



Preliminary Phytochemical Screening of *Plumbago zeylanica* L. Roots and Its Aphrodisiac Effect in Male Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PO, DO and JN designed the study, collected samples and performed laboratory analyses. Authors TO and AO provided analytical support, performed literature search and analyzed the collected data. Authors PO, TO and AO wrote the first draft of the manuscript. All authors revised and approved the final manuscript.

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Short Communication

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ABSTRACT

Aim: To perform phytochemical screening of *Plumbago zeylanica* L. root extracts and assess the claim of its use in traditional management of erectile dysfunction in Uganda through evaluation of the aphrodisiac effect of its aqueous extracts in male Wistar rats.

Study Design: This study employed both qualitative and quantitative research designs.

Place and Duration of Study: All research work were performed at the Department of Biological Sciences, Gulu University, Gulu, Uganda between August 2019 and December 2019.

Methodology: Classical phytochemical screening of aqueous and methanolic extracts of *Plumbago zeylanica* roots were performed following standard methods. Aqueous extracts were

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administered to Male Wistar rats and the effect of the extracts on the mounting and intromission frequencies were determined.

Results: Glycosides, phenols, saponins, quinones, terpenoids and steroids were present in both methanolic and aqueous extracts. Alkaloids were present in methanolic extracts only while tannins and phlobatannins were only present in aqueous extracts. Flavonoids, coumarins and anthraquinones were not detected in both extracts. Aqueous root extracts of *Plumbago zeylanica* produced pro-sexual stimulatory effects in male rats when administered at 150, 300 and 450 mg/kg body weight.

Conclusion: The results support the use of *Plumbago zeylanica* roots by indigenous people in Uganda to increase libido, treat premature ejaculation and erectile dysfunction. The extracts had low sexual enhancement in sexually inexperienced male rats and therefore, further studies using experienced animal models are needed to better apprehend the prosexual effects of *P. zeylanica* roots. The toxicity of the extracts as well as structural elucidation and pharmacological evaluation of the responsible bioactive compounds merit further studies.

Keywords: Erectile dysfunction; intromission; sexual impotence; virility; traditional medicine; Uganda.

1. INTRODUCTION

Male impotence (erectile dysfunction, ED) is a disorder that affects the sexual life of up to 30 million men worldwide [1-3]. It ascribes a repeated inability to get or maintain an erection firm enough for sexual intercourse [3]. In developed countries, treatment options for ED have progressed from psychosexual therapy and penile prostheses, through revascularization, vacuum constriction devices and intracavernous injection therapy to transurethral and oral therapy [4]. In developing countries however, inability to afford medical healthcare and little trust in western medicine has made patients to seek traditional medical treatment. In these therapies, medicinal plants are commonly used. *Mondia whitei* (Hook.f.) Skeels, *Capsicum frutescens* L., *Carica papaya* L. and *Plumbago zeylanica* L. are some of the plants used in management of ED and increasing virility in Uganda [5-9]. Though some have been investigated [10-12], the claim of using *P. zeylanica* roots for management of male sexual disorders in Uganda have not been verified. In the current study, we performed a preliminary study to identify the secondary metabolites in *P. zeylanica* roots and investigated the aphrodisiac effects of its aqueous extracts in male Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Material

Fresh *P. zeylanica* roots were harvested from naturally growing plants in Nam-Okora subcounty, Kitgum district (Uganda). The roots

were identified and authenticated by a taxonomist at Department of Botany, Makerere University, Uganda where a voucher sample (no. PO-001) was deposited at Makerere University Herbarium. The roots were washed under running tap water and shade-dried for three weeks. They were then ground into fine powder using an electric grinder [13]. Plant extracts were prepared by maceration of 100g of the powder with 300 mL of methanol or distilled water for 24 hours with occasional shaking [14].

2.2 Phytochemical Analysis of Methanolic and Aqueous Extracts of *P. zeylanica* Roots

Qualitative phytochemical screening of the extracts was done for alkaloids, glycosides, flavonoids, phenols, saponins, tannins, quinones, coumarins, terpenoids, steroids, phlobatannins and anthraquinones following standard methods [14-16].

2.2.1 Test for alkaloids

To 2 mL of the extract, 2 mL of 1% hydrochloric acid was added and steamed. Three drops of Wagner's reagent was then added to the resultant solution. Brown or reddish brown precipitate indicated the presence of alkaloids.

2.2.2 Test for glycosides

Glacial acetic acid (1 mL) was added to 2 mL of the extract in a test tube followed by 3 drops of ferric chloride solution and finally 1 mL of concentrated sulphuric acid. Presence of a brown ring at the interface confirmed the presence of cardiac glycosides.

2.2.3 Test for flavonoids

To measured 2 mL of the extract in the test tube was added two drops of sodium hydroxide solution. Appearance of a yellow colour which decolourized following addition of two drops of dilute sulphuric acid indicated the presence of flavonoids.

2.2.4 Test for phenols

Measured 1 mL of 5% ferric chloride solution was added to 2 mL of the extract in a test tube. A blue, green or dark green colour formation indicated the presence of phenols.

2.2.5 Test for quinines

Concentrated sulphuric acid (1 mL) was added to 1 mL of the extract in a test tube. Formation of a red colour showed the presence of quinones.

2.2.6 Test for saponins

Distilled water (5 mL) was added to 1 mL of the extract in a test tube. The mixture was then shaken vigorously for 2 minutes. Appearance of a stable foam lasting for 5 minutes indicated the presence of saponins.

2.2.7 Test for steroids

A volume of 2 mL of acetic anhydride was added to 1 mL of the extract in a test tube followed by 2 mL of concentrated sulphuric acid. A blue or green reddish black coloration indicated the presence of steroids.

2.2.8 Test for tannins

In a test tube containing 2 mL of the extract, 2 mL of distilled water was added followed by three drops of ferric chloride solution. Formation of a blue or green precipitate indicated the presence of hydrolysable and condensed tannins, respectively.

2.2.9 Test for terpenoids

To 5 mL of the extract, 2 mL of chloroform and 3 mL of concentrated hydrochloric acid were added. Formation of a reddish brown colour at the interface indicated the presence of terpenoids.

2.2.10 Test for coumarins

To 1 mL of the extract in a test tube, 1 mL of 10% sodium hydroxide was added. Formation of

a yellow colour indicated the presence of coumarins.

2.2.11 Test for phlobatannins

To 0.5 mL of the extract in a test tube, 3 drops of 10% ammonia solution was added. Formation of a pink precipitate indicated the presence of phlobatannins.

2.2.12 Test for anthraquinones

To 1 mL of the extract in a test tube, 4 drops of 2% hydrochloric acid was added. Formation of a red precipitate indicated the presence of anthraquinones.

2.3 Assessment of Aphrodisiac Activity of *P. zeylanica* Root Extracts

Male Wistar rats (200-250 g) were completely randomized into 4 groups of 4 rats per cage in clean aluminium cages at 23 ± 1 °C, 12:12 light: dark and 45–50 % humidity. All animals were given unrestricted access to rat pellets (Bellmill Feeds Limited, Kenya) and water *ad libitum*. Administration of extracts were as follows; Group 1 were administered with distilled water (negative control) whereas rats in Group 2, 3 and 4 were administered with the plant extract at doses of 150, 300 and 450 mg/kg bw respectively.

Female rats (200-253g) were made receptive by sequential administration of oestradiol benzoate (10 mg/100g bw) and progesterone (0.5 mg/100g bw) (Sigma Chemicals, St Louis, USA) through injections prior to pairing [17,18]. For each substance, the volume administered intravenously was 0.2 mL while the infusion time was 5 seconds. Oestrous female rats were paired with male treated rats and the sexual behaviours were monitored in a separate room. The assessed parameters included mounting frequency (MF) and intromission frequency (IF) as described by Fouche et al. [19]. Mounting and intromission frequencies were determined on day 1, 7 and 14 of the study.

2.4 Statistical Analysis

Data were reported as means \pm standard deviations of replicates. Statistical significance between means were determined using one-way ANOVA followed by Tukey test at $p < 0.05$. All statistical analyses were performed with Sigma Plot statistical software (v14, Systat Inc., USA).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening Results

Qualitative phytochemical screening of medicinal plants is an essential step to their detailed phytochemical and pharmacological investigation [20]. Preliminary analysis for the presence of bioactive secondary metabolites in the roots of *P. zeylanica* revealed that glycosides, phenols, saponins, quinones, terpenoids and steroids were present in both methanolic and aqueous extracts (Table 1). Only the methanolic extract had alkaloids. Tannins and phlobatannins were only detected in aqueous extracts while flavonoids, coumarins and anthraquinones were not detected in both extracts. The presence of tannins, steroids and alkaloids in methanolic root extracts of *P. zeylanica* was reported by previous authors [20]. However, we did not detect flavonoids while the presence of quinones and saponins were confirmed, contrary to a previous observation [20]. Earlier phytochemical analysis of *P. zeylanica* roots reported the presence of naphthoquinones: lapachol, plumbagin, 2-isopropenyl-9-methoxy-1,8-di-oxa-dicyclopenta[*b,g*]naphthalene-4,10-dione, 9-hydroxy-2-isopropenyl-1,8-dioxadicyclopenta[*b,g*]naphthalene-4,10-dione, 2-(1-hydroxy-1-methyl-ethyl)-9-methoxy-1,8-dioxadicyclopenta[*b,g*]naphthalene-4,10-dione and 5,7-dihydroxy-8-methoxy-2-methyl-1,4-naphthoquinone [21], 1,4-naphthalenedione, β -asarone, oleic acid, naphtho(2,3-*b*)furan-2(3H)-one, ethyl *p*-methoxycinnamate and *n*-hexadecanoic acid [20]. Therefore, the observed differences in the secondary metabolites recorded in this study could be due to the differences in soil chemistry, rainfall, topography and climate that affects the interaction between

plants, the environment and subsequently the phytochemical composition of plant organs.

3.2 Effect of Aqueous Extracts of *P. zeylanica* Roots on Mounting Frequency and Intromission Frequency in Male Wistar Rats

Plants have been utilized across the globe as a safe and easily accessible source of medicines to contest the recurrent deterioration in sexual behaviours [22-24]. In the current study, male treated rats in all the groups advanced towards the females upon introduction into the cages with precopulatory behaviours such as chasing and anogenital sniffing which ended in mounting, intromission and ejaculation. The rats did not show any signs of tiredness, a clear justification that the extracts exerted no sedative effect on the animals.

Aphrodisiacs are substances that arouse sexual instincts and may be categorized according to their mode of action into three groups: those that increase libido (i.e. sexual desire), those that increase potency (i.e. effectiveness of erection) and those that increase sexual pleasure [25]. They act on the central nervous system by altering specific neurotransmitters or sex hormone concentrations [25-29]. Plant-derived aphrodisiacs act on specific neurotransmitter systems. For example, intravenous administration of *Bersama engleriana* extracts to spinal male rats prevented rhythmic contractions of bulbospongiosus muscles (the main ejaculatory muscles) and the occurrence of ejaculation induced by dopamine and oxytocin [29]. Root extracts of *P. zeylanica* was previously shown to have central nervous system stimulatory action in rats [30].

Table 1. Secondary metabolites identified in methanolic and aqueous extracts of *P. zeylanica* roots

Metabolite	Methanolic extract	Aqueous extract
Alkaloids	+	-
Glycosides	+	+
Flavonoids	-	-
Phenols	+	+
Quinones	+	+
Saponins	+	+
Steroids	+	+
Tannins	-	+
Terpenoids	+	+
Coumarins	-	-
Phlobatannins	-	+
Anthraquinones	-	-

*+ means present and – means absent

In the current study, mount and intromission frequencies as useful indices of vigor, libido and potency were used to assess the effects of the plant extracts on male rat sexual behaviour. The effects of the aqueous extracts of *P. zeylanica* root on the mounting and intromission frequencies of the male Wistar rats are shown in Fig. 1 and Fig. 2. While the number of mounts (MF) reflect sexual motivation, increase in the number of intromission (IF) shows the efficiency

of erection, penile orientation and the ease with which ejaculatory reflexes are activated [31]. The increase ($p < 0.05$) in mount and intromission frequencies following administration of aqueous extracts of *P. zeylanica* at 300 and 450 mg/kg bw indicates that the extracts improved sexual vigor or libido in the male rats. Similar findings were recorded for *Myristica fragrans*, *Syzygium aromaticum* and *Monsonia angustifolia* extracts by previous authors [19,32].

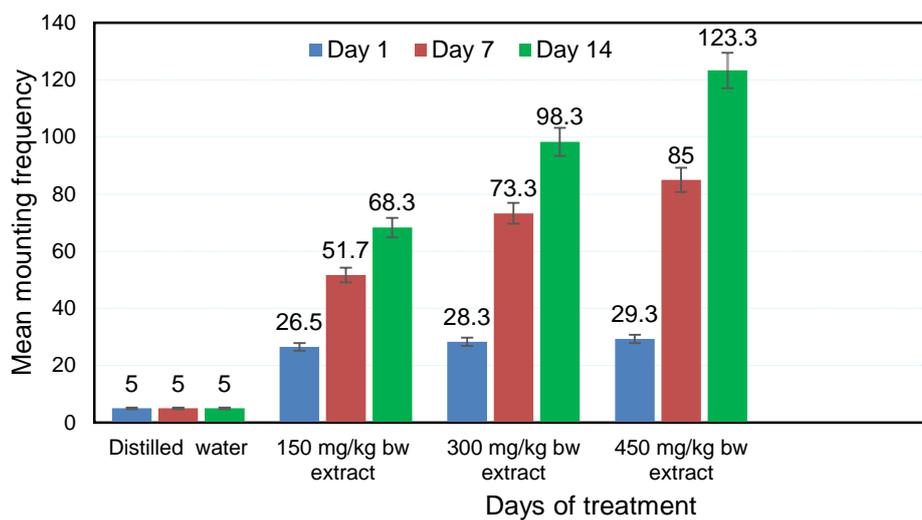


Fig. 1. Effect of aqueous extract of *P. zeylanica* roots on mounting frequency in male Wistar rats

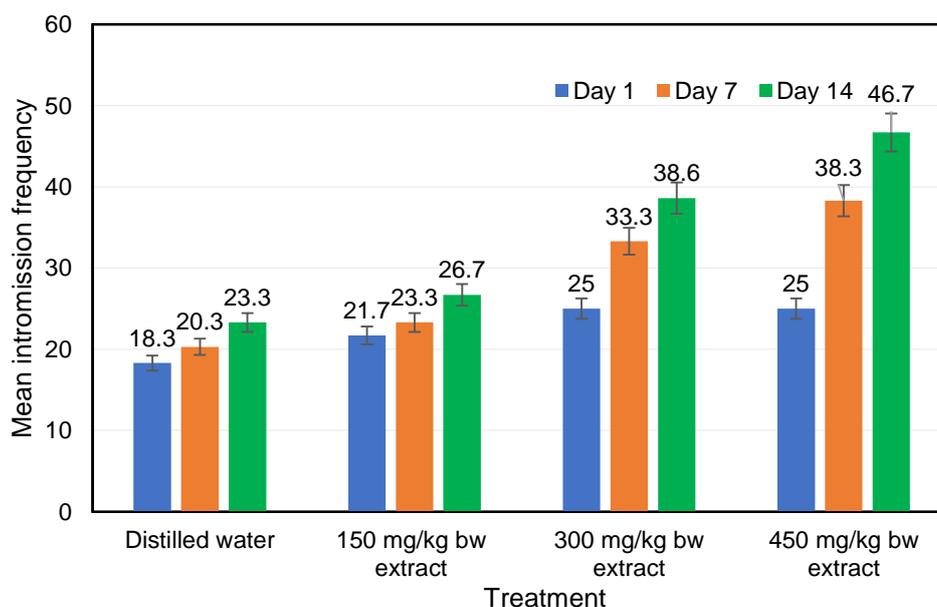


Fig. 2. Effect of aqueous extract of *P. zeylanica* roots on intromission frequency in male Wistar rats

The increase ($p < 0.05$) in intromission frequency of the male rats induced by the extracts at 300 and 450 mg/kg bw implies that penile erection was activated. Therefore, aqueous root extracts of *P. zeylanica* may increase potency by allowing or sustaining erection in males. Plant activities on penile erection have been attributed to various phytochemicals such as alkaloids and saponins which have ergogenic activities in vasodilation of blood vessels and consequently erection [31,33]. A study by Kim et al. [33] reported that relaxation of corpus cavernosum muscle was induced by saponins in *Panax ginseng* acting as nitric oxide donor through the L-arginine/nitric oxide pathway. Saponins in the extracts might have stimulated increase in body endogenous testosterone levels probably by raising the levels of luteinizing hormones, which translated into the male sexual competence observed. The steroidal nature of saponins, in part, facilitates their role as an intermediate in the steroidal pathway of androgen production [34]. Saponins bind to hormone receptors which may result in conformational changes that enhances physiological function of the hormone or bind to enzymes that are involved in the synthesis of such hormones, and thus enhance their production [24]. The disparity in the mounting and intromission frequencies observed indicate that it was not every mount by the male rats that resulted in intromission.

4. CONCLUSION

This study showed that aqueous roots of *P. zeylanica* contains glycosides, phenols, saponins, quinones, terpenoids and steroids which could be the therapeutically active compounds responsible its aphrodisiac activity. The extracts had low sexual enhancement in sexually inexperienced male rats. Further experiments using experienced animal models is needed to better apprehend the prosexual effects of *P. zeylanica* roots. The results of this study supports the acclaimed aphrodisiac use of the plant in Northern Uganda folk medicine. However, further studies need to establish the toxicity (safety) of the extracts from this plant when used in herbal medicine. The antioxidant activity, total phenolic and total flavonoid content of the extract should be established. Isolation, characterization, efficacy and safety of pure compounds from *P. zeylanica* roots should be pursued.

ETHICAL APPROVAL

Animal experiments were conducted in accordance with the National Institute of Health guide for the care and use of laboratory animals (NIH Publication No. 80-23; revised 1978) and in accordance with guidelines of Gulu University Research Ethics Committee (Approval No. GUREC-006/08/19).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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