



PHYTOCHEMICAL EVALUATION AND INITIAL TLC PROFILING OF TWO POTENTIAL INDIGENOUS MALE CONTRACEPTIVE PLANTS

*Etta Hannah Edim¹, Ekaluo Utib², Okon Archibong Essien¹

Eneobong Effiom Eneobong¹,

¹Biological Science Department, Cross River University of Technology, Calabar, Cross River State, Nigeria.

²Genetics and Biotechnology Department, University of Calabar, Calabar, Cross River State, Nigeria.

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*Correspondence for
Author

Etta, Hannah Edim

Biological Science
Department, Cross River
University of Technology,
Calabar, Cross River State,
Nigeria.

ABSTRACT

Introduction: The Phytochemical Evaluation and Initial TLC Profiling of Two Potential Indigenous Male Contraceptive Plants - *Phyllanthus amarus* (Schum and Thonn.) and *Mucuna urens*(Linn.) - was carried out. The importance of contraception in sub-Saharan Africa cannot be overemphasized. Majority of currently existing contraceptive methods tend to focus on the female body as male contraceptive trials have always proved abortive. The purpose of this

investigation is to screen and identify the phytocomponent(s) in these herbs with a view to isolate and further characterize them in order to better understand the pharmacognostic action of the phytocomponent(s). The following tests were employed in this investigation: Glacial acid test, Dragendroff's test, Foam Test, Alkaline Test, Lead acetate test, Ferric Chloride test, Fehlings Test, Hydrochloric acid test and Borntrager's test. TLC profiling was carried out using di-ethyl ether: methanol: water (16:1:4) for *M. urens*, *P. amarus* and chloroform: ethyl alcohol (16:1) to further evaluate the extracts. Results revealed the presence of cardiac glycosides, saponins, tannins, alkaloids, polyphenols and reducing sugar, in aerial parts of *P. amarus* and flavonoids, Tannins, Anthraquinones and Steroids in seed extract of *M. urens*. R_f values obtained ranged from 0.512 – 0.9 in the various solvent systems for the two herbs. Further isolation and characterization of these phyto components is being proposed.

KEYWORDS: Phytochemistry, TLC profiling, *Phyllanthus amarus*, *Mucuna urens*, Pharmacognosy.

INTRODUCTION

Plant extracts, either as pure compounds or as standardized extracts provide unlimited opportunities for new drug discoveries because of their rich store of phytochemicals. However, screening the herb formulations is an important initial step in this direction (Durapaudiyam *et al.*, 2006). Majority of birth control methods tend to focus on the female body. Recent research however have shown that the search for a successful male contraceptive is yet to come to an end. A recent publication by Bremner (2012) reports that a compound known as JQ1, which works by targeting a testis-specific protein called BRDT that is essential for fertility, may finally present as a break-through in the search for a male contraceptive. The BRDT-inhibiting molecule was given to mice and they began producing fewer sperm that did not even swim well. This same effect was recorded in separate studies by Etta *et al.*, 2009 and Etta *et al.*, 2012 on the anti-spermatogenic effects of ethanol extracts of *Mucuna urens*, also known as ox-eye beans, a common soup thickener consumed mostly in the South Eastern states in Nigeria and *Phyllanthus amarus* (stone breaker) ethanol extract on albino rats of the Wister strain respectively. Based on the results of these studies, it was concluded that these indigenous herbs be further investigated to ascertain their spermatogenic effect in the albino rat. Hence this research on the phytochemical evaluation and initial thin layer chromatography of extracts of the two plants.

Mucuna urens is a plant that belongs to the family fabaceae, commonly found in home gardens in the South eastern parts of Nigeria, West Africa, where the Efiks, Ibibios and Igbos use the seeds as a major soup condiment for thickening. In Northern Nigeria, farmers incorporate the seeds into the normal feed for farm animals due to its' rich protein content (Umoren *et al.*, 2007). It is called "Ibaba" by the Efiks/Ibibios and "Ukpor" by the Igbos and is usually sold in the local markets during its harvest season which is in the month of January (Eilitta and Carsky, 2003), though in recent times, it may be found throughout the year. The plant *Mucuna* is cultivated near trees as support for growth to enable production of many seeds per plant (Sridhar and Bhat, 2007). In other localities where *M. urens* is found, it is known as velvetbean, *pica-pica*, bengal bean, *nescafé*, *ojo de venado*, *pois mascate*, *kara benguk*, *olhos de burro*) (Esonu *et al.*, 2001). Horse eye bean, ox-eye bean and devil bean are all common English names for *Mucuna*.

Despite being nutritionally promising, *Mucuna* has been reported to contain some endogenous toxic factors. Relatively high concentration of tannins, phytic acid, cyanogenic glucoside, oxalate and gossypol have been reported in *Mucuna* (Laurena *et al*, 1994). Toxic compounds including L-DOPA (3,4-dihydroxy-L-phenylalanine), nicotine, physostigmine and serotonin have also been reported in *Mucuna*. These factors negatively affect the nutritive value of the beans through direct and indirect reactions; they inhibit proteins and carbohydrate digestibility; induce pathological changes in the intestine and liver tissues, thus affecting metabolism; inhibit a number of enzymes and bind nutrients, thus making them unavailable (Bressani, 1993). It is however believed that heat treatments reduce these anti nutritional properties of the seeds (Umoren *et al*, 2007).



Fig. 1 *Mucuna urens* seeds



Fig. 2 Pulverized seeds

Phyllanthus amarus belongs to the family Euphorbiaceae. It occurs as a weed throughout the Southern and Western part of Nigeria. It is best known by the common names stone breaker, carry-me-seed, gale wind grass, hurricane weed and quinine weed. In Nigeria, it is called Oyomokiso-amankedem (Efik), Ngwu (Igbo), Iyeke (Urhobo) and Eyin-Olobe (Yoruba). (Etta, 2008). According to folklore medical research, the leaves are used widely by the local people for the treatment of gonorrhea, genitor-urinary diseases, asthma, diabetes typhoid fever, jaundice, stomach ache, dysentery, hypertension and ringworm (Odugbemi, 2008). Other reports claim the use of the plant juice extracted from the stem for ophthalmic condition (Idika and Niemogha, 2008). It has also been reported to possess two lignans namely phyllantin and hypophyllantin obtained from the leaves. This has been noted to enhance cytotoxic responses with cultured multi drug resistant cells (Newman and Graag, 2007). Foo and Wong (1992) have reported the presence of a tannin, phyllanthusin D in the plant. Similar reports have reported the presence of quercetin and other tannins (Rajeshkumar and Kuttan, 2002); lignans (Singh *et al*, 2009) and alkaloids (Houhgton *et al*, 1996) in extracts of the herb.



Fig. 3 *Phyllanthus amarus* plant



Fig. 4 Pulverized *P. amarus*

Qualitative phytochemical evaluation of the components of these herbs was carried out after which TLC profiling towards the initial characterization of the components was also preformed. Thin Layer Chromatography was chosen over other chromatographic methods because it is a simple, quick and inexpensive procedure that can be used for the analysis of mixtures.

MATERIALS AND METHODS

Phyllanthus amarus

The plant material, *Phyllanthus amarus* was collected from the surroundings of University of Calabar and identified by Mr. Akpan of the Botanical Garden of University of Calabar. The leaves of *P. amarus* were separated from the tiny yellowish-green seeds and air-dried for 3 days to avoid denaturing of pigments, after which it was pulverized using warring commercial miller (Gateshed, tyne and wear NE83AT). Alcoholic extraction was carried out by using Soxhlet extraction apparatus (Quickefit, U.K) for 48 hours. The extraction was carried out using 99.5% (v/v) methanol as the solvent of extraction. The methanol was then evaporated out using rotary evaporator so as to concentrate the extract for further use.

Mucuna urens

The *Mucuna urens* seeds were collected from a local market and authenticated in the Department of Botany, University of Calabar by the curator. The seeds were cracked with a milling machine (LISTER, Germany) to enable separation of seed coat and the testa. This can also be done manually, but can be very stressful. The endosperms were collected and air-dried for 4 days, after which a manual grinding machine was used to grind the seeds into

powder and stored in an air-tight container at 4°C until commencement of investigations. Soxhlet extraction method, with 80% ethyl alcohol as extracting solvent, was also used for extracting the powdered herb. The *Mucuna urens* extract obtained was dried in a hot air oven at 40°C after extraction.

Phyto-chemical Screening

Qualitative phyto-chemical tests were carried out on both powdered extracts using standard procedures for Glacial acid test, Dragendroff's test, Foam Test, Alkaline Test, Lead acetate test, Ferric Chloride test, Fehlings Test, Hydrochloric acid test and Borntrager's test to identify the constituents as described by Harbome (1973), Trease and Evans (1989) and Soforowa (1996). The extracts were screened for flavanoids, steroids, alkaloids, cardiac glycoside, tannins, polyphenols, reducing sugar, saponins, and anthraquinone.

Thin Layer Chromatography

TLC profiling was carried out using di-ethyl ether: methanol: water (16:1:4) for *P.amarus* and chloroform : ethyl alcohol (16:1) for *M. urens*, to further evaluate the extracts. The development tanks were saturated using the solvents of isolation. The TLC plates (20X20cm) were activated above room temperature in an air oven for 30 minutes. 30g of silica gel 60F254 was accurately weighed out and dried in the air oven at 45°C for 10 minutes and prepared to slurry by adding 60ml distilled water and stirring continuously for 15 minutes. The activated plates were mounted on a clean spreader and coated with the prepared slurry by spreading with a spray gun vertically from one end to the other at 0.25mm in diameter and left to stand undisturbed for few minutes.

The mounted plates were then reactivated in air oven at 110°C for 30 minutes to ascertain a total removal of moisture. At this point, the plates were handled carefully from the side to avoid contamination. Spotting was done 2cm above the TLC plate using a micro pipette. The spotted samples were then labeled to avoid mistakes. Thereafter, the spotted plates were carefully immersed in the saturated development chambers. The eluted spots were visualized using two different detection methods. Visualization of *P. amarus* spots was done using day light radiation, while iodine chamber was used to visualize the *M. urens* spots. After visualization, the distance of solvent front and that of the detected spots were derived for the calculation of R_f Value (s), using the formula.

$$Rf = \frac{\text{distance moved by the solute}}{\text{distance moved by the solvent}}$$

RESULTS

Phyto-chemical Screening

Qualitative phyto-chemical analysis of the methanolic extract of *Phyllanthus amarus* leaves showed the presence of cardiac glycoside, alkaloids, saponins, tannins, polyphenols and reducing sugar while flavanoids, steroids and anthraquinone were absent, while *Mucuna urens* ethyl alcohol extract tested positive for Flavonoids, Tannin, Anthraquinone and Steroids. This extract was negative for Alkaloids, Saponin, Cardiac Glycosides, reducing sugar and polyphenols as shown on Table 1.

Table 1: Phyto chemical Components of extracts of *P. amarus* and *M.urens*

Plant Extract	Phyto- chemicals								
	Alkaloid	Saponin	Flavonoid	Reducin g Sugar	Tanni n	Cardiac glycoside	Anthra- quinone	Polyphenol s	Steroi d
<i>Phyllanthus amarus</i> (Methanol)	++ve	+ve	-ve	++ve	+ve	+ve	-ve	++ve	-ve
<i>Mucuna urens</i> (Ethyl alcohol)	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve

(++ve) **Strongly Present**, (+ve) **Present**, (-ve) **Not Present**

TLC profiling

Results of the TLC profiling of both indigenous extracts using mobile phase di-ethyl ether: methanol: water (16:1:4) for *P.amarus* and chloroform : ethyl alcohol (16:1) for *M. urens* are presented on Table 2. The values for *P. amarus* ranged from 0.917 -0.205 and 1.0 – 0.20 for *M. urens*.

Table 2: Rf values of solvent extracts of *P. amarus* and *M. urens*

Spots Rf Values	1	2	3	4	5	6
<i>P. amarus</i>	0.9178	0.8904	0.8630	0.7808	0.6849	0.2054
<i>M.urens</i>	1.0	0.30	0	0	0.60	0.20

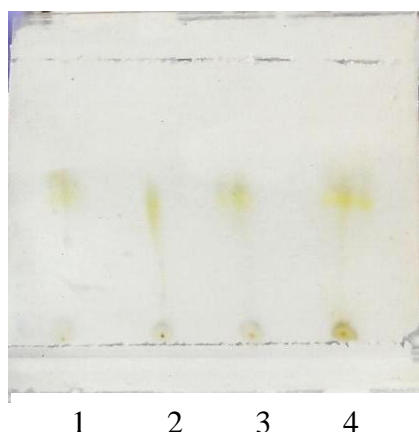


Fig. 5 TLC plate showing separation of spots.

DISCUSSION

This study presents the phyto-chemical screening and initial TLC profiling of two indigenous herbs that have been documented as having anti-fertility effects in the male albino rat of the Wister strain. Phyto-chemical screening showed a rich array of components establishing the potency of these herbs in tradi-medical practice around the world. Most of these medicinal qualities and other uses have been verified experimentally and documented by various workers, thus providing scientific evidences to support many of these claimed health benefits. The results obtained here agree with previous reports (Ujowundu *et al.*, 2010; Umoren *et al.*, 2007; Ujuala *et al.*, 2012; Houghton *et al.*, 1996; Morton, 1981; Foo, 1993; Foo and Wong, 1992 and Chevalher, 2000). Despite being nutritionally promising, *Mucuna* has been reported to contain some endogenous toxic factors. Relatively high concentrations of tannins, phytic acid, cyanogenic glucoside oxalate and gossypol have been reported in *Mucuna* (Leiner and Kakada, 1980; Laurena *et al.*, 1994). Tannins are known to be anti-nutritional and have been recorded to be very high (468.37 mg/100g) in raw *Mucuna urens* seeds but lower in the processed (cooked and roasted) seeds (36.20 and 45.87 respectively) (Umoren *et al.*, 2007). Though tannins have been known to be anti-nutritional, current information has it that their beneficial or anti-nutrition properties depend upon their chemical structure and dosage (Muller-Harvey and McAllan, 1992). Tannins are highly astringent and leave a dry feeling in the mouth if consumed unprocessed (Yisa, 2009). This account for the dry feeling in the mouth when *Mucuna urens* seed is consumed raw. The medical potentials of tannins have been well documented (Bayaj, 1998; Lu *et al.*, 2004; Akiyama *et al.*, 2001; Kolodziej and Kiderlen, 2005; Nobre-Junior and Helio, 2007; Souza *et al.*, 2006).

Recent findings indicate that tannins neither inhibit food consumption nor digestion but rather possess the potential to decrease the efficiency of converting the absorbed nutrients to new body substances (Chung *et al*, 1998), due probably to the great efficiency with which tannins precipitate proteins through interaction that occurs by hydrophobic forces and hydrogen bonding (Bennick, 2002).

The presence of flavonoids indicates the presence of polyphenolic secondary metabolites in the extract. Wound healing potentials of *Mucuna urens* has been attributed to the presence of several phytochemicals including flavonoids (Manjunatha *et al*, 2006). The seeds have also showed antibacterial activity against *B. cereus*, *E. coli*, *P. viugaris* and *Staph.* (Manyinatha *et al*, 2006). This could be attributed to its rich store of flavonoids.

The antioxidant and scavenging of free radicals ability of flavonoids may also be responsible for the anti-carcinogenic property of *Mucuna urens* reported by Ujowundu *et al*, 2010.

The laxative effects of anthraquinone have been documented (Delmulle and Demeyer, 2010). They have also been documented as not being friendly chemicals, as they cause kidney damage and gastro intestinal bleeding (Ukoha *et al*, 2011). The presence of anthroquinone in *Mucuna urens* may also be implicated for the highly toxic anti-nutritive property of unprocessed *Mucuna urens* (Umoren *et al*, 2007).

The presence of steroids in the ethanol extract of *Mucuna urens* attests to the possible efficacy of therapeurtic use of *Mucuna urens*. Steroidal compounds are of importance and interest in the body since they are related to sex hormones and could serve as potential starting materials in the synthesis of sex hormones and ensure such hormonal balance (Ukoha *et al*, 2011). Alkaloids present in *P. amarus* maybe responsible for the anticancer, anti-aging and antiviral activities of the herb (Harikumar *et al.*, 2007). Saponins have been reported to be cardiotonics, while flavanoids have anti-inflammatory activity (Evans 2002). It is also reported that fractions of *P. amarus* are acidic with pH of 4.40 – 5.70 (Sign *et al.*, 2009). These phytocomponents tested positive in the extract of *P. amarus* investigated and maybe implicated in their being used to treat diseases related to these physiological properties.

The TLC profiling presents the pharmacological properties of the extracts. The less polar components have R_f values close to 1 while the more polar components scored lower R_f values. This is because the silica gel that is on the plate is a polar substance; therefore the

compounds that are polar tend to not go up as far because likes dissolve likes, so nonpolar compound travel further up the plate than polar compounds (Julian, 2011). Nevertheless, the effects of the herb extracts on male fertility is a novel aspect of their efficacy that still needs to be investigated. Kumar *et al*, 2012, in their review on potential antifertility agents from plants, stated that it is time to increase the number of experimental studies on the vast array of unexploited antifertility plants found scattered in the green forests of the world. Hence this study is presented as a preliminary investigation of the pharmacognosy of these two herbs. Further detailed analysis by column chromatography and possibly spectrophotometric analyses is being proposed.

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