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Antimicrobial Evaluation of Ammi majus L. Extract Powder using in Vitro Methods

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ABSTRACT: In traditional medicine, the native Egyptian plant Ammi majus L. has demonstrated diverse pharmacology. Pharmacognostic and phytochemical analysis has reported various chemical constituents. Some of them have been studied for biological profile, pharmacokinetics and toxicity profile. Experimental studies report role of different parts of Ammi majus in the treatment of skin diseases, cancer and as a diuretic. In the present study we aimed to evaluate antimicrobial activity of the selected plant extract against pathogens causing oro-dental infections using in vitro methods. Zone of inhibition was taken as the measurement parameter for the activity profile using antimicrobial drug as a reference. The study results were helpful to understand and further explore the role of Ammi majus in the treatment of oro-dental infections.

KEYWORDS: Ammi majus, Antimicrobial evaluation, Oro-dental infections, In vitro studies

I. INTRODUCTION

For overall well-being and safety oral health is an essential parameter. World Health Organization (WHO) has reported some oral diseases of major concern for public health and it includes dental caries, periodontal disorders, oral cancer, etc. [1]. Patients suffering from diseases like mental retardation, Down syndrome, diabetes mellitus (type I) have been observed with higher risk of periodontal diseases [2,3]. In a recent study by Vilen et al. (2023), higher chances of hospitalization reported among patients infected with tonsillary and dental origin [4]. It also suggests early and prompt diagnosis could help to provide treatment and to hospitalization duration. The reported factors behind oro-dental infections include microbial infections by Gram-positive (Streptococcus mutans, S. sobrinus, S. aureus, S. pyogenes, B. subtilis), Gram-Negative (E. coli, Porphyromonas gingivalis, Actinobacillus sp., Prevotella sp. and Fusobacterium sp.) and C. albicans [5,6,7,8,9]. They also have role in the infectious-inflammatory processes which further progress to gingivitis or periodontitis [9].

The global burden of oral diseases demands discovery and development of therapeutics and preventive measures having established safety and potency. The available agents are associated with various limitations like undesired side effects, staining of tooth, diarrhea, alternation of the oral mircobiota, toxicity, and development of microbial resistance to the oral antibiotics [10-12]. Studies also report toxicity and risk of cancer development associated with certain products (mouthwash, gargles) used for treatment and prevention as they contain chlorhexidine, amine fluorides, cetylpyridinium chloride, etc. [13]. While plant based products or products containing natural phytoconstituents are considered as better alternative to the synthetic counterparts and some examples are reported in Table 1 [14,15].

The traditional safe and sustainable herb Ammi majus (A. majus) is a wild vegetable plant. Its different parts including leaves, stems, fruits, roots and their extracts have been reported to possess activity against some diseases [16,17]. It has broad activity profile like antimicrobial, antioxidant, antiviral, antihypertensive, antiviral, etc. [18]. The reported major phytoconstituents for A. majus are furanochromone, coumarins (khellin, visnadine, ammoidin, visnagin, visammiol, samidin, xanthotoxin, khellinin) (Figure) [19,20] as well as pyrones, saponins, flavonoids, coumarins and essential oils [21,22,23]. Also recent literature also highlights development of resistance cases among bacterial strains as a serious health concern [24,25,26,27]. This puts forward the requirement to identify novel compounds with broad activity spectrum and to control resistance cases.

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In view of this, the present work aims to investigate the antimicrobial potential of plant A. majus (extract powder) using in vitro studies to investigate antimicrobial properties.

Table 1. Examples of some reported plants with inhibitory potential for microorganisms causing oral infections

Name of microorganism	Name of plant (family)
Oral streptococci	Abies canadensis (Pinaceae) Albizia julibrissin (Fabaceae) Chelidonium majus (Papaveraceae) Ginkgo biloba (Ginkgoaceae) Juniperus virginiana (Cupressaceae) Pinus virginiana (Pinaceae) Rosmarinus officinalis (Lamiaceae) Sassafras albidum (Lauraceae) Tanacetum vulgare (Asteraceae) Thuja plicata (Cupressaceae)
S. mutans S. sobrinus	Drosera peltata (Droseraceae)
Porphyromonas spp. Preveotella spp. Actinomyces odontolitycus	Hamamelis virginiana (Hamamelidaceae)
P. gingivalis	Allium sativum (Liliaceae) Pistacia lentiscus (Anacardiaceae)
Actinomyces Fusobacterium Lactobacillus Prevotella Propionibacterium Streptococcus species	Harungana madagascariensis (Hypericaceae)
S. mutans	Breynia nivosus (Euphorbiaceae) Ageratum conyzoides (Asteraceae)

II MATERIAL AND METHODS

Chemicals and reagents

For the present work solvents (acetone, butanol, chloroform, dichloromethane, dimethyl sulfoxide, methanol, hexane), reagents/chemicals (sodium sulphate, sodium chloride) and standard compounds/drugs were used and they procured from Fisher Scientific Company. Rest of the chemicals was obtained from Sigma-Aldrich Company, USA. A. majus extract powder (300 gm, a generic brand) was obtained from Vedik Herbals, India (Figure 2).

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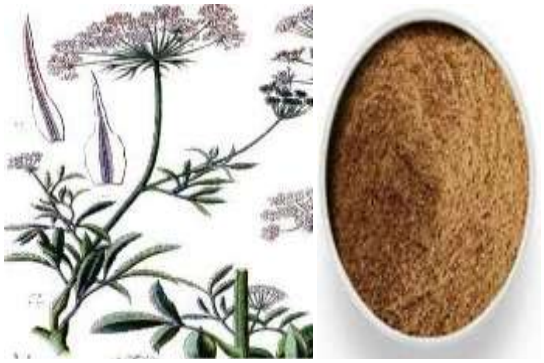

(a) (b)

Figure 2. (a) A. majus plant; (b) Extract powder of A. majus

Evaluation of Dissolving Percentage

For the selected A. majus extract powder, the dissolving percentage was determined by calculating the water-solubility index analysis [28]. The extract powder (3 gm) was dissolved in distilled water (30 ml). It was vortexed and incubated on the water-bath (at 90ºC, 30 minutes). Later it was centrifuged for 10 minutes (5000 rpm) and the supernatant was collected. It was further dried (105 ºC, 12 hours) and weighed. Following formula was used to calculate the dissolving percentage:

$$\text{Dissolving Percentage} = 100 \times \frac{\text{Weight of dried supernatant}}{\text{Initial weight of the dried extract powder}}$$

The process was repeated 3 times and the results were reported as mean ± standard deviation.

Evaluation for Antimicrobial activity

The extract powder of A. majus (1 mg/ml) was dissolved in distilled water. The solution was filtered using a sterile membrane (0.2 µm, Millipore). The selected microbial strains (namely Staphylococcus aureus (S. aureus), Pseudomonas aeruginosa (P. aeruginosa), Bacillus subtilis (B. subtilis), Candida albicans (C. albicans), and Escherichia coli (E. coli) obtained from IMTECH, Chandigarh) selected for this study were suspended in a normal saline solution.

Agar well diffusion method was used to investigate the antimicrobial properties for the selected sample [29,30]. Media plates were prepared using Mueller Hinton Agar. The microbial isolates were sub-cultured using the prescribed medium (at 37ºC, 24 hours). For each of the cultured microorganism, 100 µl of inoculums was spread on to the media plates. Following this wells with 6 mm diameter were bored in these inoculated plates. During this process aseptic conditions were maintained.

The prepared A. majus extract powder solution (25, 50, and 100 µl) was placed in the wells and allowed time of 10 minutes for diffusion. These plates were further incubated for 24 hours (37 ºC) [31-33]. For negative control, sterile distilled water and DMSO (20%) were used while for positive control chlorhexidine (0.2%) and amoxicillin (30 µg) were used.

The antimicrobial potential was measured based on the formation of zone of inhibition surrounding the well which were filled with test solution or standard/negative control/positive control. The diameter was measured using the vernier calipers in mm. All the experiments were performed in triplicates and the observations were reported as mean for the diameter of zone of inhibition ± standard deviation (SD) (Table 2).

Table 2: Antimicrobial properties of A. majus extract powder as mean for the diameter of zone of inhibition ± standard deviation (SD)

Name of microorganism	A. majus extract powder solution (100 µl)	Amoxicillin (30 µg)	Chlorhexidine (0.2%)
St. aureus	13.0 ± 0.54	2.0 ± 0.42	1.0 ± 0.53
P. aeruginosa	-	4.0 ± 0.91	2.0 ± 0.14
B. subtilis	10.0 ± 0.63	3.0 ± 0.47	5.0 ± 1.21
C. albicans	5.0 ± 0.21	4.0 ± 0.39	3.0 ± 1.14
E. coli	15.0 ± 0.92	4.0 ± 0.1	2.0 ± 1.9

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III RESULTS AND DISCUSSION

In the recent time, drug resistant bacterial infections are a serious threat to human health and reports highlight multiple deaths due to such strains [34]. Thus the study aims to perform in vitro studies for evaluating antimicrobial properties of A. majus having promising wide spectrum biological activities. For this study, the extract powder of A. majus was used.

Solubility is an important aspect to be considered while developing formulation. The dissolving percentage in water for A. majus (extract powder) was found to be 93.92 ± 0.63. This indicates the suitability to achieve desired concentration of selected herbal sample in systemic circulation and can help to achieve anticipated therapeutic properties.

During antimicrobial property evaluation, Amoxicllin and Chlorhexidine were used as reference drugs and the obtained results were compared with them. The extract powder solution at the concentration of 100 µl has shown inhibitory potential against E. coli, St. aureus, B. subtilis and C. albicans in the descending order of its potency. While it failed to show inhibition of P. aeruginosa. The results were better than the reference drugs confirming its suitability for further investigation.

IV CONCLUSION

In this work, antimicrobial property of A. majus extract powder has been evaluated using in vitro studies against pathogenic microorganism namely, St. aureus, P. aeruginosa, B. subtilis, C. albicans, and E. coli. The observed reports suggest to further investigate the potential for development of formulations using A. majus for the treatment of infections caused by E. coli, St. aureus, and B. subtilis. It may be further explored for other infectious strains.

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