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Preliminary Phytochemical Screening and Invitro Effects of Leaf Extracts of *Byrsocarpus Coccineus* Shum &Thonn on Pregnant Rat Uterus

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Research Article

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Abstract

Byrsocarpus coccineous Schum and Thonn is a scrambling shrub with prominent and numerous lenticels that has been known and used in traditional medicine to treat different ailments including induction of labour in several parts of West Africa. This study was aimed at assessing the phytochemical components and *in vitro* effects of the extracts of *Byrsocarpus coccineous* on pregnant rat uterus. Leaves of the *Byrsocarpus coccineous* were collected, air dried, pounded and extracted with ethanol, ethylacetate, N-butanol and water. The extracts obtained were used for the screening of phytochemicals and the assessment of *in vitro* activity on pregnant rat uterus. All the extracts contained carbohydrates, tannins, glycosides, except ethyl acetate extract which did not test positive for glycosides. Other substances that tested positive in all the extracts include cardiac glycosides, flavonoids, cardenolides and saponin glycosides. However, none of the extracts tested positive for alkaloids. The ethylacetate leaf extract contracted the pregnant rat uterus, while the aqueous extract was observed to relax the pregnant rat uterus *in vitro*. Papaverine and atropine blocked the depolarizing effect of the ethylacetate leaf extract. The antagonistic effect of atropine may suggest the likelihood of the extract to be acting via muscarinic receptor (M_1 or M_3).

Keywords: *Byrsocarpus coccineous, Phytochemistry, Uterotonic*

Introduction

Plants have been the source of medicinal agents since earliest times, and today have continued to play a dominant role in the primary health care of about 80% of the world's population [1]. In Nigeria, more than 70% of the estimated 140 million people are rural dwellers, who depend entirely on indigenous herbal medicines as a source of alternative health care [2]. *Byrsocarpus coccineus* schum and thonn (family connaraceae) is one of such plants that have been known and used in traditional medicine in several parts of West Africa [3]. *Byrsocarpus coccineus* is a climbing shrub with prominent and numerous lenticules, pinnate leaves, 6-9 pairs of leaflets that are larger near rounded apex. It has small white or pinkish scented flowers usually between January to March. *Byrsocarpus coccineus* is popularly known in Ghana by the Twi and Gar people as "awenda" or "awende." In Northern Nigeria, it is referred to by the Hausas as "Tsamiyar kasa or kimbar maharba." The Fulani people call it "wangarabubi or yangara-bubihi", while the Bassange people call it "Kogi." In the southern part of Nigeria the Yoruba people call it "Oke abolo" or "Mybo-apepea" [3]. Kilba people in Adamawa State call it ''mblakiki''. Timothy and his colleaques [4] detected saponins, tannins, glycosides, flavonoids, terpenes, reducing sugars and anthraquinones in N-butanol and ethylacetate leaf extracts of *Byrsocarpus coccineus.* Tannins have also been detected in the leaves, bark and roots of the plant [5]. Three flavonoids were identified and isolated as quercetin 3-0-d arabinoside, quercetin and quercetin 3-0-β-D-glucoside from the bioactive ethylacetate and N-butanol soluble part of ethanolic extract of *Byrsocarpus coccineus* [6]. Gamaniel and his colleagues [7] reported the uterotonic activity of ethanolic extract of *Byrsocarpus coccineus*. Several literatures reported the medicinal values of *Byrsocarpus coccineus* that include activities on head, ear, mouth, skin, urogenital tract and blood [8-12]. The Bassange people of Kogi state of Nigeria uses the leaf decoction of *Byrsocarpus coccineus* to aid women during child birth, while the Bassa people of Plateau state of Nigeria uses the seeds of a plant *Ricinus communis* as anti-fertility agent. This and

several other reasons prompted us to evaluate the phytochemistry and uterotonic effects of this plant.

Material and Methodology

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 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 was air dried under shade until it was completely free from n-hexane. The mark was then macerated with absolute ethanol. The same procedure as described for n-hexane was repeated. 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 were 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.

Preliminary Phytochemical Screening

The preliminary phytochemical screening for carbohydrates, Soluble starch, Tannins, Phlobatannins and Glycoside was carried out according to standard methods of Farnsworth [13], Trease and Evans [14], Brain and Turner [15] and Vishnoi and Narain [16]. The screening for cardiac glycosides, Cardenolides, Saponins, Glycoside, Flavonoids and Alkaloids was carried out according to standard methods of Silva *et al* [17], Trease and Evans [14], Sofowora [18], Markham [19], Brain and Turner [15] and Vishnoi and Narain [16].

In-Vitro Experiments

The pregnant rat was humanely killed, a piece of uterus was removed and mounted in a 25 ml capacity organ bath containing Dejalon solution connected to a microdynometer set. After allowing the uterine tissue to equilibrate for 30 minutes, different concentrations of the extracts were added and the responses recorded. The contact time in this experiment was 30 seconds. The tissue was washed three times and five minutes was allowed as rest time. The following drugs were added and responses recorded appropriately; ethylacetate extract, oxytocin, papavarine, salbutamol, atropine, acetylcholine, water extract, N-butanol extract and ethanol extract.

Results

Preliminary phytochemical screening of leaf extracts of Byrsocarpus coccineus (Tests for Carbohydrates, Tannins and Glycosides): The ethanol extract revealed moderate concentration for Molisch colour, Fehling's and combined reducing sugar test colour, whereas ketoses colour was found in low concentration. The ethylacetate extract exhibited moderate concentrations for Fehling and combined reducing sugar. N-butanol and water extracts contained moderate concentrations of carbohydrate for Molisch, Fehling's and combined reducing sugar test, while low concentration for ketoses was observed. Test for Barfoed and pentoses were present in low concentration in N-butanol but were absent in water extract. Ferric chloride solution test for ethanol, ethylacetate and N-butanol extracts indicated high concentration for tannins and moderate concentration for water extract. All the extracts contained moderate concentrations of tannins when HCl test was carried-out. Only water extract indicated low concentration with Goldbeater's skin test for tannins. Phlobotannins were not detected in all the extracts. Ethanol, N-butanol and water extracts contained moderate quantity of anthraquinones. Ethanol extract has low concentration for combined anthraquinones (Table 1).

Preliminary phytochemical screening of leaf extracts of Byrsocarpus coccineus (Tests for Cardiac Glycosides, Alkaloids, Flavonoids, Cardenolides and Saponin Glycosides): Both ethanol and ethylacetate extracts contained low concentration of cardiac glycoside for Salkowski, Libermann and terpenoids tests. N-butanol extract indicated high concentration of cardiac glycoside for Salkowski test, negative for Libermann test and low concentration for terpenoids. All the extract tested negative for alkaloids (Table 2). Ethanol extract revealed

Table 1: Preliminary phytochemical screening of leaf extracts of *Byrsocarpus coccineus*
(Tests for carbohydrates, Tannins and Glycosides)

Phytochemical test	*Extracts A*	*B*	*C*	*D*
Carbohydrates				
Molish	++	-	++	++
Barfoed	-	-	+	-
Fehling	++	++	++	++
CR sugar	++	++	++	++
Ketosis	+	-	+	+
Pentoses	-	-	+	-
Tannins				
$FeCl_3$	+++	+++	+++	++
HCl	++	++	++	++
Goldbeater	-	-	-	+
Phlobatannins	-	-	-	-
Glycosides				
Free AC	++	-	++	++
Combined AC	+	-	-	-

A = Ethanol extract, B = ethylacetate extract, C = N-Butanol extract, D = Water extract,
CR = Combined Reducing, AC = Anthraquinone, + = present in low concentration,
++ = present in moderate concentration, +++ = present in high concentration, - = absent

Table 2: Preliminary phytochemical screening of leaf extracts of *Byrsocarpus coccineus* (Tests for Cardiac Glycosides,Alkaloids, Flavonoids, Cardenolides and Saponin)

Phytochemical test	*Extracts A*	*B*	*C*	*D*
Cardiac Glycosides				
Salkoski	+	+	+++	++
Lieberman BT	+	+	-	-
Terpenoids	+	+	+	+
Alkaloids				
Mayer's R	-	-	-	-
Dragendroff's R	-	-	-	-
Flavonoids				
Shinoida	++	+++	++	+
$FeCl_3$	+++	+++	+++	++
				+
Lead ethanoate	++	-	+	++
NaOH	-	-	-	-
Cardenolides				
Keller-Killiani	+	+	++	++
Saponin Glycosides				
Frothing	+	-	-	++
				+
Fehling	++	++	++	++

A = Ethanol extract, B = ethylacetate extract, C = N-Butanol extract, D = Water extract, CR = Combined Reducing, AC = Anthraquinone, + = present in low concentration, ++ = present in moderate concentration,
+++ = present in high concentration, - = absent, R = reagent, BT = Burchard test

Table 3: Effect of Ethanol and Ethyl acetate leaf extracts of *Byrsocarpus coccineus* on pregnant rat uterus

Organ bath¥ Conc. (µg/ml)	*Quantity of Extract in organ bath (mg)*	*Responses* (mm)*
Ethanol leaf extract		
4	0.1	-
8	0.2	-
16	0.4	-
32	0.8	-
Ethylacetate leaf extract		
4	0.1	3.0
8	0.2	6.0
16	0.4	8.0
32	0.8	9.0
40	1.0	91.0
Ethylacetate leaf extract		
80	2.0	36.0
160	4.0	34.0
320	8.0	19.0
400	10.0	3.0

*= Depolarising effect observed ¥ = 25 ml organ bath - = no response

Table 4: Effect of N-butanol and aqueous leaf extracts of *Byrsocarpus coccineus* on pregnant rat uterus

Organ bath¥ Conc. (µg/ml)	*Quantity of Extract in organ bath (mg)*	*Responses* (mm)*
N-butanol leaf extract		
4	0.1	-
8	0.2	-
16	0.4	-
32	0.8	-
40	1.0	-
N-butanol leaf extract		
40	1.0	
80	2.0	-
160	4.0	-
320	8.0	-
400	10.0	
Aqueous leaf extract		
4	0.1	-
8	0.2	-
16	0.4	-
32	0.8	-
Aqueous leaf extract		
40	1.0	-
80	2.0	19.0
160	4.0	17.0
320	8.0	19.0

- = no response *= Repolarising effect observed ¥ = 25 ml organ bath

Table 5: Effect of Ethyl acetate leaf extract of *Byrsocarpus coccineus* and some agonists and antagonists on pregnant rat uterus

S/No.	*Drug organ bath concentration (µg/ml)*	*Response (mm)*
Agonistic study		
1	EAE (80)	33
2	Oxytocin (16)	90
3	Ach (0.32)	24
4	Salbutamole* (0.08)	-
5	EAE (80) + Oxytocin (16)	36
6	EAE (80) + Ach (0.32)	35
Antagonistic study		
7	EAE (80) + Papaverine (0.08)	-
8	EAE (80) + Atropine (0.08)	-
9	EAE (80) + Salbutamole* (0.08)	-
10	Oxytocin (16) + Papaverine (0.08)	95
11	Ach (0.32) + Atropine (0.08)	-

- = Depolarising response blocked, EAE = Ethyl acetate extract, ( ) =, Quantity in 25 ml organ bath, *= No response

moderate concentration of flavonoids for Shinoida and lead ethanoate test. High concentration of flavonoids for ferric chloride test and negative for sodium hydroxide test was observed. Ethylacetate extract indicated high concentration of flavonoid for Shinoida and ferric chloride test. N-butanol extract showed moderate concentration of flavonoids for Shinoida test, high concentration for ferric chloride test, low concentration for lead ethanoate test and negative result for sodium hydroxide test. Water extract revealed low concentration of flavonoids for Shinoida test, high concentration for ferric chloride test, moderate concentration for lead ethanoate test and negative for sodium hydroxide tests. Ethanol and ethylacetate extracts revealed low concentration of cardenolides, while N-butanol and water extracts indicated moderate concentrations. Ethanol extract indicated low concentration of saponin glycoside with frothing test, while water extract indicated high concentration of saponin glycosides with frothing test. All the extracts indicated moderate concentrations for saponin glycosides with the Fehling solution A and B tests (Table 2).

In vitro effect of Ethanol and Ethylacetate leaf extracts of Byrsocarpus coccineus on rat uterus:

The result of the effect of ethanol leaf extract of *Byrsocarpus coccineus* on pregnant rat uterus is shown in Table 3. There was no effect on the pregnant rat uterus at the tested concentrations of 4 to 32 µg/ml. Ethylacetate leaf extract of *Byrsocarpus coccineus* was able to depolarize the pregnant rat uterus at concentrations between 4 to 40 µg/ml dose dependently, whereas there was decreased activity with increase in concentration up to 400 µg/ml extract used. The effect of 80 µg/ml ethylacetate leaf extract was a depolarization of 36 mm magnitude, while 400 µg/ml of ethylacetate leaf extract was able to contract the rat uterus by only 3 mm (Table 3).

In vitro effect of N-butanol and aqueous leaf extracts of Byrsocarpus coccineus on rat uterus

The result of the effect of N-butanol leaf extract of *Byrsocarpus coccineus* on pregnant rat uterus is shown on Table 4. There was no effect on the pregnant rat uterus at the tested concentrations of 1 mg/ml and 10 mg/ml. Aqueous leaf extract of *Byrsocarpus coccineus* did not affect the pregnant rat uterus at the concentration up to 40 µg/ml. However, at concentrations between 40 and 320 µg/ml of the aqueous leaf extract, there was a hyperpolarizing effect. At 80 µg/ml, 160 µg/ml and 320 µg/ml there was a relaxation of the rat uterus by 19 mm, 17 mm and 19 mm magnitude respectively (Table 4).

In vitro effect of Ethylacetate leaf extract of Byrsocarpus coccineus and some agonists / antagonists on pregnant rat uterus: The effect of 80 µg/ml of ethylacetate leaf extract, 16 µg/ml of oxytocin and 0.32 µg/ml of acetylcholine on the pregnant rat uterus showed a contraction of 33 mm, 90 mm and 24 mm magnitude respectively. Salbutamol (0.08 µg/ml) did not show any response on the pregnant rat uterus. The concomitant administration of ethylacetate extract (80µg/ml) with oxytocin (16 µg/ml) showed a contraction of 36 mm, similarly the administration of ethylacetate (80 µg/ml) with 0.32 µg/ml of acetylcholine contracted the pregnant rat uterus by 35 mm magnitude (Table 5). The effect of ethylacetate leaf extract (80 µg/ml) on concomitant administration with papaverine (0.08 µg/ml), atropine (0.08 µg/ml) and salbutamol (0.08 µg/ml) blocked the initial depolarizing effect of ethylacetate leaf extract on the pregnant rat uterus. The effect of concurrent administration of oxytocin (16 µg/ml) and papaverine (0.08 µg/ml) on the pregnant rat uterus did not show any antagonism. However, atropine (0.08 µg/ml) was able to block the effect of 0.32 µg/ml acetylcholine on the pregnant rate uterus (Table 5).

Discussion

The presence of carbohydrate in the ethanol, ethylacetate, N-butanol and aqueous extracts did not quite agree with the report of Timothy *et al* [4] in which carbohydrate was found to be present only in ethylacetate leaf extract. The discrepancy may likely be as a result of the inability of Timothy and his colleagues [4] to carry out differential methods of detecting carbohydrate in the leaf extract that could have been more sensitive to detect the bioactive compounds. The presence of tannins and glycosides in all the extracts observed in this study agrees with the report of Timothy *et al* [4] and Dalziel [5] in which tannins was found to be qualitatively present. The presence of cardiac glycoside, alkaloids and cardenolides observed in this study to the best of our knowledge has never been reported in our

community especially in the report of Timothy *et al* [4] in which similar study was conducted.

In this study, ethanol leaf extract of *Byrsocarpus coccineus* did not show any effect on the pregnant rat uterus which contradicts the reports of Amos *et al* [10] and Okunji *et al* [11] in which the ethanol leaf extract showed significant contraction on the pregnant rat uterus. The variation in the study site as well as environmental changes in which the plant was sampled could be the likely explanation for the variation. The significant uterotonic effect of Ethylacetate leaf extract of *Byrsocarpus coccineus,* and the relaxing effect by the water extract observed in the present study, to the best of my knowledge have not been reported elsewhere. The absence of uterotonic or hyperpolarizing effect of N-butanol leaf extract of *Byrsocarpus coccineus* observed in this study could be attributed to the inability of the N-butanol solvent to extract the bioactive compound(s) responsible for the uterotonic activity. Studies aimed at evaluating the uterotonic effect of N-butanol to the best of our knowledge is currently not available. However, Timothy *et al* [4] and Ahmadu *et al* [12] reported the antibacterial properties of N-butanol leaf extract of *Byrsocarpus coccineus.* Aqueous leaf extract of *Byrsocarpus coccineus* did not show any effect on the pregnant rat uterus at 40 µg/ml. However, the activity at 80 up to 320 µg/ml was hyperpolarization which also has not been previously reported elsewhere.

The effect of ethylacetate leaf extract on pregnant rat uterus was significantly greater than the effects of acetylcholine and salbutamol. Oxytocin contracted the rat uterus better than ethylacetate extract in this study. However, the effect of concurrent administration of ethylacetate with oxytocin and acetylcholine did not differ significantly. Acetylcholine potentiated the effect of ethylacetate on the rat uterus. Papaverine was able to block the uterotonic effect of ethylacetate leaf extract but did not block the effect of oxytocin on the rat uterus. Interestingly, the effect of ethylacetate leaf acetate and acetylcholine was blocked by atropine, suggesting that the action of ethylacetate leaf extract may be acting via similar mechanism with acetylcholine (i.e through muscarinic receptor). To the best of our knowledge, no study was conducted that evaluated the effect of antagonist on ethylacetate leaf extract induced contraction of the pregnant rat uterus.

Conclusion

Byrsocarpus coccineus leaves have been found to contain a lot of bioactive phytochemical compounds which may be responsible for the observed uterotonic effects of the ethyl acetate leaf extract on pregnant uterus of albino rat. This study provides scientific justification for the traditional use of this plant in augmenting delivery when women are at terms.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.