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Artículo Original

Antimicrobial and uterine smooth muscle activities of *Albizia ferruginea* extracts.

Actividad antimicrobiana y sobre la músculatura lisa uterina de extractos de *Albizia ferruginea.*

AGYARE, C.*, KOFFUOR, G. A.*, MENSAH, A. Y., AGYEMANG D. O.

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1. College of Health Sciences, Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutics, KNUST, Kumasi, Ghana

*Corresponding Authors.

Agyare C.: Tel. +233-51-60374, Email address: chrisagyare@yahoo.com

Koffuor, G. A.: Tel. +233 27 740 0312, Email address: gkoffuor@yahoo.com or

gakoffuor.pharm@knust.edu.gh

Resumen

Albizia ferruginea es una planta medicinal muy utilizada en Africa. El tamizaje fitoquímico de un extracto de etanólico de las hojas y la corteza del tallo de esta planta indica la presencia de saponinas, taninos y esteroles. Los extractos tuvieron una actividad antimicrobiana significativa sobre los organismos seleccionados. Estos también mostraron efectos contráctiles dependientes de la dosis utilizada sobre los músculos lisos uterinos de ratas Sprague – Dawley grávidas y no grávidas. Esta planta podría presentar efectos colaterales de inducción de aborto cuando se use durante el embarazo.

Palabras clave: *Actividad antimicrobiana, actividad musculatura lisa uterina, Albizia ferruginea, extracto de hojas, extracto de corteza del tallo*

Abstract

Albizia ferruginea is a very useful medicinal plant in Africa. Phytochemical screening of an ethyl alcohol extract of the leaves and the stem bark of this plant indicates the presence of tannins, saponins, and sterols. The extracts had significant antimicrobial activity on selected organisms. They also showed dose-dependent contractile effects on isolated uterine smooth muscles of non – gravid, and gravid Sprague – Dawley rats. This medicinal plant could therefore have a side effect of inducing abortion when used during pregnancy.

Key words *Antimicrobial activity, uterine smooth muscle activity, Albizia ferruginea, leaf extract, stem bark extract*
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Introduction

Albizia ferruginea (Emil & Perr) Benth (Fam. Mimosaceae) is a perennial plant found in deciduous forests in parts of Africa (1). In Ghana, it is known locally as *Awiemfo semina* (Twi). It can grow up to 150 feet high and 9 feet girth with a thick, dark reddish-brown bark, peeling in small rough scales and exuding red juice (gummy exudate). The branches are flat and spreading with slightly ground leaf rachis when young. The leaves are bipinnate with pinnae up to seven pairs (2).

In Nigeria, the powdered root bark is taken with salts to relieve constipation. A decoction of the stem bark has been used medicinally for treating dysentery and wounds in Ivory Coast and Upper Volta (Cotê d'Ivoire and Burkina Faso). The stem bark is also used to treat sickle cell anaemia and gonorrhoea (1).

Though *Albizia ferruginea* has been used therapeutically in various herbal preparations, they may present some side effects. A decoction of the stem bark of *Albizia ferruginea* has abortifacient effect among others (1, 2). Our aim therefore is to justify its folkloric use as an antimicrobial agent and also to establish that it has a side effect of causing abortion when used by pregnant women.

Material and Methods

Preparation of *A. ferruginea* leaf and stem bark extracts

The leaves and stem bark of *A. ferruginea* were collected in May, 2004 from the Bobiri Forest Reserve (Kubease, Ashanti Region) and authenticated at the Forestry Department of Forest Research Institute of Ghana, by Mr. Alfred Boakye. The voucher specimen of the *A. ferruginea* labeled AFS1 has been deposited at the Faculty of Pharmacy, KNUST in Kumasi, Ghana.

The plant materials were sun-dried (separately) and powdered. 100 g of the leaf powder was soxlet-extracted with 70 %v/v ethyl alcohol. The liquid obtained was gradually dried into a solid mass (9.3 g). Quantities of this mass was taken and dissolved in distilled water to form the *A. ferruginea* leaf extract for use. The same procedure was carried out with 120 g of the stem bark. 10.2 g of dry solid mass was obtained. This was used to prepare the *A. ferruginea* stem bark extract.

Phytochemical screening

Part of the dried powdered materials was subjected to preliminary phytochemical testing for the major chemical groups (3).

Antimicrobial activity

The agar diffusion method was used for determining the antibacterial and antifungal activities of the extract (4). Methanol 96% (AnaLar grade) was used as the solvent for the extracts and showed no antimicrobial activity against the test organisms. Sterile distilled water was used as solvent for the reference drugs (chloramphenicol and clotrimazole).

Microorganisms

The test organisms listed in Table 2 were obtained from the Microbiology Laboratory, Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana.

Uterine smooth muscle activity

Drugs

Acetylcholine bromide (Ach, Sigma, Germany) and oxytocin (MSD, Herts) were used as reference agonist drugs with their corresponding antagonists; atropine (Merck, Germany), and indomethacin (Alliance, UK). Stilbestrol (Sigma, Germany) was used to induce oestrus.

Isolated rat uterus preparation

A non - gravid female Sprague-Dawley rat recieved stilbestrol (0.1 mg/kg, s.c.) 24 h before it was euthanized, dissected, and the uterine horns removed. The purpose for injecting stilbestrol is to bring the rat into oestrus so as to enhance the sensitivity of the uterus (uterine smooth muscle) to stimulatory chemical substances. Each horn was cut open longitudinally to form a sheet of muscle (5).

Experimental conduct and design

The isolated rat uterus was mounted in a 10 ml organ bath containing aerated De Jalon's physiological solution at 32^{o}C and allowed 30 min to equilibrate with the *in vitro* environment (6). With a contact time of 30 s, and a 3 - minute time cycle, graded dose-responses for oxytocin, ACh, *A. ferruginea* leaf, and stem bark extracts, and these in the presence of atropine (0.05 µg/ml), and indomethacin (0.25 µg/ml) were obtained.

Table 1: Antimicrobial activity of ethyl alcohol extracts of *A. ferruginea*. Values are inhibition (mm) and an average of triplicate

Drug	Zone of Inhibition (mm) of the listed micro-organisms						
	S.A	B.S	E. C	P. A	C. A	A. N	P. N
A. ferruginea leaf extract (1% w/v)	15.0	18.0	16.0	12.0	17.0	15.0	17.0
A. ferruginea stem bark extract (1% w/v)	13.0	17.0	17.0	12.0	15.0	15.0	16.0
Chloramphenicol	22.0	23.0	21.0	14.0	-	-	-
Clotrimazole	-	-	-	-	21.0	18.0	20.0

S.A – *Staphylococcus aureus*; B.S - *Bacillus subtilis*; E.C – *Escherichia coli*; P.A – *Pseudomonas aeruginosa* ; C.A – *Candida albicans*; A.N – *Aspergillus niger* P.N – *Penicillium notatum*

In another experiment, the gravid rat uterus was isolated (procedure as above) but without earlier injection of stilboestrol. The effects of oxytocin, ACh, *A. ferruginea* leaf, and stem bark extracts, and these in the presence of atropine (0.05 µg/ml), or indomethacin (0.25 µg/ml) were obtained.

Statistical analysis

One-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparisons test with instat® software (Graph Pad Inc USA). Statistical estimates were made at confidence interval of 95 %. $P < 0.05$ was considered significant.

Results and Discussion

Phytochemical screening

Both the *A. ferruginea* leaf and stem bark extracts contain tannins, sterols, and saponins. The percentage yields were as follows: *A. ferruginea* leaf extract, 9.3 % and *A. ferruginea* stem bark extract, 8.5 %.

Antimicrobial activity

The ethyl alcohol extract of *A. ferruginea* leaves and stem bark showed remarkable activity against all the test organisms (Table 1). The extracts were less active against *Pseudomonas aeruginosa* which is highly resistant to available orthodox antibiotics (7). The petroleum ether extract was less active against the test organisms (data not shown). The antimicrobial activity was more pronounced with the leaf extract compared to the stem bark. The antimicrobial activity exhibited by *A. ferruginea* suggests that the folkloric uses of the plant as wound healing, treatment of gonorrhoea and dysentery may be based on the antimicrobial activity.

Effect on isolated rat uterus preparations

The uterus is characterized by a high sensitivity to oxytocic agents and cholinergic agonists. Both the non-gravid and gravid uteri contracted in the presence of the reference drugs and the extracts in a dose dependent manner (Figs. 1 and 2). The EC_{50} values obtained for oxtocin, ACh, *A. ferruginea* leaf, and stem bark extracts on the non-gravid uterus were 0.01 µg/ml, 0.12 µg/ml, 66.2 µg/ml and 566.2.0 µg/ml respectively, and that obtained on the gravid uterus were 0.004 µg/ml, 0.07 µg/ml, 50.2 µg/ml and 209.6 µg/ml, respectively. These show that *A. ferruginea* leaf extract had a more potent effect than *A. ferruginea* stem bark extract but both were less potent than oxytocin, or Ach.

The contractile responses to oxytocin, Ach and the *A. ferruginea* extracts were more pronounced with smaller doses of the drugs on the pregnant uterus preparation than on the non-pregnant. The number of oxytocin and Ach receptors increase in the myometrium during pregnancy resulting in an increased sensitivity of the uterus to smooth muscle agonists**.** This would make pregnant women more susceptible to uterine contractions (8).

Except oxytocin, atropine (0.05 µg/ml) inhibited the response obtained for acetylcholine, *A. ferruginea* leaf and stem bark extracts significantly ($P < 0.01$) but indomethacin (0.25 µg/ml) inhibited significantly ($P < 0.01$) only the oxytocin response (Fig. 3) in both the isolated non – gravid and gravid uterus preparations. This suggests a possible similarity in the mechanism of action between Ach and *A. ferruginea* than between Ach and oxytocin. Muscarinic M_2 and M_3 receptors are present in rat uterus (8, 9). Ach (a muscarinic agonist) exerted its effect through a direct action on cell membrane causing depolarization. The influx of sodium ions into the cell leads to the contractile effect on the uterus**.**

Figure 1: Dose response curves for, oxytocin, Ach, *A. ferruginea* leaf, and stem bark extracts in an isolated non-pregnant rat uterus. Values plotted are means ± SD (n=4)

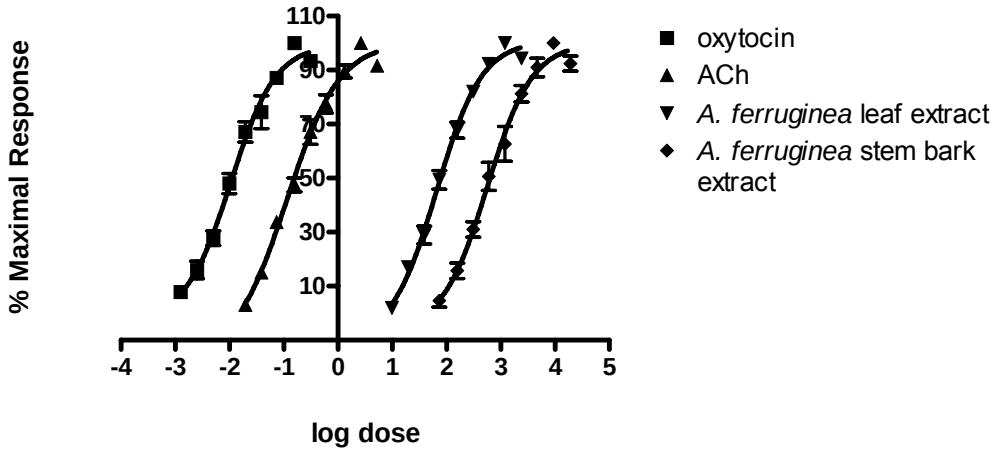


Figure 2: Dose response curves for oxytocin, ACh, A. ferruginea leaf, and stem bark extract in an isolated pregnant rat uterus. Values plotted are means ± SD (n=4)

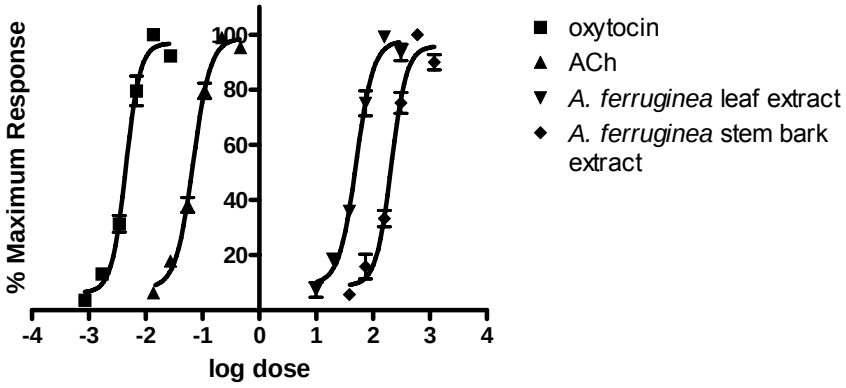
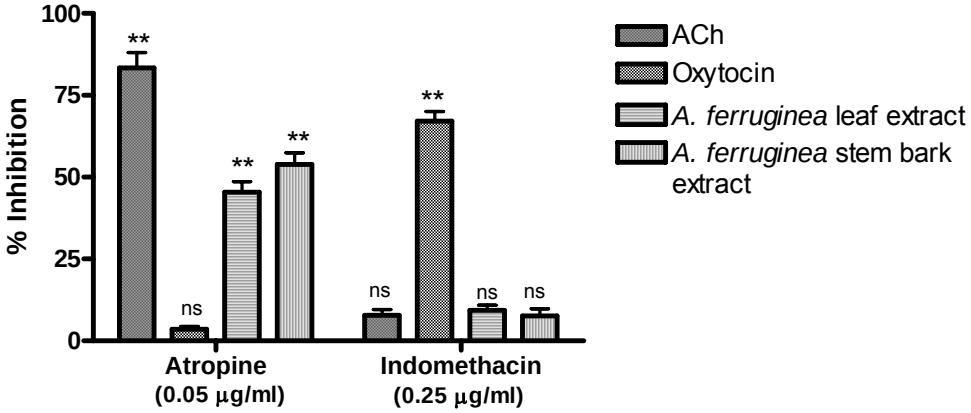


Figure 3: Percentage inhibitions of oxytocin, Ach, *A. ferruginea* leaf, and *A.* stem bark extracts by atropine, and indomethacin on the isolated rat uterus preparations. ** implies P < 0.01; ns implies P > 0.05 (n=8).



Prominent effects of muscarinic agonist within the digestive tract include stimulation of salivation and acid secretion, increased intestinal tone and peristaltic activity, and relaxation of most sphincters. This muscarinic receptor activity may account for its ability to relieve constipation as stimulation of these receptors result in increased gastrointestinal motility (10).

Conclusion

An ethyl alcohol extract of *A. ferruginea* is significantly active against bacteria (Gram positive and Gram negative) and on the fungi studied. It also has contractile effect on uterine smooth muscle (non-pregnant and pregnant) and hence should be not be used by pregnant women.

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