***Original Research Article***

***ANTIFERTILITY EFFECTS OF POUZOLZIA MIXTA IN FEMALE RATS***

**ABSTRACT**

*Pouzolzia Mixta* (*hypoleuca*), is a small multi- stemmed shrub tree from the *Urticaceae* family found throughout Southern Africa. The dried shrub’s root powder has been the traditional contraception for Zimbabwean women for centuries. Traditionally believed to possess postcoital anti-fertility activity, the dried ground powder is taken in mealie meal porridge the morning after sexual intercourse, a concoction called “*Mukutura*” which is believed to prevent pregnancy or as an infusion in beverages a practice which can be equated to the use of “morning after levonorgestrel pills” in conventional medicine. Apart from poor adherence issues, current conventional emergency contraception methods have reported adverse side effects and significant failure rate which results in unwanted pregnancies and illegal abortions. There is a need therefore to explore traditional safe and effective emergency contraception alternatives. Our present study explores the biosafety and efficacy of *Pouzolzia Mixta* as a herbal emergency contraception. Phyto screening of the root extract for active metabolites was done using classical wet chemistry techniques. The oral toxicity was assessment using a modified OECD TG 425 method. The anti-fertility effect was assessed biochemically on hormonal changes using nulliparous laboratory Wistar rats. The studies confirmed the abundance of secondary metabolites of pharmacological relevance in the lyophilised root extracts. The serum biochemical assays demonstrated hormonal aberrations in estrogenic and oxytocin biomolecules of anti-implantation relevance. The toxicity studies confirmed the biosafety of the root extract at 4000mg/kg body weight in laboratory animal models. Our studies concluded that *P Mixta* root extracts do possess potential antifertility activity and are toxicologically safe according to the Hodge and Steiner toxicity classification.

**Key words**: *Pouzolzia mixta*, anti-fertility, anti-implantation, oxytocin.

# Introduction

## *Poulzozia Mixta*

*Pouzolzia mixta* is a small tree or multi-stemmed shrub growing up to 4 m high. These shrubs grow on rocky hillsides, in open woodland, wooded grassland, and along riparian thickets in bushveld. The bark is dark reddish-brown and smooth; branchlets have a velvety surface. Watery latex present. The leaves are simple, spirally arranged and ovate with a tapering apex and lobed base, 3-veined from the base with smooth margins. The leaves have contrasting upper and lower surfaces, the upper surface dark green and the lower surface silvery white-felted. Leaves tend to stick together and to clothing. The flowers are small and greenish with separate male and female flowers on the same plant. They grow in dense clusters in the axils of leaves. The fruit is a very small nut enclosed in the remains of the flower. Soap-nettle fibres from the bark are used to make rope, string and fishing nets1. The leaves are cooked as a green vegetable often with that of *Obetia tenax*. The crushed leaves are used as a soap substitute to wash hands and clothes.

The use of plants for medicinal and fertility regulating purposes has been practiced in Zimbabwean societies for centuries1,2. Fertility was very highly regarded and associated with the preservation of the tribe; contraception was not openly discussed. However, contraception was practiced, and some plant contraceptives continue to be used until today. In the ongoing search for more convenient safe and effective products to increase reproductive choice, there is a need to explore methods that utilize natural products1.The perennial shrub *Pouzolzia mixta* (hypoleuca) with local names: soap brush (English), *isikhukhukhu* (Ndebele) or *munanzva* (Shona), belongs to the Urticaceae family. It is claimed to have antifertility activity. Its native distribution extends from northern South Africa, Botswana, Zimbabwe, and then further north as far as Malawi 1,2. The root is taken orally as a nutraceutical by women as a powder in porridge and as an infusion or decoction the morning after sexual intercourse. Therefore, this plant may possess postcoital anticeptive activity,3 *Pouzolzia mixta* is also used in Zimbabwe by women for treating painful uterus, for expulsion of retained placenta, to treat infertility in women, to treat venereal diseases and to dilate the birth canal. Indeed, despite the availability of synthetic contraceptive medications, alternatives are still needed that may have less side effects and to increase the contraceptive supermarket The antifertility effects of *Poulzozia mixta* roots have not been scientifically validated. The aim of this study therefore was to investigate antifertility activity of crude extracts of *P.mixta* in female rats



*Figure 1: Poulzozia mixta plant and the leaves*

## Contraception, Emergency contraception/ post coital contraception, secondary metabolites and ant-fertility.

Contraception is the use of medicines, surgery and devices to prevent pregnancy. These ranges from different types which are reversible and permanent methods. Barrier methods of contraception are reversible methods which includes male and female condoms, contraceptives sponges, spermicides, diaphragms and cervical caps. Hormonal methods introduce artificial hormones to regulate ovulation hence preventing pregnancy. Hormonal methods include oral daily contraceptives, contraceptives patch, vaginal rings and implants. There are also sterilization methods which permanently inhibits the male and females’ systems fertility. Examples of sterilization methods includes female tubal ligation and male vasectomy. There are also natural methods of contraception which have been used to prevent pregnancy but are less reliable and effective, Examples of natural methods of contraception includes female fertility days tracking, withdrawal and lactational amenorrhea (LAM) method.4,5,7 Emergency contraception also known as postcoital contraception are methods used as emergency procedures to prevent pregnancy after contraception method failure, unprotected sexual intercourse and sexual assault. Ulipristal acetate pills, levonorgestrel pills and copper intrauterine device are the currently available emergency contraceptives with levonorgestrel morning after pills being commonly used. These levonorgestrel morning after pills can only be used once a cycle and are only effective if taken before ovulation hence contraception challenges as they are being taken by victims without the knowledge of their menstrual cycles. They are also associated by many side effects which include nausea, emesis, abdominal pains, headaches, menstrual irregularities, fatigue, dizziness and breast tenderness. Women who weigh more than 75 kg or have a body mass index greater than 25kg/m^2 have a higher risk of unintended pregnancies when using oral levonorgestrel pills. The limited mode of action and too many side effects is of major concern as they are appearing not to be very reliable and safe. Copper uterine device which is stated to be the most effective emergency contraception methods has many contraindications which include copper allergies, dysmenorrhea, menorrhagia, Wilson’s disease, pelvic inflammatory disease and cervical cancers hence only a limited number of females can be inserted the device.7 Development of an effective and safe emergency contraception is therefore imperative. The use of indigenous plants for anti-fertility has been prevalent in Zimbabwean rural areas for aeons.

*Pouzolzia Mixta* has other therapeutic effects, but its antifertility activity is of major interest as the bioactivity and safety profiles are not well characterized. The activity of anti-fertility the plant is a consequent of its richness in secondary metabolites. Secondary metabolites are organic compounds produced by plants which are not directly involved in growth, development, reproduction but are crucial for environmental adaptation. Phytoscreening studies showed the presence of flavonoids, saponins, phenols, tannin, glycosides and terpenoids. However high concentrations of flavonoids and tannins were detected which we believe are behind the estrogenic activity and anti -implantations properties demonstrated by the plant extract .Flavonoids are hormones either structure similarity to estrogen hence can bind to estrogen receptors and influence the female menstrual cycle .Flavonoids also have a high influence in the metabolism of estrogen in the liver hence have potential in the hormonal regulation .Flavonoids also having a similar shape to estrogen can also affect uterine receptivity on uterus lining eventually affecting implantation. Tannins are also effective at disturbing implantation as they stop supply of growth factors necessary for implantation. Tannins antioxidant effect can also create an unfavorable environmental condition for implantation and embryo development. The estrogenic and anti-implantation activity of the secondary metabolites are promising in the development of an emergency contraception from the *Pauzolzia* plants extracts.

## Traditional herbal contraceptives

Traditional use of medicinal plants and their extracts have become widely known among society for various diseases including fertility related problems. Considering women healthcare, it has become important to use herbal antifertility agents which can interfere with the natural procedure of reproduction in women. Modern research includes the use of various plant extracts having antifertility action in various ways. Numerous herbs have been tested for their contraceptive activity on different animal models.8 These herbal contraceptives are found to be ecofriendly, can be easily available and affordable even in rural areas. They are more effective but less potential than synthetic drugs. Synthetic or chemical based drugs can interfere with the endocrine system and produce reproductive, neurological, developmental and metabolic effects in the body.9 These compounds may have negative effect on the synthesis, secretion, transport and activity of natural hormones. They disturb the normal hormone levels either by inhibiting the production and metabolism of hormones or by blocking hormonal action. It has been shown observed through various experimental on Animal model that herbal compounds have shown minimal side effects in comparison the chemically synthesized contraceptives which usually contain various combinations of hormones. So, the value of traditional knowledge for herbal contraception needs to be highlighted to the mass in order to make them more acceptable and practiced.10

# Materials and methods

## Materials, equipment and facilities

All chemicals, associated reagents, equipment and facilities for the *in-vivo* laboratory animal toxicity investigations and the bioactivity assays were obtained from the University of Zimbabwe,

Faculty of Medicine and Health Sciences laboratories, and the faculty of veterinary science laboratories.

## Animal use Approval

Prior to the investigations, animal use and research ethics approvals were obtained from the Joint Parirenyatwa Research Ethics Committee (JREC) which is the local research Institutional Review board for the University of Zimbabwe.

## *Pouzolzia mixta* Plant material collection and preparation

*Poulzozia mixta* was collected from Gutu Mupandawana area of Zimbabwe, 230 km southwards from the City of Harare. The plant material was authenticated by the National Herbarium and Botanical Garden in Harare, Zimbabwe. The roots were thoroughly washed using clean water to remove debris and other contaminants, shade dried at room temperature to constant weight for three weeks and then pulverized using mortar and pestle. The pulverized material was ground into a fine powder using a coffee grinder. The phyto-extraction was done by adding 300g of each plant sample powder into 1500ml of 70% (v/v) hydro-ethanolic mixture in a 2-litre sterile amber bottle and macerated for 3 days, with 3 minutes physical shaking twice a day. A muslin cloth was used to obtain a filtrate from each solution, which was further clarified by filtration using Whatman filter paper number 1. The filtrate was then evaporated under vacuum and low pressure (Rotavapor® R-300, Buchi, Switzerland), followed by lyophilization (Lyovapor l-200, Buchi, Switzerland) under 140Pa pressure and -50 °C. The lyophilized extracts were stored in an airtight sample bottle until required.

## Phytochemical Screening of *Pouzolzia Mixta* Extract

In a 200ml round bottomed flask, 10g of the lyophilized hydro-ethanolic extracts of *Poulzozia Mixta*  were dissolved in 100ml of distilled water and subjected to various phyto-screening techniques to confirm the presence or absence of relevant phytoconstituents of pharmacological interest that we wanted.11

### Detection for alkaloids by the Iodine test

The Iodine test was used to determine the presence of alkaloids. In this assay, to 3ml of the lyophilized extract solution, a few drops of iodine solution were slowly added along the sides of the test tube. The absence of alkaloids was noticed as there was no appearance of a blue colour, which must disappear on boiling and reappears on cooling .12

### Detection of tannins by the Braymer’s test

The simplified Braymer’s test was used to detect the presence of tannins. To 1ml lyophilised extract solution, 3 drops of a 10% Ferric chloride solution were added. The presence of tannins was confirmed by conversion of the solution to a blue green colour.13

### Detection of flavonoids by the Ammonia test

Flavonoids were detected by means of the Ammonia test where 5ml dilute ammonia solution was added to 5ml of the lyophilised solution followed by a few drops of conc. H2SO4. The emergence of a yellow colour indicates the presence of flavonoids.13

### Detection of Glycosides by the Keller-Killani test

The presence of glycosides was done by the Keller-Killani test . To 1mL of the lyophilised solution, 1.5mL glacial acetic acid was added and a few drops of 5% ferric chloride were added as well as conc. H2SO4 (along the side of test tube). The presence of glycosides was confirmed by the emergence of a blue-coloured solution in mixture acetic acid layer.13

### Detection of Phenolic compounds by the Gelatin test

Phenolic compounds were detected using the Gelatin test. In this assay, 2ml the lyophilised extract solution was added to 5ml of a 1% gelatin solution and 5 drops of a 10% NaCl were further added. Phenolics were identified by the appearance of a white precipitate.14

### **Detection of saponins by the simplified foam** test

The simplified foam test was used to determine the presence of saponins. In this assay 2ml of the extract was added to 20ml distilled water. The mixture was shaken in a graduated cylinder for 15 minutes. The presence of saponins would be confirmed by the formation of form with a head height of at least 1cm.15

### **Detection of Phytosterols by the Salkowski test**.

Sterols were detected using the Salkowski test. After the addition of chloroform and sulfuric acid, the presence of sterols was confirmed by the alteration of the colour from dark red to green

## Acute oral toxicity of *Poulzozia Mixta*

The acute oral toxicity evaluation of *Poulzozia Mixta* lyophilized extract was done using a modified OECD technical guideline 425 (The up and down test). Female nulliparous Wistar rats (4) were used, which were acclimatized to the test environment for 1day prior to the commencement of the test protocols. 16

In our test, sequential ordered progressions of doses were orally administered to the animals at 6-hour intervals. The animals were divided into 2 groups of 2 female rats each; the first group (group 1) received distilled water and served as the control group. The second (group 2) received incremental doses of the *Poulzozia Mixta* solution. The selected animals were marked so as to facilitate individual identification. The experimental animals were fasted for 24 hours with water prior to dosing. Initial starting doses were chosen based on related toxicological studies. The first animal received a dose of 300mg/kg body weight, which was below a randomly selected estimated LD50. When animals survived the dose, the next dose was doubled, subject to our observations of the test animals over a period of 6hours. The extractwas orally gavaged in a water solution in 4 different sets of doses of: 300,500, 1000, and 4000mg/kg body weight. The female rats were observed by a veterinary specialist for morbidity and mortality twice daily. In the absence of mortality, the rats were observed for any visible changes and clinical signs and symptoms of toxicity every 1 hour, and up to 12 hours on day 2. Animals were observed over 48 hours for behavioral changes and mortality.17

## Contraceptive activity

A total of 9 female rats, irrespective of stage of estrus cycle, were divided into 3 experimental groups of 3 animals/group. The rat treatment groups were as follows: Group 1- Control (CON = normal saline orally); Group 2- 300 mg/kg body weight *P.mixta* ethanolic extract orally (EtPM) and Group 3 -300mg/kg body weight aqueous extract orally(AqPM).18 The animals were treated for seven days then allowed to cohabit with males of proven fertility at a ratio of 1male:2 females on the 8th day. Animals were examined in a room with camera for successful mating. Animals with mucus plug were separated from male partners and this was considered day one of gestation (Desta, 1994). The mated animals were distributed into the treatment groups.19 Animal treatments were continued until day 10 of pregnancy. On the 10th day, female rats were euthanized by deep sodium pentobarbital (65mg/kg IP; Sigma) anesthesia. A caesarean dissection was performed on each female rat to expose the uterine horns. The uteri were examined for pregnancy status.

### Postcoital antifertility activity

Animals with regular oestrus cycle were mated as described above without pre-treatment with extract. Animals with confirmed successful copulation were separated from the male and treatment with saline (CON) or *P. mixta* (EtPM) at 300mg/kg was commenced on day-1 of pregnancy for 10 days.19, 20 On the 10th day, female animals were euthanized and dissected to examine pregnancy status and implantation sites.

### Data analysis

All experimental data were expressed as mean ± standard error of mean (SEM). Statistical comparison was performed using GraphPad InStat software version 3 using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparison test. Values were considered significantly different when P<0.005

# Results and Discussion

## Phytoscreening of Poulzozia *Mixta*

Preliminary phytochemical screening Qualitative phytochemical analysis of the extract revealed the presence of flavonoids, polypeptides, tannins and glycosides in both extracts. Alkaloids were not detected.

*Table 1 : Qualitative screening of Poulzozia Mixta secondary metabolites*

|  |  |  |
| --- | --- | --- |
| Test | Presence in hydro-ethanolic extract | Presence in distilled water extract |
| Alkaloids | - | - |
| Phytosterols | ++ | ++ |
| Flavonoids | +++ | + |
| Saponins | ++ | + |
| Phenolic compounds | ++ | + |
| Tannins | +++ | + |
| Glycosides | ++ | + |

*(-): shows the absence of the phytochemical, (+): showsthe presence of the phytochemical (+++): shows strong presence of the phytochemical, (++): shows moderate presence of the phytochemical*

## Acute toxicity

No behavioral changes and no mortalities were observed in all control and treated (EtPM) mice at both doses.

*Table 2: Acute oral toxicity study of Poulzozia Mixta behavioural Observations*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Observed parameter | Dose of *C. Poulzozia Mixta* in mg/kg body weight | | | | |
|  | **300mg** | **500mg** | **1000mg** | **4000mg** | **Control** |
| Food intake | Normal | Normal | Normal | Normal | Normal |
| Water intake | Normal | Normal | Normal | Normal | Normal |
| Death | Alive | Alive | Alive | Alive | Alive |
| Breathing | Normal | Normal | Normal | Normal | Normal |
| Diarrhea | Not observed | Not observed | Not observed | Not observed | Not observed |
| Urination | Normal | Normal | Normal | Normal | Normal |
| Skin colour | Normal | Normal | Normal | Normal | Normal |
| Drowsiness | Not observed | Not observed | Not observed | Not observed | Not observed |
| Erection of Fur | Not observed | Not observed | Not observed | Not observed | Not observed |

## Contraceptive activity and postcoital antifertility activity

Oral treatment with extract for 7 days before and oral treatment with extract for 10 days after confirmation of mating resulted in both aqueous and ethanolic P. mixta extracts at 300mg/kg b.wt inhibiting implantations in female rats. AqPM was more effective in inhibiting implantation compared to EtPM as all the rats in group 2 did not show any sign of pregnancy

*Figure 2: antifertility effects of the extracts*

### Estrogenic activity

Oral treatment with P.mixta extracts (AqPM and EtPM) at 300mg/kg b.wt caused a significant increase in weights in immature rats caused by increased uteri weight. However, uteri from EtPM treated immature rats showed a greater increase in weight compared to AqPM treated rats. Thus, EtPM exhibited greater estrogenic activity compared to AqPM. However, both extracts resulted in earlier opening of vaginas compared to controls

Preliminary phytochemical analysis of this plant showed presence of flavonoids, polypeptides, tannins and glycosides with undetected alkaloids and carbohydrates.20 The plant was safe in rats up to a dose of 4000mg/kg b.wt as determined by acute toxicity studies. Results of the present study demonstrated that *P. mixta* possesses primarily postcoital, estrogen with modest contraceptive activity. In order for implantation to occur, especially in the rat, the exact equilibrium of estrogen and progesterone hormones must be attained to create a milieu ideal for implantation Thus, *P. mixta* showed contraceptive activity with inhibition of implantation and fertility rates, ethanolic extract being more effective than the aqueous extract. The steroidal activity demonstrated in our study may be due to flavonoids detected in the phytochemical study. And indeed, the nonpolar ethanolic extract demonstrated a higher estrogenic activity than the polar water extract. Where previous studies have demonstrated high contraceptive activity associated with estrogenic activity our studies demonstrated modest contraception despite the potent estrogenic activity. Instead, we have demonstrated a highly effective postcoital and oxytocic activity. Postcoital activity is the mechanism used in emergency contraception to prevent pregnancy after unprotected sexual intercourse. Indeed, in agreement with our study, previous studies have demonstrated an association between postcoital activity with potent estrogenic and oxytocic activities. The rat endometrium is sensitive to blastocyst signals in the morning of day 5 but can experience failure of blastocyst implantation due to hostile uterine environment or hypermotility. Hypermotility may result in accelerated movement resulting in early arrival of the embryo to a non-receptive uterus, and failure of the blastocysts to implant.21 We propose that *P.* *mixta* estrogenic activity created unfavorable endometrial microenvironment while the oxytocic activity caused hyper motility of the myometrium also preventing implantation. According to some investigators, prolonged phytoestrogen therapy might present with health hazards similar to classic estrogens. With use of P. mixta in emergency contraception, exposure to phytoestrogens would not be prolonged and therefore deleterious effects of prolonged use may not be an issue.

We conclude that *P. mixta* has potential as an emergency contraceptive due to the demonstrated postcoital activity whose mechanism could be attributed to antiimplantation, estrogenic and oxytocic activity. The potential of this medicinal plant as a useful source of antifertility agent warrants further investigation.

# Conclusion

The aerial lyophilised hydro-ethanolic extracts of *Poulzozia mixta* were shown to possess considerable post-coital and contraceptive activities. The observed activities were attributable to the presence of secondary metabolites including phenolic compounds, flavonoids, and phytosterols. These contribute to the underlying mechanisms behind the plant’s proven antifertility effects and therapeutic activities in *Poulzozia mixta*. Our biosafety and bioactivity studies therefore authenticate the use of *Poulzozia Mixta* as a potential emergency contraception in traditional medicine.

# Disclaimer (Artificial intelligence)

The Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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