentially extracted using petroleum ether, ethylacetate, methanol and distilled water respectively with the soxhlet extractor (fig.1) (gradient extraction). The extracts were concentrated *in vacuo*, weighed and properly labeled and stored in the refrigerator at 4°C. until use (Trease and Evns, 2002). All work was carried out in accordance with the general guidelines for methodologies on research and evaluation of traditional medicine (Geidam *et al*. 2007; WHO, 2000).

4.Thin layer chromatography (TLC)

The *C.zambesicus* organic and distilled water fractions were subjected to thin layer chromatographic examination according to the standard proce-

dures (Smith and Feinberg, 1962; Harbon, 1973; Trease and Evans, 2002).

Analytical silica-gel G60 (merck) was dissolved in distilled water and this was smeared on glass plate using the Shandan applicator (spreading jig). This was allowed to dry and activated in an oven at 100-110°C. for 30minutes before use(Smith and Feinberg,1962, Trease and Evans, 2002).

A line was drawn about 3cm. from the bottom of the activated plate and using the capillary tube, a spot of the extracts dissolved in their various solvents were made on the line. The sporting was repeated thrice and at each application, a time interval was allowed for drying of the sport.

The solvent system was constituted in the chromatographic tank and was allowed so that the solvent vapor of the solvent saturates the tank to homogeneity.

The spotted plate was then taken and placed inside the homogenized tank and the lid placed back. This was allowed so that the solvent ascends the plate by capillarity to about three quarter way of the plate and thus causes the chromatographic separation. After this, the chromatogram was removed from the tank and left to dry, after which it was put in another chromatographic tank containing a locating agent (iodine crystals) to locate the spots. After the spots

were indicated, it was removed from the tank and the various distances moved by the spots and the solvent front were measured and recorded. The R_f (rate of flow) values were however calculated using the relation:

$$R_f = \frac{\text{distance moved by spot(cm) from origin}}{\text{distance moved by solvent (solvent front) (cm) from origin}}$$

Smith and Feinberg,1962., Abdul, 1990 and Trease and Evans, 2002).

5. Result

The result of extraction of the crude extracts of *Croton zambesicus* Muell Arg. Stem bark is presented in fig.1. The methanol soluble fraction of the extracts is more in quantity (52.023g), followed by ethylacetate fraction (50.56g), then distilled water fraction (26.4g) and lastly petroleum ether fraction (17.197g) respectively. The thin layer chromatographic examination of the extracts is presented on tables 1a&1b. These reveals the number of discrete spots, i.e. 8, 9, 5 and 3 spots for petroleum ether, ethylacetate, methanol and distilled water extracts respectively. The distances traveled by each spot and R_f values of the constituent compounds or spots are also presented here on the same table.

Croton zambesicus Muell Arg. Dried powdered stem bark (400g)

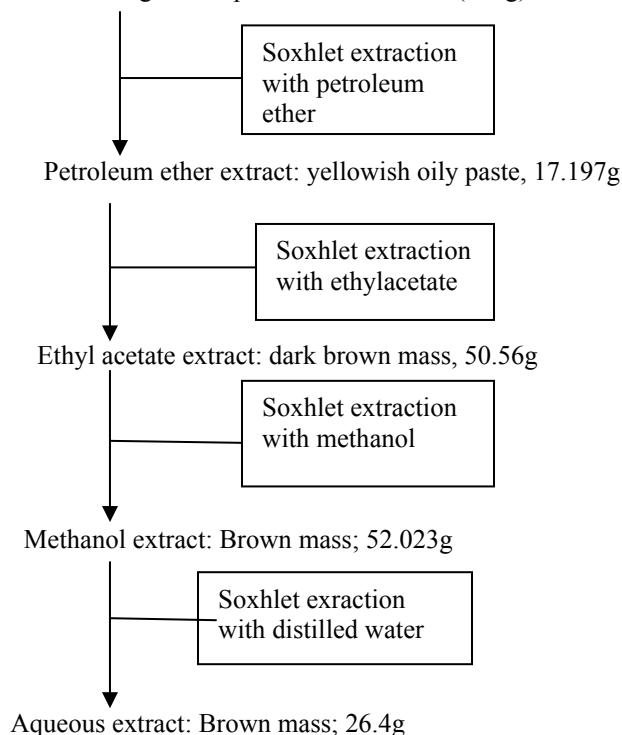


Fig. 1: Schematic Diagram of Gradient Extraction of *Croton zambesicus* Stem Bark in Organic and Aqueous Solvents.

Table 1a. Thin layer chromatography (TLC) examination of *Croton zambesicus* Muell Arg. Stem bark. Extract preparations

Petroleum ether extract			Ethylacetate extract		
No. of spots	dist. (cm);	R _f values	No of spots,	dist. (cm)	R _f values
8	12.7	0.77	9	12.9	0.78
	9.9	0.60		10.0	0.60
	7.5	0.45		7.5	0.45
	7.0	0.42		7.0	0.42
	5.9	0.36		5.9	0.36
	5.0	0.30		4.6	0.28
	3.4	0.20		3.4	0.20
	2.0	0.12		2.0	0.12
				1.3	0.7

Solvent system: hexane: ethylacetate (for petroleum ether and ethylacetate extracts)

1 : 0.5

Solvent front: 16.6cm

Running time: 1hr.3minutes.

Locating agent: iodine vapor

Analytical silicagel: G60 (Merck) 0.50mm thick; activated at 100-110°C for 30min. before use.

Table 1b. Thin layer chromatography (TLC) examination of *Croton zambesicus* Muell Arg. Stem bark

Extract preparations

Methanol extract			Distilled water extract		
No. of spots;	dist. (cm);	R _f values	No. of spots;	dist. (cm);	F _r values
5	15.4	0.94	3	11.4	0.70
	14.6	0.90		7.5	0.46
	12.0	0.73		5.0	0.31
	8.0	0.49			
	4.8	0.29			

Solvent system: chloroform: methanol: water (methanol and distilled water extracts)

3 : 3 : 1

Solvent front: 16.3cm

Running time: 2 hrs.

Locating agent: iodine vapor

Analytical silica gel : G60(Merck) 0.50mm thick activated at 100-110°C for 30 minutes before use.

6. Discussion

The thin layer chromatographic analysis of *C.zambesicus* stem bark extracts [petroleum ether extract(PEE), ethylacetate extract (EAE), methanol extract (MTE) and aqueous extract(AQE)] as presented in tables 1a and 1b reveals the number of constituent compounds present in each extract fraction. This is because according to Zoag and Sharma, (1972), a substance can be directly identified by its R_f value by comparison to the R_f of a standard or pure compound. However, in the present study, no comparison has been made to ascertain which compound they may be/are, but it indicates the number of

possible compounds present in each extract fraction, since there was separation resulting in discrete spots as indicated in tables 1a&1b showing the distances traveled by each spot in centimeters (cm) and thus R_f values calculated as shown. Thus the number of spots only have been used to indicate the number of constituent compounds suspected to be present in extract, i.e. PEE = 8, EAE = 9, MTE = 5 and AQE = 3.

It can also be observed that 6 of the spots (compounds) of PEE and EAE had the same R_f values (0.60, 0.45, 0.42, 0.36, 0.20 and 0.12) which corresponds to the distances traveled 9.9/10, 7.5, 7.0, 5.9, 3.4 and 2.0. this compounds are likely to be the

same compounds, since the extraction was done by exhaustive continuous soxhlet extraction in which both the solvents are non polar, more so that petroleum ether was used followed by ethylacetate, it is possible that some of compounds soluble in PEE might have still come out in EAE. However, the remaining 2 compounds of PEE with R_f s 0.77 and 0.30 corresponds to distances 12.7 and 5.0 and 3 of EAE with R_f 0.78, 0.20 and 0.7 corresponding to distances 12.9, 3.4 and 1.3 are different from each other meaning different constituent compounds. Similarly, on considering R_f values for compounds of MTE and AQE in table 1b, it can be seen that no two compounds had the same R_f or distances of travel, therefore they are all different compounds from each other.

In conclusion therefore, the number of possible compounds present in the PEE and EAE is eleven (11) while in the MTE and AQE is eight (8).

I also would like to suggest that further work be carried out on this plant part and the usage of standard compounds for good comparison in order to ascertain the real compounds present in this plant part as it has been reported to have broad spectrum antibacterial property and also its wide application in folk-low medicine.

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