ORIGINAL ARTICLE

Evaluation of *Euphorbia nivulia* seeds for their secondary metabolites and therapeutic potential

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Abstract

Introduction: The phytochemical analysis, antimicrobial activity, and *in vitro* antidiabetic assay of extracts obtained from the seeds of mature Indian Dudhi plant also known as Milk hedge (*Euphorbia nivulia*) were examined in this study. **Materials and Methods:** Using aqueous methanol extracts of the seeds of *E. nivulia*, phytochemical components were investigated. Further using chromatography, different components were separated and its antimicrobial activity was investigated. **Results and Discussion:** Two components from the seed extract were isolated using column chromatography. Four different types of bacterial cultures were utilized for evaluating the antimicrobial activity. The results indicated that seed extracts are more sensitive toward Gram-negative organisms. **Conclusion:** Thus, the presence of vial phytochemicals in the seed extract could render it therapeutic potential which can be utilized for the treatment of different diseases including microbial infections.

Key words: Antimicrobial activity, Dudhi seeds, *Euphorbia nivulia*, phytochemicals, thin-layer chromatography

INTRODUCTION

Since ancient times, people have used plants as sources of chemicals, for therapeutic and recreational purposes and for poisoning.[1,2] Based on the knowledge of medicinal plants, human being has been investigating throughout the globe a traditional medicine since long time. From many generations due to observations and research on animal character, this knowledge got enhanced. Most of the time, this information is only orally inherited and is, therefore, in risk of being disoriented in favor of allopathic medicine. However, it represents for the local population a possibility of simple and cheap treatment. In addition, it is a origin of imaginably dominant contemporary pharmaceutical compound for the local residents and it may be a uncomplicated and low cost treatment for them.[3] Numerous plant-derived substances have demonstrated physiological and behavioral activity against insect pests and they can provide new sources for the development of natural pesticides.[4,5] Ancient people utilized chemicals extracted from plant origin as animal poisons, vermifuges, and insect repellents. The presence of toxic substances as secondary metabolites in plants emerges from an extensive and remarkable process known as coevolution: Changes that take place in 2 or many generations as an end result of the populations particular action of the community on one another.[5] *Euphorbia nivulia* in India commonly known as Dudhi is used in Ayurveda medicine for the diseases such

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as tumors, jaundice, asthma, bronchitis, and leprosy, its juice is particularly used as purgative.[6] Hence, many chemical components from *E. nivulia* latex have been isolated and exhibited antimicrobial activity.[1-3,7,8] Our present investigation explains the separation of active phytochemicals from seed extracts of Dudhi and its antimicrobial activity on Gram-positive and Gram-negative microorganisms.

MATERIALS AND METHODS

Reagents and Chemicals

All chemicals, reagents used were of analytical grades. Standard microbial strains and culture plates used and various biochemical reagents were purchased from Sigma-Aldrich.

Collection of Plant Material and Extraction of Secondary Metabolites

Dudhi plant also known as milk hedge plant is common in India. Very fresh seeds were obtained from Nuggikeri, Dharwad, in the month of September. The seeds were washed in fresh water separately for removing the adherent particles and it was air dried in dark condition. A very fine powder of seeds (4 g) done by mixer grinder [Figure 1]. The secondary metabolites were extracted using methanol at room temperature and concentrated under vacuum and reduced pressure using a rotary vacuum evaporator.[8,9] The extract was then stored in the centrifuge tubes. Using well method, antimicrobial activity of crude methanol extract from seed was performed, which has shown good sensitivity against many microorganisms.

Microorganisms Used

In our study, we used four microbial cultures procured from National Centre for Cell Science Pune, India. Gram-positive strains included *Staphylococcus aureus*, *Bacillus cereus*, and *Bacillus subtilis* and for Gram-negative strain *Escherichia coli* was used. The concentration of 0.5 mg per ml was used for this study.

Figure 1: Dudhi seed powder

Identification of Phytochemical Components

Using standard biochemical procedures, phytochemical components such as phenols, soluble starch, flavonoids, saponins, steroids, quinones, tannins, reducing sugar, and alkaloids were identified from *E. nivulia* seed extract.[9,10]

Test for phenols

The plant extract (2 ml) was dissolved in 5 ml of distilled water. To this, add few drops of neutral 5% ferric chloride solution were added. Dark green color indicated the presence of phenolic compounds.

Test for soluble starch

0.25 ml of plant extract was boiled in 1 ml of 5% KOH solution. Cooled and acidified with few drops of H_2SO_4. Occurrence of yellow coloration indicates the presence of soluble starch.

Test for flavonoids

Filter 100 mm of plant solution with water (3 ml). To this, add 2 ml of 10% of NAOH to which the solution turns yellow. Add few drops of diluted HCl to which the yellow color turns colorless. Coloration from yellow to colorless indicates the presence of flavonoids.

Test for saponins (frothing test)

Filter 1 ml of plant extract with 5 ml of water. To this filtrate, add 3 ml of distilled water. Shake the test tube vigorously for 5 min. No frothing was seen indicates the absence of saponins.

Test for steroids

To 200 µlt of plant, 2 ml of acetic acid was added. Ice bath for 5 min. Add few drops of concentrated H_2SO_4. Appearance of blue, bluish-green, or violet indicates the presence of steroids.

Test for quinones

Filter 200 µlt of plant with 10 ml of benzene. To the filtrate, add 5 ml of 10% ammonia. Shake vigorously. Appearance of red or pink or violet color indicates the presence of quinones.

Test for tannins

Filter 500 µl of plant with 10 ml of water. To the filtrate of 2 ml, add few drops of ferric chloride. Appearance of blue-black, green/blue-green precipitate indicates the presence of tannins.

Test for reducing sugar

About 2 ml of plant extract was hydrolyzed by boiling with 5 ml dil. HCL. This is further neutralized with sodium hydroxide solution. To this, few drops of Fehling's solution were added and heated on water bath for 2 min. Appearance of reddish-brown precipitate of cuprous oxide indicates the presence of reducing sugars.

Test for alkaloids

Wagner's test was carried out which gave a reddish-brown precipitate indicating the presence of alkaloids.

Separation of Components

To check the number of components, thin-layer chromatography (TLC) method applied and further using column chromatography components were separated using hexane, ethyl acetate, and methanol solvent in different ratios.

RESULTS AND DISCUSSION

The polar methanol crude seed extracts of *E. nivulia* exhibit antimicrobial activity.[8,11] The polar fractions show a very good antibacterial activity as shown in Table 1. Based on our result, both polar methanol extracts have broad-spectrum antimicrobial activity against *E. coli* and *Bacillus*.

Further phytochemical components such as phenol, soluble starch, flavonoids, saponins, steroids, quinones, tannins, reducing sugar, and alkaloids were identified from the Dudhi seed extract as shown in Table 2. Seed extract showed the presence of phenol, soluble starch, flavonoids, and alkaloids.[9,10]

Further, seed crude extracts were subjected to column chromatography, using neutral silica gel eluted with hexane, acetone, and ethyl acetate to obtain two pure components from the seed. Further using TLC purity of all components was checked.[12]

These separated pure extracted components were used for testing antimicrobial activity using different bacterial strains and the result is shown in Table 3. Four microorganisms were used for the process, that is, *B. cereus*, *E. coli*, *S. aureus,* and *B. subtilis*.

Observation

Seed fractions 1 and 2 were not sensitive against *E. coli* and *S. aureus,* whereas more sensitive against bacterial strains such as *B. cereus* and *B. subtilis* as shown in Table 3 and Figure 2.

Table 1: Antimicrobial activity of crude seed extract

Microorganisms	Dudhi seed (mm)
Escherichia coli	0.4
Bacillus	0.5

Table 2: Qualitative phytochemicals analysis

S. No.	Name of the phytochemical	Dudhi seed extract
1.	Phenol	+
2.	Soluble starch	+
3.	Flavonoids	+
4.	Saponins	–
5.	Steroids	–
6.	Quinones	–
7.	Tannins	–
8.	Reducing sugar	–
9.	Alkaloids	+

Table 3: Some of the components extracted are antimicrobial

Dudhi seed extract no.	*B. cereus* Dia in mm	*E. coli* Dia in mm	*S. aureus* Dia in mm	*B. subtilis* Dia in mm
Dudhi seed 1	0.1	0	0	0.2
Dudhi seed 2	0.4	0	0	0.4

S. aureus: *Staphylococcus aureus*, *B. cereus*: *Bacillus cereus*, *B. subtilis*: *Bacillus subtilis*, *E. coli*: *Escherichia coli*

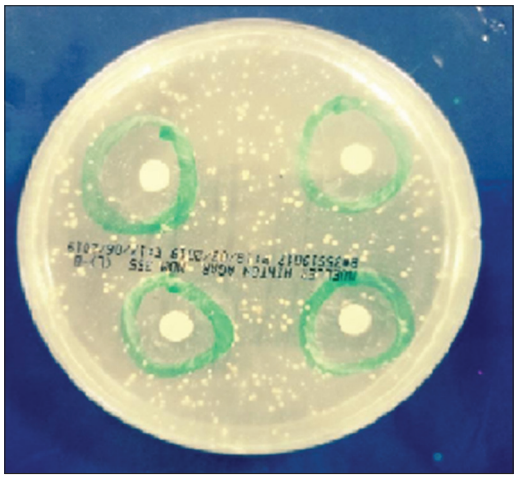

Figure 2: Antimicrobial activity of Dudhi seed fraction

CONCLUSION

Around the world, *Euphorbia* species plant extracts are very well known for its medical applications. As per its plant

part, region, season, species, and method of extraction, chemical contents may vary. Our present study reveals Dudhi seed extract showed presence of phenol, soluble starch, flavonoids, and alkaloids and shows antimicrobial activity. The results indicate that seed samples are more sensitive toward Gram-negative organisms such as *B. cereus and B. subtilis.* The presence of important phytochemicals can be used for different ailments.[11] For diseases like microbial infection treatment, these seed extracts and compounds could be used.[13]

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