# In Vitro Effect of Stigma-5-En-O-B-Glucoside Isolated from Ethylacetate Leaf Extract of *Byrsocarpus Coccineus* Shum &Thonn on Pregnant Rat Uterus

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## **Research Article**

Please cite this paper as Wazis CH<sup>1</sup>, Timothy SY<sup>1\*</sup>, Anuka JA<sup>2</sup>, Zezi AU<sup>2</sup>, Danjuma NM<sup>2</sup>, Hussaini IM<sup>1</sup>, In Vitro Effect of Stigma-5-En-O-B-Glucoside Isolated from Ethylacetate Leaf Extract of *Byrsocarpus Coccineus* Shum &Thonn on Pregnant Rat Uterus. IJPTP, 2014, 5(1), 941-943.

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# Abstract

**Objectives:** The purpose of this study was to identify actual The aim of this study was to assess the activity of isolated compound from ethylacetate leaf extract (Stigma-5-en-O- $\beta$ -glucoside) of *Byrsocarpus coccineous* Schum and Thonn on pregnant rat uterus. The activity of the isolated compound was tested using a uterine tissue in a 25 ml capacity organ bath. The isolated compound contracted the uterine tissue of the pregnant rat similar to that of oxytocin and this amply justifies the traditional use of this plant in augmentation of labour.

**Keywords:** Byrsocarpus coccineous, Stigma-5-en-O-βglucoside, rat uterus.

# Introduction

Plants, animals, soil microbes and fungi have provided valuable medicines for mankind. It is estimated that less than 10% of the world's genetic resources have been studied seriously as source of medicines <sup>1</sup>. About 74% of the 119 plant- derived drugs commonly in use in one or more countries were discovered as a result of chemical studies directed at the isolation of the active constituents of plants used in traditional medicine <sup>2</sup>. Notable examples include the cardiac glycosides from *Digitalis purpurea* used in the treatment of cardiac failure, the anti-hypertensive agent and tranquilizer (reserpine), from the East Indian snakeroot, *Rauwolfia serpentine*, the analgesic codeine and morphine from *Papaver* 

somniferum. Others include physostigmine a drug used for the treatment of glaucoma extracted from Calabar beans (Pysostigma venanosum) which was used in South-East Nigeria as an "ordeal poison", dtubocurarine a skeletal muscle relaxant isolated from curare and emetine an antidiarrhoeal active ingredient extracted from epecacuanha Byrsocarpus coccineus schum and thonn (family connaraceae) is one of such plant that has been known and used in traditional medicine in several parts of West Africa<sup>3</sup> to manage oro-pharngeal, dermatological, urogenital tract and haematological problems <sup>4-8</sup>. The invivo and invitro uterotonic effects of crude ethylacetate leaf extract of Byrsocarpus coccineus on pregnant rat uterus has been reported by Wazis *et al*<sup>9</sup> and Wazis *et al*<sup>10</sup>. The uterotonic activity of the bioactive principle Stigma-5-en-O- $\beta$ -glucoside isolated from the ethylacetate leaf extract of Byrsocarpus coccineus by Wazis et al <sup>11</sup> to the best of our knowledge has not been reported elsewhere. This study was embarked upon in order to assess the in vitro uterotonic effect of the isolated compound Stigma-5-en-O-β-glucoside from the ethylacetate leaf extract.

# **Material and Method**

# Identification, collection and authentication of plant materials

Samples of the plant material *Byrsocarpus coccineus* were collected from Idu, Abuja during the month of April 2009 under the guide of a professional plant collector Mr.Yakubu Habi of the Department of Medicinal Plant Research and Traditional Medicine of the National Institute of Pharmaceutical Research and Development Abuja where a voucher specimen number (3452) was assigned and deposited at the herbarium for future reference.

# Processing and extraction of the powdered plant material

The leaves of *Byrsocarpus coccineus* were carefully separated from the other morphological parts of the plant and washed clean with water, air dried under shade for seven days pounded with pestle and mortar into fine particles. Six kilograms of the powdered leaves of *Byrsocarpus coccineus* was extracted by marceration with 6 L of N-hexane to

### International Journal of Pharmacy Teaching & Practices 2014, Vol.5, Issue 1, 941-943.

cover the powder. The set up was closed tightly for 72 hours with occasional agitation and stirring. Afterwards the mark was filtered, squeezed for remaining N-hexane and were air dried under shade until it was completely free from n-hexane. The mark was then macerated with absolute ethanol. The same procedure as indicated for n-hexane was repeated. Hexane was recovered by rotary evaporator. Ethanol extract was then subjected to evaporation using rotary evaporator. Two hundred grams of dried and crude ethanol extract of Byrscocarpus coccineus leaf was suspended in 150 ml of distilled water and successively extracted with 500 ml x 3 ethylacetate and 500 ml x 3 N-butanol. At every stage of the partitioning and before switching over to the next organic phase, it was ensured that the organic phase exhaust the aqueous phase of the needed ingredients and this was indicated by colour change in the organic phase. In addition it was also ensured that the next partitioning organic solvent does not interfere with the previous one; the two organic phases are used though successively but strictly exclusively. The ethyl acetate and N-butanol pooled fractions were filtered and evaporated to dryness separately at reduced pressure under rotary evaporator and the dried fractions stored in the desiccator until constant weight was obtained. The percentage yields for ethanol, ethylacetate, N-butanol and aqueous leaf extracts of Byrsocarpus coccineus were 16.7%, 3.9%, 1.2% and 1.0% respectively.

#### **Fractionation of the Ethyl Acetate Extract**

The ethylacetate leaf extract was fractionated using column and thin layer chromatography at which a pure compound stigma-5-en-O- $\beta$ -glucoside was obtained. The structure and molecular weight elucidation was carried out by mass spectra data acquired using Nanospray ionization techniques.

#### Results

Table 1 showed the effect of the pure sample (stigma-5-en-O- $\beta$ -glucoside) isolated from the ethylacetate leave extract of *Byrsocarpus coccineus* on an isolated pregnant rat uterus. At 10 $\mu$ /ml, 20 $\mu$ g/ml, 40 $\mu$ g/ml, 80 $\mu$ g/ml and 100 $\mu$ g/ml the responses obtained were 10mm, 2mm, 3mm, 2mm, and 1mm respectively. The effect of the pure sample (Figure I) on isolated pregnant rat uterus was dose independent as observed from this study while the responses obtained from oxytocin was dose dependant.

#### Discussion

The depolarizing effects of the isolated compound Stigma-5en-O- $\beta$ -glucoside <sup>10</sup> from the ethylacetate soluble parts of ethanolic extract of *Byrsocarpus coccineus* leaf that was observed to decrease with increasing concentration did not correlate with the activity of oxytocin on the pregnant rat uterus. The fact that ethylacetate leaf extract in which the compound was isolated contracted the pregnant rat uterus and the effect was blocked by atropine <sup>9, 10</sup>, may suggest the likely mechanism of action to be a muscarinic agonist. The isolated compound may likely be acting via similar mechanism. Interestingly, both the ethylacetate extract and the Stigma-5en-O- $\beta$ -glucoside showed a decrease in depolarizing response with increase in dose. These may suggest that at higher doses

Table 1 Effect of stigma-5-en-O-β-glucoside		
Volume of drug	Organ bath	Response
injected (ml)	concentration	( <i>mm</i> )
Oxytocin (1.0 x	IU/ml	
10 <sup>-2</sup> IU/ml)		
0.1	4.0 x10 <sup>-5</sup>	05
0.2	8.0 x10 <sup>-5</sup>	17
0.4	1.6 x10 <sup>-4</sup>	23
W1 (1000	μg/ml	
μg/ml)		
0.25	10	10
0.50	20	02
1.0	40	03
2.0	80	02
2.5	100	01

W1 = stigma-5-en-O- $\beta$ -glucoside



stigma-5-en-O-ß-glucoside Chemical Formula: C<sub>35</sub>H<sub>60</sub>O<sub>6</sub> Exact Mass: 576.44 Molecular Weight: 576.85

Fig.1 The structure of the pure sample (W1)

there is an evidence of desensitization of muscarinic receptor at the target tissue similar to that of suxamethonium. Faizi and colleagues <sup>12</sup> isolated a similar compound Stigma-5-en-3-O- $\beta$ -glucoside and its acetyl derivative from oil cakes of *Brassica rapa* but was not tested on rat uterus. The effect of this compound was tested and found useful for the treatment of human immunodeficiency virus (HIV) infection at which the compound has already been patented <sup>13, 14</sup>. The data on W1 (Stigma-5-en-3-O- $\beta$ -glucoside) isolated by Wazis and his colleagues <sup>11</sup> also agrees closely with those found in literatures <sup>15, 16</sup>. However, the activity of this isolated compound on the pregnant rat uterus reported in this study has significantly contributed to knowledge.

#### Conclusion

The isolated compound Stigma-5-en-O- $\beta$ -glucoside contracted the pregnant uterine tissue and this justifies the traditional use of this plant in some parts of Nigeria for the augmentation of labour.



International Journal of Pharmacy Teaching & Practices 2014, Vol.5, Issue 1, 941-943.

# Acknowledgement

Authors are sincerely thankful to Mr John Kono and Mr Ossey of Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Science, Ahmadu Bello University, Zaria for their technical assistance and support.

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## **AUTHORS' CONTRIBUTIONS**

Authors contributed equally to all aspects of the

study.

### PEER REVIEW

Not commissioned; externally peer reviewed.

## **CONFLICTS OF INTEREST**

The authors declare that they have no competing

interests.