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Evaluation of Hydroalcoholic Extract of Leaves of *Buddleja asiatica* for its Abortifacient Activity on *Poecilia sphenops*

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Abstract:

Background: *Buddleja asiatica* (Scrophulariaceae), native to sub-Himalayan regions, contains flavonoids, saponins, lignans, and terpenoids with traditional uses for respiratory ailments and folklore abortifacient properties. Induced abortion seeks safer herbal alternatives to synthetic drugs like mifepristone and misoprostol due to side effects. **Aim:** The main aim of this study is to evaluate abortifacient activity of hydroalcoholic leaf extract of *B. asiatica* in pregnant *Poecilia sphenops* (molly fish) and compare with standards. **Method:** Leaves were shade-dried, extracted with 70% hydroalcohol, and screened for phytochemicals (glycosides, carbohydrates, saponins, flavonoids, phenolics confirmed present). In this experimental model, young female *P. sphenops* were reared individually under controlled aquatic conditions until adulthood. Sexually mature males were introduced for mating and subsequently removed, after which females were monitored for pregnancy. On the day of experimentation, 24 confirmed pregnant females were selected and randomly divided into four groups, each maintained in separate tanks. Group I served as the control. Group II (Standard I) received mifepristone (20 mg/kg), Group III (Standard II) received misoprostol (80 µg/kg), and Group IV (Test) received the test extract of *Buddleja asiatica* (500 mg/kg). All treatments were administered along with food pellets. Abortifacient activity was assessed by recording the time to abortion, the percentage of dead fry, and the percentage of live fry, employing this model as an ethical alternative to conventional rodent-based research. **Result:** The extract induced abortion at 34.17±3 hours (vs. 25.33±1.54 for mifepristone, 23.5±1.38 for misoprostol; control: none), with 68.05±13.98% dead fries and 15.45±5.03% live fries (comparable to standards: 81.92±2.86 for mifepristone, 84.14±2.20% dead for misoprostol, as well as 15.85±2.20 for misoprostol, 18.07±2.86 % live for mifepristone), which was statistically highly significant ($p < 0.01$ vs. control). **Conclusion:** Hydroalcoholic extract of leaves of *B. asiatica* shows significant abortifacient activity via phytochemicals disrupting pregnancy, comparable to standards, validating traditional use and needing further mechanistic/safety studies.

Major Finding: The extract contained glycosides, carbohydrates, flavonoids, phenolics, and saponins. *B. asiatica* demonstrated significant abortifacient activity compared with the control and produced outcomes comparable to standard drugs, though with slightly delayed abortion onset.

Keywords: Abortifacient Activity, *B. asiatica*, Fish Model, Hydroalcoholic Extract, Phytochemicals

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1. Introduction

Abortion is defined as the termination of a pregnancy by the removal or expulsion of an embryo or fetus from the uterus. The term "abortion" is commonly used without qualification and may refer either to induced abortion or to spontaneous abortion (miscarriage)[1]. Spontaneous abortion is an early pregnancy loss that

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occurs without deliberate intervention, often without an identifiable cause or overt symptoms, and typically before 20 weeks of gestation[2]. Several clinical forms of spontaneous abortion have been described, including early pregnancy loss and threatened abortion. In contrast, induced abortion is intentionally undertaken for medical, social, or personal reasons, and may be performed as a medical (pharmacological) or surgical procedure depending on the gestational age and clinical status[3]. Common reasons for induced abortion include inability to care for a child, concerns regarding maternal health, lack of social or financial support, career considerations, and pregnancies resulting from incest or sexual violence.

Unsafe abortion remains a major public health problem worldwide and has been identified as a significant cause of maternal morbidity and mortality by the World Health Organisation (WHO)[4-7]. Ensuring access to safe abortion requires effective and acceptable medical methods. Although several pharmacological agents are currently available for medical termination of pregnancy, their use can be associated with adverse effects and contraindications. In this context, herbal medicines have attracted considerable interest as potential complementary or alternative options. Plant-based abortifacient agents, if proven effective and safe, may provide additional choices, particularly in resource-limited settings.

Herbal medicines are preparations derived directly from plants and used for the prevention or treatment of disease within traditional and contemporary medical systems. The use of medicinal plants dates back at least 5,000 years, as documented in early written records[8]. Herbal medicine (herbalism) relies on whole plants or plant extracts, which may be administered orally, topically, or by other routes. Historically, many modern drugs originated from plants, such as morphine, which was isolated from the seed pods of the opium poppy approximately 200 years ago[9]. Despite the development of numerous synthetic drugs, issues such as antimicrobial resistance, side effects, and high cost continue to drive interest in phototherapeutics.

Globally, medicinal plants are used for a wide range of conditions, including arthritis, wounds, burns, and fatigue, often at a lower cost than conventional therapies. It is estimated that 75-80% of the world's population relies, at least in part, on traditional herbal medicines due to their cultural acceptability, perceived compatibility with the human body, and relatively fewer side effects[10]. In several countries, including Germany and France, standardised herbal preparations are integrated into mainstream healthcare, and in the United States, herbal products are widely marketed, often as dietary supplements[11].

India has a long-standing tradition of herbal medicine, particularly within systems such as *Ayurveda*, *Siddha*, and *Unani*, and is rich in biodiversity with numerous medicinal plants, especially in the Himalayan and sub-Himalayan regions. In many rural and remote areas, plant-based remedies are commonly used where access to modern healthcare and pharmaceuticals is limited[12]. Moreover, several conventional allopathic drugs have been derived from Indian medicinal flora. In settings with poor healthcare infrastructure, women may resort to herbal preparations for inducing abortion, especially when safe medical or surgical abortion services are unavailable or unaffordable. However, many marketed abortifacient drugs and unsupervised herbal remedies carry the risk of significant side effects and complications[13]. There is therefore a need to systematically evaluate plant-based abortifacient agents for efficacy and safety.

The genus *Buddleja* (family *Scrophulariaceae*) comprises approximately 150 species, many of which are distributed in the sub-Himalayan tracts of Asia, as well as in Africa and the Southern regions of the United States[14]. Species of *Buddleja* are generally deciduous shrubs characterised by simple leaves and aromatic tubular flowers[14,15]. Members of this genus contain diverse bioactive phytoconstituents and have been reported to exhibit various pharmacological activities. *Buddleja asiatica* (*B. asiatica*), in particular, has been used traditionally by local communities in mountainous regions for multiple purposes, including as an abortifacient[16]. Given the widespread reliance on herbal remedies and the need for safer abortifacient options, scientific evaluation of such traditional claims is warranted.

In this context, the present study was designed to investigate the abortifacient potential of *B. asiatica* leaves using appropriate experimental models, to provide a scientific basis for its traditional use and assess its suitability as a candidate for safer plant-based abortifacient therapy.

2. Materials and Methods

2.1 Collection of Samples

The leaves of *B. asiatica* were collected from the Himalayan region and transported to the Netaji Subhas Chandra Bose Institute of Pharmacy.

2.2 Preparation of Leaves Extract[17]

The collected leaves of *B. asiatica* were thoroughly washed with distilled water to remove surface impurities and subsequently shade-dried for 7-10 days. The dried material was then accurately weighed, coarsely powdered, and macerated in 70% hydroalcoholic solvent for 48 hours with intermittent shaking. Following maceration, the extractive solution was filtered, and the solvent was evaporated to dryness under controlled conditions to obtain the crude extract. The resulting concentrated extract was transferred to an airtight container and stored at 4 °C until further experimental use.

2.3 Phytochemical Screening of the Sample[18,19,20]

To identify the presence of bioactive phytochemical constituents, qualitative phytochemical screening was performed on the crude extract using standard procedures.

2.4 Animals

A novel experimental model using young female *P. sphenops* (molly fish) was employed to evaluate the abortifacient activity of the extract. *P. sphenops* is a widely distributed freshwater and brackish-water teleost, originally native to Mexico and Colombia, but now abundant in India due to its low cost, ease of maintenance, and high reproductive efficiency. The species is viviparous, with embryonic development occurring internally within the maternal ovary, resulting in the birth of fully formed fry. Female mollies (average size ~12 cm) are larger than males (~8 cm) and can be distinguished by the absence of a gonopodium, a modified anal fin present in males that functions as an intromittent organ for sperm transfer. Pregnancy in females can be reliably identified 12-15 days post-fertilisation by the appearance of a darkened and enlarged gravid spot, along with a significant abdominal distension (up to 50% increase in body weight). The species exhibits a gestation period of 40-60 days, producing approximately 20-100 live fry per brood. Notably, *P. sphenops* lacks parental care, often preying upon its own offspring, which facilitates accurate assessment of reproductive outcomes in controlled laboratory settings.

2.5 Preparation of Drug-loaded Food Pellets[21]

Commercially available fish feed was finely triturated and thoroughly mixed with the latex powder. For the preparation of the test formulation, 0.20 g of fish feed was combined with 0.20 g of *B. asiatica* leaf powder. A small quantity of Tween 80 was added as a binding agent to facilitate the formation of granules (beads) of the test diet. The resulting beads were then dried in a hot air oven for a few minutes, with drying time adjusted according to the moisture content under aqueous conditions.

2.6 Experimental Procedure

In this model, young female *P. sphenops* (1-2 months old) were initially maintained in separate holding tanks until sexual maturity was attained. Water quality parameters were controlled throughout the study, with pH maintained between 7.5 and 8.5 and temperature between 24°C and 28°C. At approximately six months of age, mature females (1.5-2.0 g) were introduced into breeding tanks with mature males (approximately one year old) in a 1:2 male-to-female ratio for a mating period of 0-3 days. Following this period, males were removed, and the females were observed from Day 4 to Day 19 for signs of pregnancy.

On Day 20, visibly pregnant females (2.0-2.5 g) were selected and randomly allocated into three groups (n=6 per group). Each female was housed in an individual tank to avoid confounding behavioural interactions. Group I served as the untreated control and received no intervention. Group II, designated as Standard I, received the reference abortifacient agent Mifepristone (20mg/kg), administered along with standard food pellets. Group III, designated as Standard II, received a second reference abortifacient agent, Misoprostol (80mcg/kg). Group IV, designated as Test, received the test drug (500mg/kg) along with food pellets. All treatments were administered for a continuous 72-hour observation period.

Throughout the study, the following reproductive endpoints were recorded: Time to abortion, number of dead fries delivered, and number of live fries produced. These parameters were used to assess and compare abortifacient activity across the groups.

2.7 Statistical Analysis

Data are expressed as Mean ± SEM. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) to compare differences among experimental groups (control and treatment groups), followed by Dunnett's test. A value of $p < 0.01$ was considered statistically significant.

3. Result and Discussion

3.1 Phytochemical Investigation

Phytochemical screening of the hydroalcoholic extract of *B. asiatica* leaves revealed the presence of multiple classes of bioactive constituents (Table 1).

3.2 Assessment of Abortifacient Potential

The data presented in the table demonstrate that the hydroalcoholic extract of *B. asiatica* leaves exhibits significant abortifacient activity in *P. sphenops*. Observations recorded across the three treatment groups were systematically compared with those of the untreated control group. For enhanced clarity, all measured reproductive parameters relevant to abortifacient assessment were analysed and compared in a structured manner.

The primary parameter evaluated was the "Time to abortion", which represents the most critical indicator of abortifacient efficacy. As expected, the untreated control group did not exhibit abortion, as no abortifacient agent was administered. In contrast, the treatment groups receiving mifepristone and misoprostol demonstrated markedly reduced abortion times of 25.33 hours and 23.5 hours, respectively. The hydroalcoholic extract of *B. asiatica* also induced abortion, with a recorded mean abortion time of 34.17 hours (Table 2, Figure 1).

The second parameter assessed was the "% of dead fries" produced. The *B. asiatica* extract resulted in a substantial proportion of dead fry (68.05%), while misoprostol and mifepristone produced higher percentages of 84.14% and 81.92%, respectively, further supporting their comparatively stronger abortifacient effects (Table 2, Figure 2).

The third and most critical parameter, "% of live fries", was also assessed. As expected, the untreated control group, which did not receive any abortifacient intervention, produced 100% live fry. In contrast, the standard abortifacient agents, mifepristone and misoprostol, resulted in markedly reduced percentages of live fry, at 18.07% and 15.85%, respectively. The hydroalcoholic extract of *B. asiatica* produced 15.45% live fry, demonstrating a response comparable to that of the standard drugs and further indicating its potential abortifacient efficacy (Table 2, Figure 3).

The present study evaluated the abortifacient activity of the hydroalcoholic leaf extract of *B. asiatica* using *P. sphenops* as an experimental model and compared its effects with established pharmacological abortifacients, mifepristone and misoprostol. The findings clearly demonstrate that the plant extract possesses significant abortifacient potential, supporting its traditional use in indigenous medicinal systems[22].

A complete absence of abortion in the untreated control group confirmed that *P. sphenops* does not undergo spontaneous pregnancy termination under

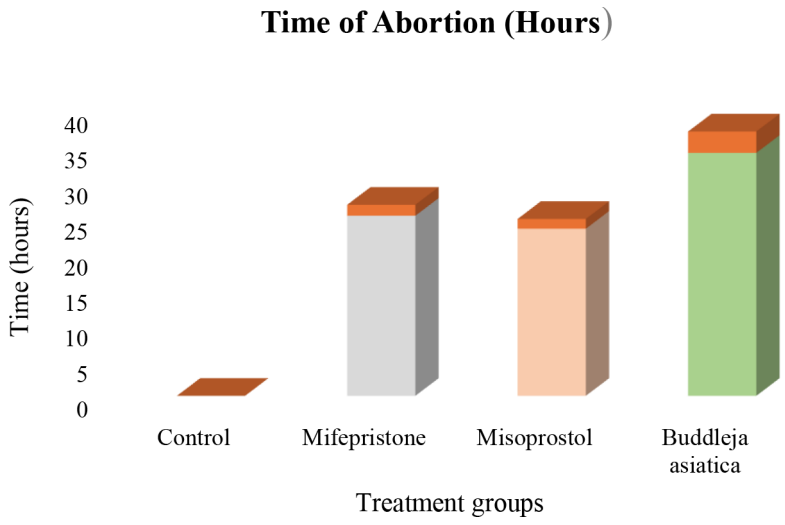
Table 1. Preliminary phytochemical investigation results of the hydro-alcoholic extract of *B. asiatica* leaves

Sl. No.	Phyto constituents	Test Name	Result
1	Glycoside	Fluorescence test	+
2	Carbohydrates	Molisch's test	+
3	Saponin	Foam test	+
4	Flavonoids	Alkaline test	+
5	Phenolic	Ferric test	+

Table 2. A comparative analysis of abortifacient activity among mifepristone, misoprostol, and *Buddleja asiatica* treated groups

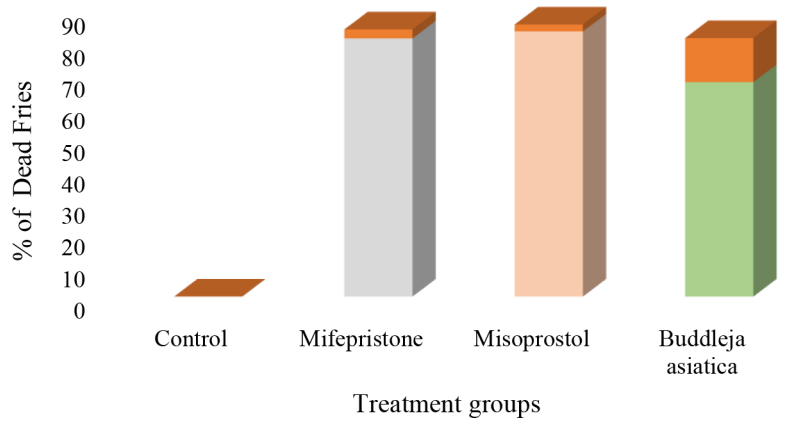
Treatment received by animals	Time of abortion (hr)	% of dead fries	% of live fries
Control	00	00	100
Mifepristone	25.33±1.54**	81.92±2.86**	18.07±2.86**
Misoprostol	23.5±1.38**	84.14±2.20**	15.85±2.20**
Buddleja asiatica	34.17±3**	68.05±13.98**	15.45±5.03**

All the values are Mean ± SEM, n=6, n means subjects, **p<0.01 vs control



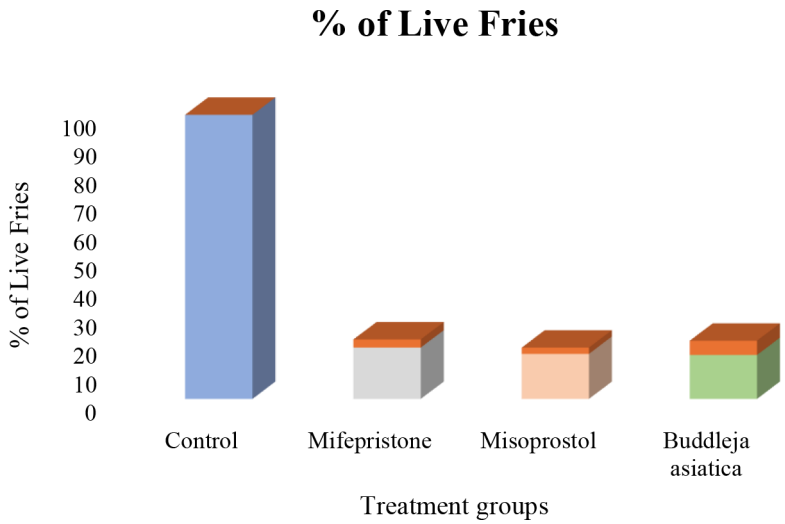
[All the values are Mean ± SEM, n=6, n means subjects]

Figure 1. Comparison among control, mifepristone, misoprostol, and test drug on time of abortion (hours).



[All the values are Mean ± SEM, n=6, n means subjects]

Figure 2. Comparison among control, mifepristone, misoprostol, and test drugs on % of dead fries.



[All the values are Mean ± SEM, n=6, n means subjects]

Figure 3. Comparison among control, mifepristone, misoprostol, and test drugs on % of live fries.

experimental conditions, establishing a reliable baseline. In contrast, the standard drugs produced rapid abortion, 23.5 hours for misoprostol and 25.33 hours for mifepristone, which aligns with their known mechanisms involving progesterone receptor antagonism and prostaglandin-mediated uterine contractions. The extract of *B. asiatica* induced abortion at 34.17 hours, demonstrating significant activity, albeit with a slightly delayed onset compared to synthetic agents.

The percentage of dead fry further substantiated the abortifacient effect. While misoprostol and mifepristone yielded 84.14% and 81.92% dead fry, respectively, the extract produced 68.05%, indicating strong efficacy, though somewhat lower than the pharmacological standards. Notably, the percentage of live fry from the *B. asiatica* group (15.45%) closely resembled that of mifepristone (18.07%) and misoprostol (15.85%), reinforcing the extract's potential to disrupt normal gestation outcomes effectively. Collectively, these outcomes highlight that the extract acts in a manner comparable to recognised abortifacients.

The biological activity of *B. asiatica* may be attributed to its diverse phytochemical profile, including saponins, flavonoids, phenolics, carbohydrates, and glycosides detected in the extract. These classes of compounds have been widely reported to exhibit antifertility, uterotonic, and embryotoxic activities[23-27]. Saponins, for example, are known to alter uterine tone and stimulate prostaglandin release; flavonoids possess estrogenic or anti-progesterogenic activity capable of disrupting reproductive hormonal balance; alkaloids and tannins have been associated with smooth muscle contraction and uterine irritation, while certain glycosides exert direct embryotoxic or placental toxicity[25-27]. Together, these constituents may act synergistically, producing a multifaceted abortifacient response, though possibly with slower kinetics than single-target synthetic drugs.

The slightly prolonged abortion time observed with the extract suggests that the phytochemicals act through combined but gradual mechanisms rather than a single dominant pathway. Nonetheless, the overall efficacy closely parallels that of mifepristone and misoprostol, indicating that *B. asiatica* may represent a viable natural alternative for fertility regulation[28], particularly in settings where access to modern healthcare is limited.

4. Conclusion

The hydroalcoholic leaf extract of *B. asiatica* demonstrated significant abortifacient activity in *P. sphenops*, as evidenced by reduced abortion time, high percentages of dead fry, and minimal live fry production compared with the untreated control. Although the onset of abortion was slightly delayed relative to standard agents such as mifepristone and misoprostol, the overall efficacy of the extract was comparable. These findings support the traditional claims regarding the abortifacient properties of *B. asiatica* and highlight its potential as a natural alternative for fertility regulation. Further studies focused on isolating active constituents, elucidating underlying mechanisms, and establishing safety profiles are warranted to advance its potential therapeutic application.

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Author Contribution

Mr. SR, Dr. MM, Ms. SG, and Mr. PR contributed to the conception, design, and execution of the research work. Mr.IB, Ms. PS, Mr. MM and Mr. PS contributed to manuscript writing, discussion, and editing. All authors have read and agreed to the published version of the manuscript.