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Original Article

Preliminary Phytochemical Screening and Evaluation of *in vitro* Anthelmintic Activity of *Blepharis maderaspatensis* Leaf

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ABSTRACT

Background: Helminth infections continue to be a major public health and the veterinary problem, especially in the tropical and subtropical regions. In recent years, the resistance is commonly used anthelmintic drugs has increased which has reduced their therapeutic effectiveness. Because there is a need to explore alternative sources of antiparasitic agents. Medicinal plants that contain a wide range of secondary metabolites and considered a promising source for the development of new anthelmintic compounds. *Blepharis maderaspatensis* is a medical plant traditionally used in indigenous medicine for treating inflammatory conditions and wound healing, but its anthelmintic activity has not been studied extensively. **Purpose:** The aim of this study was to perform a preliminary phytochemical analysis of *Blepharis maderaspatensis* leaf extracts prepared by using solvents with different polarities and to evaluate the *in vitro* anthelmintic activity using Phereetima posthuma as the experimental model. **Materials and Methods:** The shade dried leaf powder was extracted sequentially using petroleum ether, chloroform, ethyl acetate, ethanol and distilled water. Qualitative phytochemical screening was carried out using standard chemical tests to identify the major secondary metabolites present in the extracts. The *in vitro* anthelmintic activity was evaluated by recording the time taken for paralysis and death of the adult earthworms. The three worms were used in each treatment group and all experiments were performed in triplicate. Piperazine citrate was used as the standard reference drug. **Results:** The phytochemical analysis showed the presence of flavonoids, alkaloids, tannins, phenolic compounds, glycosides, steroids, terpenoids and anthraquinones in different extracts. Among all, the ethanolic extract showed the highest anthelmintic activity, cause the paralysis at 12.08 sec and death at 20.50 sec. The chloroform extract showed moderate activity, while the ethyl acetate extract had the least effect. Interestingly, ethanolic extract produced faster paralysis compared to piperazine citrate, suggesting strong neuromuscular interface. **Conclusion:** The results of this study show the *Belpharis maderaspatensis* has significant *in vitro* anthelmintic activity, with the ethanolic extract showing better effectiveness than the other extracts. This activity may be due to the combined action of different phytoconstituents present in the plant. Further studies, including isolation of active compounds, dose response evaluation and *in vivo* studies are needed to confirm its therapeutic potential.

Keywords: Anthelmintic Activity, Anthelmintic Resistance, *Blepharis maderaspatensis*, Medicinal Plants, Pheretima Posthuma, Phytochemical Screening.

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INTRODUCTION

Helminthiasis remains one of the most common parasitic diseases that affecting both humans and animal populations around world-wide. Parasitic helminths cause the long term of infections and particularly in the tropical and subtropical regions where sanitation and hygiene infrastructure are inadequate (Hotez, 2008). Soli-transmitted helminths such as *Ascaris Lumbricoides*, *Trichuris trichiura* and hookworms infect hundreds of millions of people globally and represent a major group of neglected tropical diseases (Buonfrate *et al*., 2023). Persistent infections are associated with the several adverse health outcomes including anemia, malnutrition, impaired cognitive performance, delayed physical development and especially among children living in endemic regions (Brooker *et al*., 2006).

In veterinary medicine and gastrointestinal nematode infections significantly affect live stocks productivity. These infections can lead to weight loss and reduced feed conversion efficiency, decreased milk production and increased susceptibility to secondary infections, ultimately resulting in considerable

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economic losses the agricultural sector (Mavrot *et al*., 2015). The environmental resilience of helminth eggs and larvae further complicates and control strategies and the infective stages can survive in soil for the long period of time and continue the cycle of transmission (Buonfrate *et al*., 2023). Therefore, chemotherapy remains the primary approach for the management and control of helminth infections in both the human and veterinary health systems (Geary, 2005).

Synthetic anthelmintic agents such as benzimidazoles, imidazothiazoles and macrocyclic lactones have been widely used for many years to treat the helminth infections (Wolstenholme *et al*., 2016). Although these drugs were initially highly effective frequent and sometimes indiscriminate use has resulted in the rapid development of resistance among parasite populations. Several mechanisms of resistance have been reported in helminths and including mutations in the drug binding targets such as beta-tubulin, increased expression of drug efflux transporters and alteration in the neuromuscular receptor sensitivity then enhanced metabolic detoxification pathways that reduce drug susceptibility (Kotze, 2016). Increasing reports of resistance in several helminth species particularly those affecting livestock have created serious challenges for effective parasite control (Ng'etich *et al*., 2023). This growing concern has encouraged researchers to explore alternative or complementary therapeutic approaches. Natural products derived from the medical plants are now widely recognized as valuable sources of the biologically active compounds with potential antiparasitic activity (Newman and Cragg, 2020)

Medicinal plants produce a variety of secondary metabolites that function as ecological defense compounds against herbivores, pathogens and environmental stress (Upadhyay *et al*., 2024). Many of the phytochemical possess pharmacological properties that can glycoproteins present in the cuticle of nematodes causes structural damage and impaired nutrient absorption (Williams *et al*., 2014). Alkaloids may interfere with the neuromuscular transmission which can ultimately lead to paralysis of the parasite (Graham *et al*., 1999). Flavonoids have also been shown the inhibit mitochondrial respiration and reduce ATP production and thereby affection parasite motility and the survival. In additions terpenoids and phenolic compounds can destabilize cellular membranes and induce oxidative stress in parasitic organisms (Tang *et al*., 2024). The presence of multiple phytochemical groups within plant extracts may produce synergistic or a additive biological effects that can enhance anthelmintic activity and reduce the likelihood of resistance development (Gorter *et al*., 2015).

Blepharis maderaspatensis (Family: Acanthaceae) is a medicinal herb commonly found in South India and several other tropical regions. Traditionally, the plants has been used in different systems of the medicine for the treatment of inflammatory conditions are wound healing, UTI disorder and certain infectious disease conditions (Shedoeva *et al*., 2019). Ethnobotanical records and pharmacognostic studies have reported the presence of various bioactive phytoconstituents in these plants and including flavonoids and henolic compounds, steroidal constituents and glycosides (Riaz *et al*., 2023). However, despite the presence of these pharmacologically active compounds and systematic scientific studies evaluating the anthelmintic potential ok this plant remains limited. Considering the increasing interest in plant-based therapeutic agents and the presence of secondary metabolites with known antiparasitic properties and further investigation of this species appears necessary (Belga *et al*., 2024).

Therefore, the present study was carried out to the investigate phytochemical composition and anthelmintic potential of *Blepharis maderaspatensis* leaves. This study aimed to perform qualitative phytochemical screening of solvent extracts prepared using solvents with different polarities and to evaluate their *in vitro* anthelmintic activity using *Pheretima posthuma* as an experimental model. In addition of the relative effectiveness of the different solvent extracts was compared to better understand the relationship between phytochemical constituents and the observed biological activity.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh leaves of *Blepharis maderaspatensis* were collected from naturally growing populations in Tamil Nadu, India during the appropriate vegetative season to obtain plant material with optimal phytochemical content. The collected plant specimens were taxonomically identified and authenticated by a qualified botanist through detailed examination of macroscopic morphological characteristics and including leaf arrangements and floral structure, other diagnostic features typical of members of the family *Acanthaceae* (Tripp *et al*., 2021). After authentication, a voucher specimen was prepared and properly labelled and deposited in the departmental herbarium for future reference and verification of plant identity. The preparation and preservation of voucher specimens are considered important steps in pharmacognostic studies and accurate botanical identification ensures the reproducibility and reliability of experimental findings (Upton *et al*., 2019).

Processing of Plant Material

The freshly collected leaves were thoroughly washed with distilled water to remove soil particles and dust then other unwanted materials. The cleaned plant material was shaded and dried at an ambient temperature between 25-30ºC under the well ventilate conditions. Shaded drying was used to prevent photo and thermal degradation of thermolabile phytochemical constituents that may occur under direct sunlight (Masih and Singh, 2011). After complete drying process and the leaves were powdered using a mechanical grinder to obtain a coarse powder.

The powdered material was then passed through a suitable sieve to obtain a uniform particle size and which helps to improve solvent penetration during the extraction. The processed plant powder was stored in air tight container and protected from the light and moisture until further uses. Proper drying and storage conditions are essential for maintaining phytochemical stability and preventing microbial contamination before extraction (Devi and Kumar, 2023).

Preparation of Extraction

Rationale for sequential extraction

Plant materials contain a wide range of phytochemical constituents that differ in polarity and solubility, molecular weight. Sequential extraction using solvents with gradually it increasing polarity helps in the systematic separation of these compounds based on their physicochemical properties. This method allows efficient extraction of a broad range of phytoconstituents and also it enables comparison of biological activity among different solvents and its extracts (Houghton and Raman, 1998).

Extraction Procedure

The powdered leaves of *Blepharis maderaspatensis* were subjected to successive solvent extraction using solvents arranged in increasing order of polarity. Initially, the extraction was carried out using petroleum ether to obtain the non-polar fraction and followed by chloroform as a relatively low polarity solvent and ethyl acetate as an intermediate polarity solvent, ethanol for moderately polar compound and finally distilled water to extract highly polar constituents (Offeman *et al*., 2006).

For each extraction steps, plant powder was soaked in the respective solvent and the mixture was allowed to stand with intermittent shaking to enhance solvent penetration and facilitate the dissolution of phytochemical constituents. After an adequate extraction period and the mixture was filtered to separate the liquid extract from the remaining plant residue. The filtrates obtained from each solvent system were then concentrated to dryness and stored in airtight containers until further experimental evaluation.

In addition to the solvent extracts and the dried leaf powder was also tested separately to determine whether the crude plant material itself could exhibit intrinsic anthelmintic activity without solvent mediated concentration of active constituents. Sequential extraction is widely used in phytochemical studies because non polar solvents generally isolate lipophilic compounds such as terpenoids and sterols, where solvents with intermediate and higher polarity extract compounds such as flavonoids, phenolics, glycosides and other polar phytoconstituents (Houghton and Raman, 1998).

Preliminary phytochemical screening

Qualitative phytochemical analysis of the prepared extracts is carried out to the identify the major classes of secondary metabolite present in the plant material. Standard chemical tests described in pharmacognostic literature were used to detect phytoconstituents such as alkaloids, flavonoids and tannins, glycosides, phenolic compounds, terpenoids and steroids. These tests are based on characteristics color changes or precipitate formation that occurs when specific reagents react with particular classes of the phytochemicals. The screening procedures were performed according to the established methods described in classical pharmacognosy references (Thangaraj, 2015).

In vitro Anthelmintic Activity

Selection of Experimental Model

Adult Indian earthworms (*Pheretima posthuma*) were selected as the experimental model for evaluating anthelmintic activity. Earthworms are widely used in preliminary anthelmintic studies because their anatomical and physiological characteristics are similar to those of intestinal parasitic nematodes. Moreover, these organisms are sensitive to several standard anthelmintic drugs and which makes them suitable surrogate models for the initial screening of potential anthelmintic agents (Kalmobe *et al*., 2017). The neuromuscular coordination and the cuticular structure of earthworms closely resemble those of parasitic helminths and allowing reliable observation of paralysis and mortality during experimental evaluation (Gardner, 1976).

Experimental Design

The experimental study included several treatment groups corresponding to the different extracts obtained from *Blepharis maderaspatensis*. These groups consisted of petroleum ether extract, ether extract, chloroform extract, ethyl acetate extract, ethanolic and aqueous extract and crude dried powder of the lant material. In addition, a standard drug group containing piperazine citrate and a control group containing distilled water were included for comparison.

Three worms were used in each experimental group. To improve the reliability of the observations and the minimize random variation and the entire experiment was carried out in triplicate. Mean values for each parameter were calculated from three independent experimental trials. A fixed concentration of each extract was maintained across all treatment group to ensures the proper comparability of the observed anthelmintic activity (Kumarasingha *et al*., 2016).

Evaluation Parameters

The anthelmintic activity of the plants extracts was evaluated by observing two main parameters; time to paralysis and time to death of the worms. Time to paralysis was defined as the period required for the complete cessation of spontaneous movement of the worm and except when stimulated by stronger anthelmintic activity of the tested extracts (Davuluri *et al*., 2019).

Statistical Expression of Data

All experimental observations were expressed as mean values obtained from three independent experimental trials. As the study was preliminary in nature and involved a relatively small sample size and descriptive comparison of the recorded parameters was used to assess the relative effectiveness of the different extracts.

Comparative Reference Standard

Piperazine citrate was used as the reference standard drug for comparing the anthelmintic activity of the plant extracts. Piperazine produces its pharmacological effects and including flaccid paralysis of helminths through Gamma-Aminobutyric Acid (GABA) mediated neuromuscular blockade and which facilitates the expulsion of parasites from the host intestine (Becher, 2000).

Experimental Reliability Considerations

Several precautionary measures were taken to maintain the experimental consistency and reduce variability in the observations. Worms of approximately similar size were selected to minimize biological variation among the test organisms. Freshly collected earthworms were used in each experimental trial to ensure the vitality and responsiveness (Gibbs *et al*., 1996). All experiments were carried out under similar environmental conditions and observations were recorded carefully and consistently to reduce subjective bias while determining the paralysis and death end points (Suthar, 2013).

RESULTS

Preliminary Phytochemical Screening

Qualitative phytochemical investigation of the different solvent extracts of *Blepharis maderaspatensis* leaves revealed the presence of several classes of secondary metabolites. Among the extracts and ethanolic extract showed the widest range of phytochemical constituents and including flavonoids, alkaloids, tannins, phenolic compounds. Steroids, glycosides, anthraquinones and terpenoids. This indicates that ethanol was an effective solvent for the extracting a broad spectrum of bioactive compounds from the plant material (Alamgir, 2018).

The extracts obtained using chloroform and petroleum ether mainly contained steroidal and terpenoid compounds. This observation is consistent with the relatively low polarity of these solvents and their ability to dissolve lipophilic phytoconstituents (Alamgir, 2018). In contrast, the aqueous extract primarily showed that the presence of polar constituents such as glycosides and the carbohydrates and while fewer non-polar metabolites were detected.

The crude dried powder of the plant materials and also showed detectable levels of several phytochemical classes, suggesting that biologically active compounds are naturally present in the plant matrix even before solvent extractions. The presence of diverse phytochemical constituents in the extracts may contribute to the biological activity observed during the pharmacological evaluation, as that combined action of the multiple secondary metabolites can produce synergistic or additive effects (Tables 1 and 2) (Geilfus, 2019).

In vitro Anthelmintic Activity

The *In vitro* anthelmintic activity of the different extracts of *Blepharis maderaspatensis* was evaluated using adult Indian earthworms (*Pheretima posthuma*) as the experimental model. The anthelmintic effectiveness of the extracts was determined by measuring the mean time required to induce paralysis and death of the worms. In this experimental model and shorter times taken to produce paralysis and death indicate stronger anthelmintic activity of the tested extract (Singh *et al*., 2013).

Table 1: **Preliminary Phytochemical Screening of Different Extracts of *Blepharis maderaspatensis* Leaves.**

Sl. No.	Phytochemical	Dry Sample	Ethyl Acetate	Petroleum Ether	Chloroform	Ethanol	Water
1.	Terpenoids	+	+	+	+	+	+
2.	Flavonoids	+	+	+	+	+	+
3.	Steroids	+	+	+	+	+	+
4.	Anthraquinones	+	+	+	+	+	+
5.	Glycosides	+	+	+	+	+	+
6.	Carbohydrates	+	-	-	+	-	+
7.	Alkaloids	+	+	+	+	+	+
8.	Phenols	+	+	-	+		+
9.	Tannins	+	+	+	+	+	+
10.	Proteins	+	+	+	+	+	+

Legend: (+) Presence of phytochemical constituent; (-) Absence of Phytochemical constituent.

Table 2: Effect of Different Extracts of *Blepharis maderaspatensis* Leaves on Paralysis and Death Time of *Pheretima posthuma*.

Sl. No.	Groups	Treatment	Time for Paralysis (sec)	Time for Death (sec)
1.	Group I	Distilled Water (Control)	Nil	Nil
2.	Group II	Dried Powder	255±10.69	340±7.57
3.	Group III	Aqueous Extract	2766.33±77.42	3213.66±21.30
4.	Group IV	Ethanol Extract	12.08±1.20	20.89±0.91
5.	Group V	Chloroform Extract	21.30±0.65	32.98±1.13
6.	Group VI	Ethyl Acetate Extract	64.58±0.71	67.04±0.73
7.	Group VII	Petroleum Ether Extract	32.95±0.96	48.53±0.93
8.	Group VIII	Piperazine Citrate (Standard)	1342.66±24.31	3623.66±28.26

Values are expressed as Mean+ SEM (*n*=3).

Comparative Activity Analysis

Comparative evaluation of the recorded paralysis and the death times showed clear differences in the anthelmintic potency of the various extracts. Among all the tested samples and the ethanolic extract produced the fastest onset of both paralysis and death in *Pheretima posthuma*. The paralysis time observed for the ethanolic extract was significantly shorter that that recorder for the standard drugs piperazine citrate and indicating a strong neuromuscular effect of the worms and suggesting notable anthelmintic potential (Gagman *et al*., 2022).

The chloroform extract showed moderate activity and producing paralysis and the death times that were shorter than those of several other extracts but still longer than those observed with the ethanolic fraction. The petroleum ether extracts also demonstrated moderate efficacy, whereas the ethyl acetate extract exhibited comparatively weaker activity. This observation suggests that compounds of intermediate polarity may contribute less to the anthelmintic activity of the plant.

The dried crude powder showed moderate activity and it indicating that the active constituents are naturally present within the plant material. However, solvent extraction appears to enhance the availability and concentration of these bioactive compounds (Hait *et al*., 2024). The aqueous extract displayed the least activity and which may be due to the lower efficiency of highly polar solvents in extracting key anthelmintic phytoconstituents.

Figures illustrating the comparative paralysis time and death time of *pheretima posthuma* treated with the different extracts of *Blepharis maderaspatensis* are presented in Figures 1-3, respectively. These graphical representations further highlight the superior anthelmintic activity of the ethanolic extract compared with the other solvent fractions.

Figure 1: Adult Indian Earthworms (*Pheretima Posthuma*).

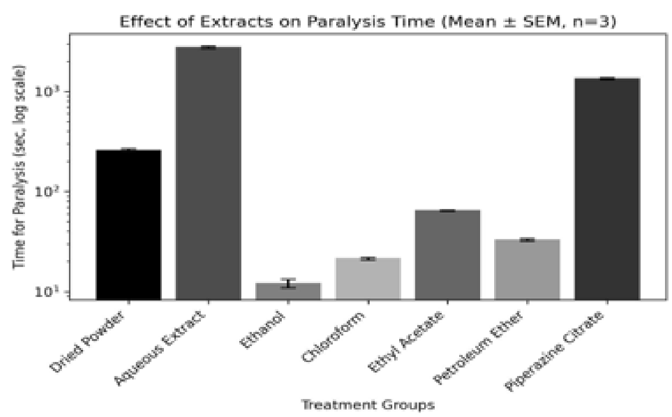

Figure 2: Comparison of Paralysis Time of Indian Earthworms (*Pheretima Posthuma*) Treated with Different Extracts of *Blepharis maderaspatensis* Leaf.

DISCUSSION

Results obtained in the current study emphasize the high impact of solvent polarity on the effectiveness of *Blepharis maderaspatensis* in terms of its anthelmintic effects. This can be explained by the higher ability of ethanol to extract bioactive ingredients with moderate polarity, such as flavonoids, alkaloids, and phenolics, which have antiparasitic effect, while less activity was recorded for both non-polar and highly polar fractions. The rapid paralysis of nematodes after exposure to the tested substances shows that the action is aimed at inhibition of neuromuscular and metabolic processes. For instance, the effects can be mediated via neurotoxicity caused by alkaloids, damage to the cuticle integrity induced by tannins, and reduction of mitochondrial activity by flavonoids, all resulting in paralysis and death of the worm. The

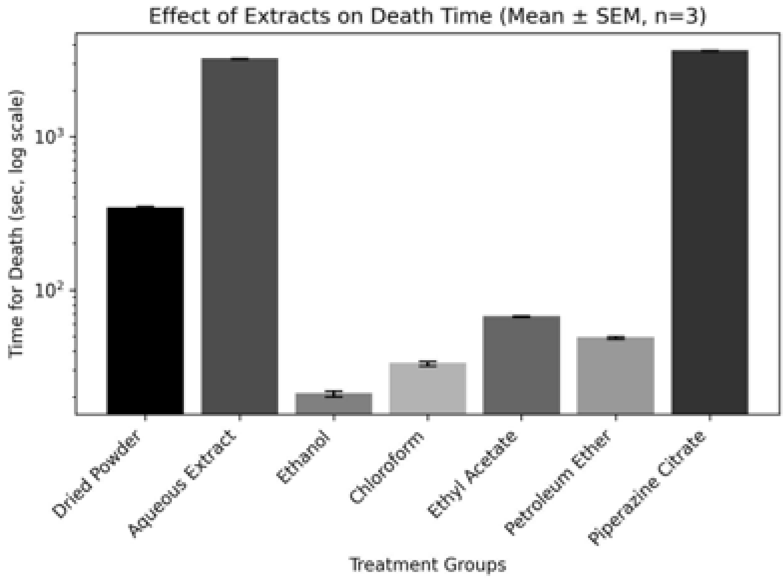

Figure 3: Comparison of Paralysis Time of Indian Earthworms (*Pheretima Posthuma*) Treated with Different Extracts of *Blepharis maderaspatensis* Leaf.

existence of a variety of phytochemicals may be responsible for a synergy mechanism that involves more targets compared to synthetic single-target compounds. The modest effectiveness of leaf crude powder also indicates the presence of phytoconstituents, although they become more effective after being isolated due to higher availability.

CONCLUSION

The current study provides the experimental evidence supporting *in vitro* anthelmintic activity of *Blepharis maderaspantensis* leaves. Among the different solvent fractions evaluated and the ethanolic extract produce the fastest paralysis and mortality in *pheretima posthuma* theat indicating greater efficacy compared with the other extract ant the even showing faster paralysis than the reference during piperazine citrate (Kumarasingha *et al*., 2016). The variation in activity observed among the different solvent extracts and highlights the importance of solvent polarity in concentrating pharmacologically active phytoconstituents. The strong activity of the ethanolic fractions suggest that moderately polar secondary metabolites and including flavonoids, alkaloids, tannins and phenolic compounds may play a significant role in the observed biological effect (Wink, 2010).

The results also indicate that the anthelmintic effect of *Blepharis maderaspatensis* may result from the combined action of the multiple phytochemicals acting through different mechanisms. These multi component interactions may interfere with neuromuscular coordination and the metabolic process and structural integrity of helminths, ultimately leading to the paralysis and death. Such a multi target mode of action may offer certain advantages over the single target synthetic drugs and particularly in addressing the growing issues of anthelmintic resistance (Gorter *et al*., 2015).

Although the present study represents a preliminary *in vitro* evaluation and the rapid activity of the observations in the ethanolic extract highlights the pharmacological potential of this plant. These findings provide a scientific basis for further research aimed at isolating active phytoconstituents and evaluating their pharmacological properties using more advanced experimental models (Newman and Cargg, 2020).

PHARMACOLOGICAL SIGNIFICANCE

The higher prevalence of the resistance to conventional anthelmintic drugs has created an urgent need for alternative therapeutic approaches. Natural products derived from the medicinal plants remain an important source of structurally diverse bioactive compounds with the potential antiparasitic activity. The strong activity observed in the ethanolic extract of *Blepharis maderaspatensis* suggested the plant could serve as a promising source of lead compounds for future anthelmintic drug discovery.

In addition to its potential as a source of new pharmacologically active compounds and the plant may also serve as a useful model for bioassay guided fraction. Studies aimed at the identifying the specific active constituents responsible for the observed activity. Furthermore, plant dried compounds with mechanisms of action may complement existing therapies and help overcome challenges associated with drug resistance. Considering its traditional medicinal used and the biological activity demonstrated in this study. *Blepharis maderaspatensis* represents a valuable candidate for the further pharmacological research and preclinical evaluatin (Mosisa *et al*., 2021).

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None.

ABBREVIATIONS

ATP: Adenosine Triphosphate; **GABA:** Gamma-Aminobutyric Acid; **sec:** seconds; **SEM:** Standard Error of the Mean; **UTI:** Urinary Tract Infection.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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