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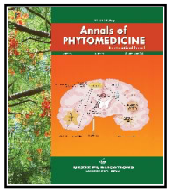

Original Article : Open Access

Efficacy of *Ipomoea staphylina* Roem and Schult. against dental and urinary infections

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Abstract

The study was conducted to assess the efficacy of *Ipomoea staphylina* Roem and Schult. against dental and urinary infections employing several strains such as *Staphylococcus aureus*, *S. saprophyticus*, *Escherichia coli*, *Chlamydia trachomatis*, *Citrobacter freundii*, *Acinetobacter baumannii*, *Mycoplasma limousine* and *Candida albicans*. The activities were performed using aqueous, ethanol and petroleum ether extracts to determine the high inhibitory percentage and maximum zone of inhibition. In antiurolithiatic activity, the ethanol extract produced a maximum inhibitory percentage of 94.25%. The ethanolic extract of *I. staphylina* resulted in maximum zone inhibition of 0.25 ± 1.3 in 100 µl concentration against *E. coli* and *C. albicans*. Highest zone of inhibition (0.20 ± 1.0 cm) in 100 µl concentration against *S. saprophyticus* was noted. In our present study, *I. staphylina* was potent against selected microbial pathogens, and hence could be beneficial in treating dental care and disorders of the urinary tract system thus providing a base for the design of future trials to determine its complete efficacy and safety for clinical use.

1. Introduction

Traditional medicines have supplied an alternative for various illness and knowledge. There are many valuable medicinal herbs in India's traditional medical system, but there is lack of scientific evidence to support their efficacy. In order to produce novel medicines, it is necessary to assess these plants based on their biological effects and chemical components (Dinnimath *et al.,* 2017).

Nephrolithiasis is currently managed medically, however, the treatment for a kidney is either expensive or not without adverse effects. Invasive treatments for nephrolithiasis provide a high risk of significant consequences and a high financial burden on the health care system (Doddola *et al*., 2008). The use of antiurolithiatic medications may also be expensive and require thorough research.

In light of the fact that antibiotics can occasionally cause negative host side effects such as hypersensitivity, immune suppression and allergic reactions. 80% of the World's population now relies primarily on plant based remedies. Earlier, dental infections were known to mostly afflict children (51.9%), but in recent times, they are increasingly more common in adults (Kassebaum *et al*., 2015). According to the Ministry of Health and Family Welfare, Government of India and WHO, the prevalence of dental infections was reported to be greater among Indians (49.6%) which leads to teeth loss, foul tasting, orofacial anomalies, temporomandibular joint disorders, dental fluorosis, malocclusion, orofacial trauma and oral cancers (Shah *et al.,* 2007; Balaji, 2018). With the purpose of lowering infectious dental illnesses, it is interesting to create alternative antibacterial medications from medicinal plants.

The second most common type of infection in the body, urinary tract infections (UTIs) cause major health risks to 150 million people each year, causing mild burning urination, bacteremia, sepsis, urinary obstruction, neurological conditions that result in urinary retention, renal failure, renal transplantation, pregnancy, presence of foreign bodies like calculi, indwelling catheters or other drainage devices and even death (Muthulakshmi and Gopal Krishnan, 2017; Flores *et al*., 2015). Patients with diabetes tend to be easily affected by UTIs (Meiland *et al.,* 2002). Over 75-90% of urinary tract infections are caused in adults by *E.coli* and the *S.aureus, S. saprophyticus, C. tracomatis* and *M. limousine* are less common offenders (Hooton, 2012). Controlling these bacterial and fungal agents in the urinary tract remains crucial. Herbal remedies create substitute treatments for uropathogens against those infecting individuals.

The genus *I. staphylina* has been utilized for food, medicinal, religious ceremonies and as an aesthetic plant. Several disorders, including diabetes, hypertension, diarrhoea, constipation, tiredness, arthritis, rheumatism, hydrocephaly, meningitis, renal problems and inflammations are treated with the 600-700 species of *I. staphylina* in many parts of the world. The species *I. staphylina* indicates antibacterial, analgesic, spasmolytic, spasmogenic, hypoglycemic, hypotensive, anticoagulant, antiinflammatory, psychotomimetic and anticancer properties. The prevalent biologically active components noticed in plant extracts include alkaloids, phenolic compounds and glycolipids (Meira *et al.,* 2012).

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2. Materials and Methods

2.1 Sample Preparation

The plant material *I. staphylina* was collected from Tamilnadu, India and identified by the Botanical Survey of India, Southern Circle, Coimbatore. The plant material was shade dried and 10 g of the sample was taken as a powder. 2 g of the plant material were used to prepare three different extracts using water, petroleum ether and ethanol, respectively. The prepared sample was subjected to maceration for 3-4 days and the residue was then filtered to obtain respective extracts.

2.2 Antiurolithiatic activity: Spectrophotometric estimation of calcium oxalate method

The samples were packed in an egg semipermeable membrane tied with thread at one end and were suspended in a conical flask containing 150 ml 0.1 M Tris buffer each, at another end covered with aluminum foil. All were kept in an incubator for three days and heated to 37°C; content was removed from the membrane and transferred into test tube l of 0.02 Mμ. 4 ml of 1 N H_2SO_4 and 60-80 $KMnO_4$ were added. The colour change from dark pink to colourless was observed after 2 h and was measured at 620 nm spectrophotometrically. The inhibitory percentage was calculated.

2.3 Antimicrobial activity: Dental pathogens and UTI

2.3.1 Preparation of the bacterial inoculum

Stock cultures were prepared and maintained at 4°C on slopes of nutrient agar and potato dextrose agar. Active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes of 50 ml nutrient broth. Bacterial cultures were incubated with agitation for 24 h and at 37°C on shaking incubator and fungal cultures were incubated at 27°C for 3-5 days. Each suspension of test organism was subsequently stroke out on nutrient agar media and potato dextrose agar.

A single colony was transferred to nutrient agar media slants were incubated at 37°C for 24 h and potato dextrose slant were incubated at 27°C for 3-5 days. These stock cultures were kept at 4°C. For use in experiments, a loop of each test organism was transferred into 50 ml nutrient broth and incubated separately at 37°C for 18-20 h for bacterial culture.

2.3.2 Well diffusion method

The antibacterial activity of crude extract extracts were determined by Well diffusion method (Bauer *et al.,* 1966). The 2-20 µl of nanoparticle extract was poured into the wells. After that, the plates were incubated at 37°C for 24 h. Assay was carried into triplicates and control plates were also maintained. The zone of inhibition was measured from the edge of the well to the zone in mm.

The tested cell suspension was spread on Muller Hinton agar plate and potato dextrose agar. Disc were put into the agar medium using sterile forceps. Plant extract were poured on to wells. Then plates were incubated at 37°C for about 24 h and control was also maintained. The diameter of inhibition zone was noted in cm.

3. Results

In *I. staphylina* extract, % inhibition was directly proportional to the concentration of the water. In 50 µl concentration, the inhibitory activity of the extract was significantly higher in selected solvents such as water (89.25%), ethanol (94.25%) and petroleum ether (92.32%), respectively, revealed in Table 1 and Figure 1.

Table 1: Antiurolithiatic activity of *I. staphylina* using different extracts

S.No.	Concentration of water	% of relative inhibitory activity (water)	% of relative inhibitory activity (ethanol)	% of relative inhibitory activity (petroleum ether)
1.	10 µl	29.48	16.61	26.29
2.	20 µl	44.82	40.00	49.77
3.	30 µl	49.01	51.32	60.27
4.	40 µl	79.55	61.32	87.24
5.	50 µl	89.25	94.25	92.32

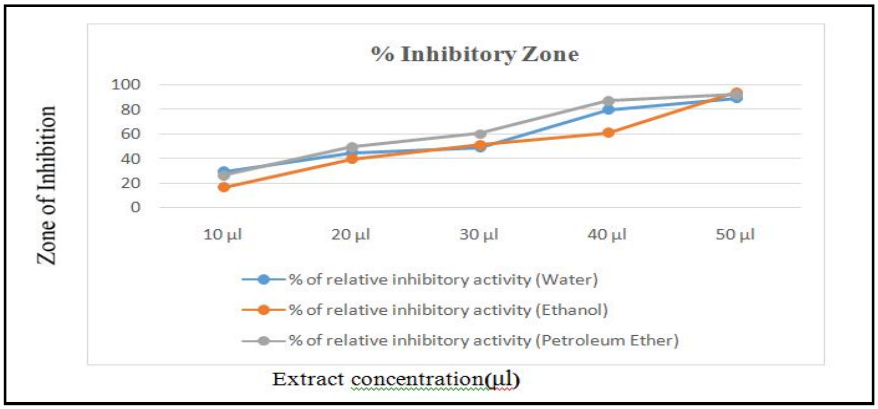

Figure 1: % Inhibitory level of *I. staphylina* in different extracts.

From the present study, it was evident that *I. staphylina* exhibited significant antimicrobial dental pathogen activity against both gram-positive and gram-negative bacterial and fungal agents such as *S. aureus, E. coli* and *C. albicans* from concentrations of 25 µl, 50 µl, 75 µl and 100 µl. *E. coli* and *C. albicans* produced the maximum zone of inhibition at 100 µl with 0.25 ± 1.3 cm, whereas *S. aureus* noted the least zone of inhibition at 25 µl with 0.07 ± 0.2 cm of diameter.

Table 2: Antimicrobial activity of different extracts of *I. staphylina* against dental pathogens

S.No.	Organisms concentration (µl)	*S.aureus* (Gram-positive bacteria)			*E. coli* (Gram-negative bacteria)			*C. albicans* (Fungi)		
		Water (cm)	Ethanol (cm)	Petroleum ether (cm)	Water (cm)	Ethanol (cm)	Petroleum ether (cm)	Water (cm)	Ethanol (cm)	Petroleum ether (cm)
1.	25 µl	0.07 ± 0.2	0.09 ± 0.3	0.10 ± 0.1	0.08 ± 0.9	0.09 ± 0.4	0.10 ± 0.5	0.12 ± 0.8	0.13 ± 0.5	0.11 ± 0.6
2.	50 µl	0.09 ± 0.5	0.09 ± 0.4	0.11 ± 0.3	0.09 ± 1.2	0.18 ± 0.8	0.12 ± 0.7	0.16 ± 0.9	0.18 ± 0.9	0.12 ± 0.7
3.	75 µl	0.11 ± 0.8	0.14 ± 1.0	0.13 ± 0.5	0.14 ± 1.5	0.22 ± 1.0	0.13 ± 0.8	0.19 ± 1.4	0.22 ± 1.0	0.15 ± 0.8
4.	100 µl	0.12 ± 1.0	0.21 ± 1.5	0.19 ± 1.0	0.19 ± 1.7	0.25 ± 1.3	0.20 ± 1.0	0.19 ± 1.4	0.25 ± 1.3	0.18 ± 1.0
5.	Standard (Chloramphenicol)	0.10 ± 1.4	0.15 ± 1.0	0.16 ± 1.1	0.18 ± 1.8	0.20 ± 1.2	0.18 ± 1.2	0.19 ± 1.8	0.19 ± 1.4	0.14 ± 1.0

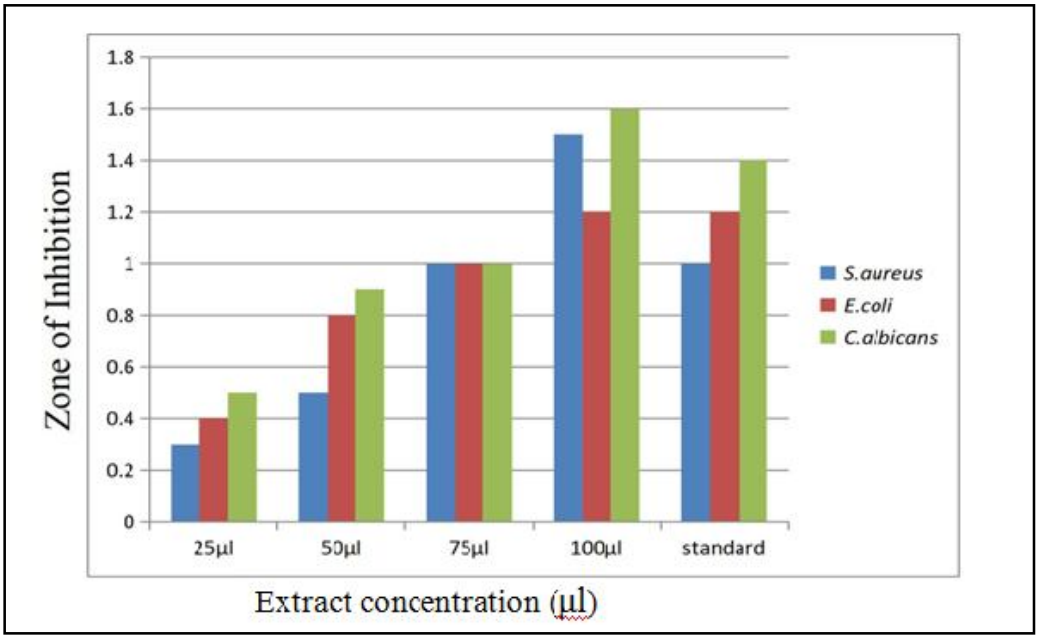

Figure 2: Antibacterial activity of different extract of *I. staphylina* against dental pathogens.

In UTI activity, the maximum zone of inhibition was remarkable in *S. saprophyticus* with 0.20 ± 1.0 cm of 100 µl concentration of water extract (Table 3). In the ethanolic extract, *M. limousine* exhibited a higher inhibitory zone with 0.20 ± 0.9 cm of 100 µl concentration (Table 4), whereas petroleum ether extract revealed the highest inhibitory zone in *M. limousine* with 0.17 ± 1.0 cm 100 µl concentration (Table 5). Among the water, ethanol and petroleum ether extracts, the ethanolic extracts evidenced the highest zone of inhibition.

Table 3: UTI activity of aqueous extract of *I. staphylina* in different organisms

S.No.	Organisms (µl)	*S. saprophyticus* (cm)	*C. trachomatis* (cm)	*M. liomusis* (cm)	*A. baumannii* (cm)	*C. freundii* (cm)
1.	25 µl	0.13 ± 0.6	0.08 ± 0.5	0.09 ± 0.1	0.10 ± 0.3	0.08 ± 0.2
2.	50 µl	0.17 ± 0.8	0.09 ± 0.6	0.14 ± 0.3	0.13 ± 0.4	0.16 ± 0.4
3.	75 µl	0.18 ± 1.0	0.11 ± 0.8	0.17 ± 0.7	0.16 ± 0.5	0.18 ± 0.5
4.	100 µl	0.20 ± 1.0	0.16 ± 1.0	0.18 ± 0.8	0.17 ± 0.6	0.19 ± 0.6
5.	Standard (Chloramphenicol)	0.15 ± 1.2	0.14 ± 1.2	0.18 ± 0.7	0.15 ± 0.5	0.16 ± 0.5

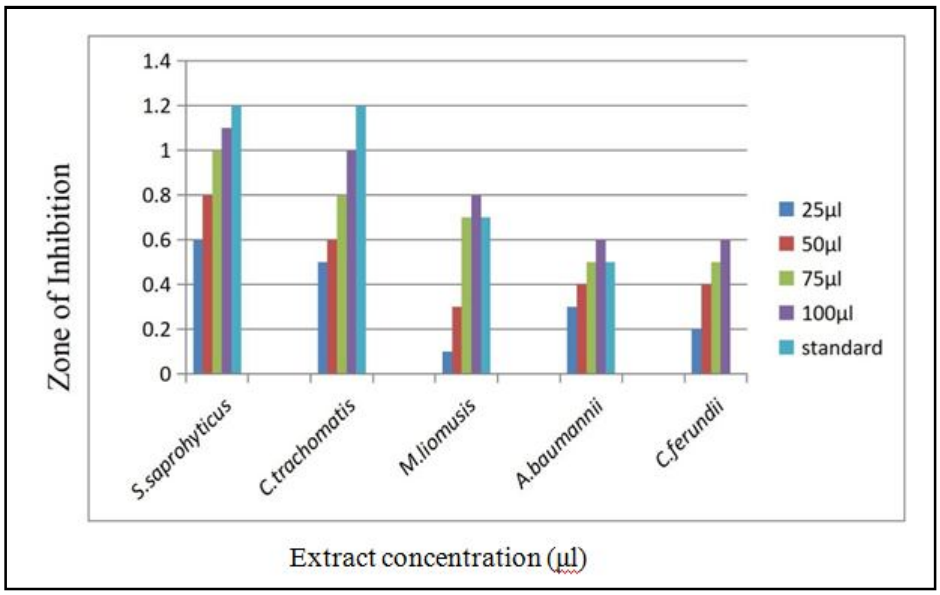

Figure 3: UTI activity of water extract of *I. staphylina*in different organisms.

Table 4: UTI activity of ethanolic extract of *I. staphylina*in different organisms

S.No.	Organisms (µl)	*S. saprophyticus* (cm)	*C. trachomatis* (cm)	*M. liomusis* (cm)	*A. baumannii* (cm)	*C. freundii* (cm)
1.	25 µl	0.09 ± 0.3	0.10 ± 0.3	0.09 ± 0.4	0.08 ± 0.2	0.09 ± 0.2
2.	50 µl	0.11 ± 0.5	0.11 ± 0.4	0.11 ± 0.5	0.09 ± 0.3	0.12 ± 0.4
3.	75 µl	0.14 ± 0.2	0.14 ± 0.6	0.18 ± 0.7	0.13 ± 0.7	0.14 ± 0.5
4.	100 µl	0.15 ± 0.8	0.19 ± 0.8	0.20 ± 0.9	0.16 ± 0.8	0.17 ± 0.8
5.	Standard (Chloramphenicol)	0.14 ± 0.8	0.16 ± 1.2	0.16 ± 1.0	0.15 ± 1.0	0.16 ± 0.9

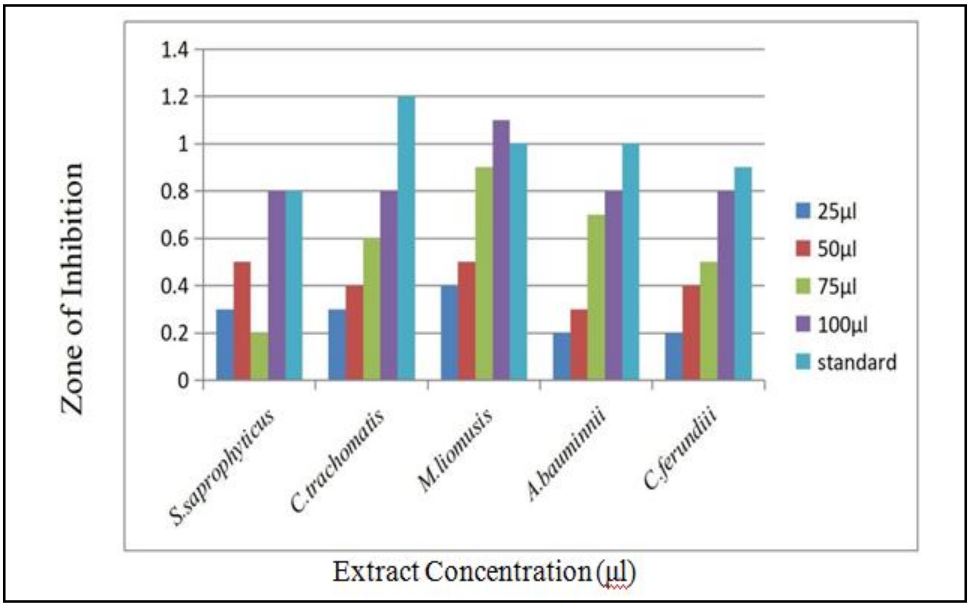

Figure 4: UTI activity of ethanolic extract of *I. staphylina* in different organisms.

Table 5: UTI activity of petroleum ether extract of *I. staphylina* in different organisms

S.No.	Organisms (µl)	*S. saprophyticus* (cm)	*C. trachomatis* (cm)	*M. liomusis* (cm)	*A. baumannii* (cm)	*C. freundii* (cm)
1.	25 µl	0.11 ± 0.7	0.06 ± 0.6	0.08 ± 0.4	0.07 ± 0.5	0.08 ± 0.4
2.	50 µl	0.09 ± 0.6	0.08 ± 0.7	0.12 ± 0.7	0.08 ± 0.6	0.12 ± 0.6
3.	75 µl	0.13 ± 0.8	0.11 ± 0.9	0.15 ± 0.9	0.09 ± 0.7	0.14 ± 0.8
4.	100 µl	0.15 ± 1.0	0.13 ± 1.0	0.17 ± 1.0	0.13 ± 0.9	0.16 ± 1.0
5.	Standard (Chloramphenicol)	0.11 ± 0.6	0.12 ± 1.1	0.12 ± 1.0	0.10 ± 1.0	0.13 ± 1.2

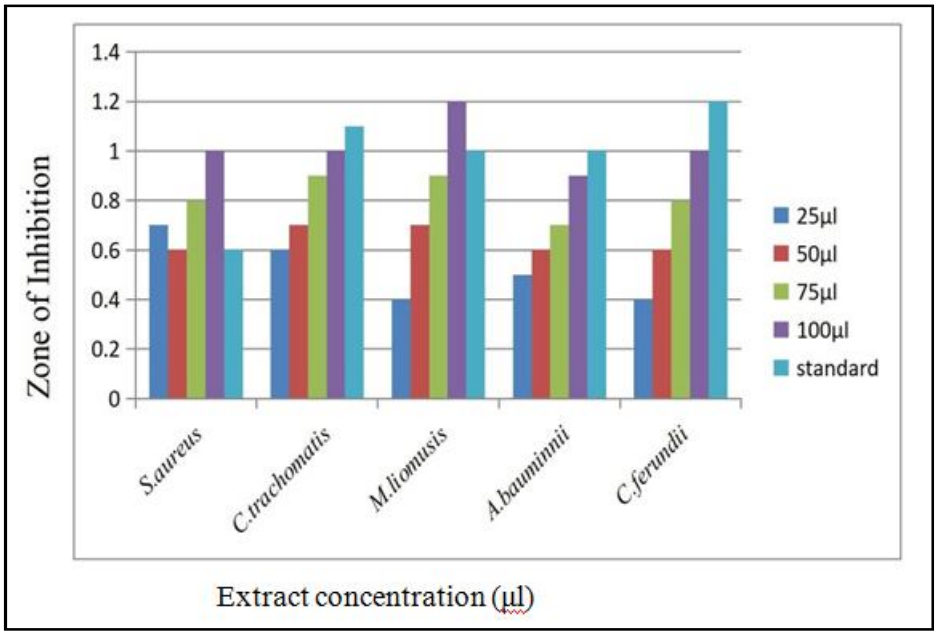

Figure 5: UTI activity of petroleum ether extract of *I. staphylina* in different organisms.

4. Discussion

The most common kind of urinary stone illness is urolithiasis. Crystal nucleation, growth and aggregation are the primary factors in pathogenic biomineralization (Chaudhary *et al*., 2010; Patel *et al*. 2012). Our findings concur with reports of Namdeo *et al*. (2021), which prove that the ethanolic extract of *I.digitata* exert beneficial inhibitory effect on the formatting precipitation of calcium and potassium *in vitro*. Therefore, 94.25% of relative inhibitory activity of the ethanolic extract of the plant was reported in 50 µl concentration. As the concentration of the drug increases, there is an increase in inhibition of the calcium oxalate crystals (Jagtap *et al.,* 2019). Ethanolic extract showed maximum efficiencies in the dissolution of calcium oxalate crystals in *Gossypium herbaceum* (Niharika *et al.,* 2018). Our study is in concordance to the above reports where maximum inhibitory activity was noted in the ethanolic extract.

Several plants and plant derived antimicrobial components are used in folklore therapeutics for the treatment of periodontal disorders and oral hygiene. The development of methods for the extraction and evaluation of plant antimicrobials in *Streptococcus* species include bacteria inhibitory components (Tichy and Novak, 1998). Accepting this statement, *S. aureus* possessed maximum inhibitory effect in our study. The secondary metabolites of plant possess medicinal properties and these medicinal qualities are used in management of various forms of oral problems and dental extraction (Ashu Agbor and Naidoo, 2015). This correlates with our work, where gram positive and gram negative bacteria such as *S. aureus* and *E. coli* record the maximum inhibitory content at higher concentration of plant extract, which can be used against dental pathogen. Alviano *et al*. (2008) observed maximum inhibitory activity at higher concentration of ethanol extracts.

Promising antibacterial activity against UTI was noted in plant extracts tested against various gram-negative bacteria including *P. aeruginosa*, *E. coli*, *S. flexeneri*, *Mycoplasma* sp. (Khan *et al.,* 2011). This associates with the study performed in *M. liomiusis* where ethanolic and petroleum ether extract produced highest inhibitory activity against UTI. Antibacterial activity of aqueous and ethanolic extracts of *I. triloba* on diarrhoeal isolates was based on maximum

inhibition zone diameters when exposed to the treated extract (Alozie *et al.,* 2022). The above findings support our work, where ethanolic extract of *I. staphylina* extract recorded highest inhibitory zone with a diameter of 0.2 ± 0.9 cm in 100 µl of concentration.

UTIs are caused by both gram-positive and gram-negative bacteria, as well as by certain fungi. The most common organisms are *E. coli*, followed by *Klebsiella pneumonia, S. saprophyticus, Proteus mitabilis*, and *Candida* sp. (Flores *et al.,* 2015). In our work, selected plants displayed higher efficiencies toward dental pathogens and UTI diseases in various solvent extracts over the standard, which can be further studied to conclude the name of the compound, responsible for the inhibitory effect.

5. Conclusion

There is a need for alternative treatment strategies that are safe, effective, and cost effective when compared to current treatment methods because of high prevalence of periodontal and urinary diseases in developing nations like India. The unfavorable side effects from prolonged use of currently used antibacterial agents and financial considerations. The medicinal plants work well as a substitute in this regard. The current preliminary research shows that *I. staphylina* possess numerous medicinal properties against urolithiasis, dental pathogens and urinary infections. Hence, the plant may be considered as a reliable source of supplements for treating urolithasis. However, detailed investigations are warranted for potential use in the future to treat dental and urinary infections using contemporary research techniques.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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