

Original Article

CHARACTERIZATION & EVALUATION OF ANTIBACTERIAL, ANTIFUNGAL ACTIVITY OF ENVIRONMENT FRIENDLY *CAPPARIS DECIDUA* MICROEMULSION

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ABSTRACT

Objective: The present study was undertaken with the objective to develop microemulsion from the ethanolic extract of the plant, *Capparis decidua* and evaluate its potency against microorganisms (bacteria & fungi).

Methods: The solubility of the extract was tested in various solvents to determine the oil phase to be used in the microemulsion system. Microemulsion formulations were developed from the plant extract and their physico-chemical studies were carried out as per standard parameter.

Results: The prepared microemulsion was tested for its antimicrobial and antifungal activities. Preliminary screening of the microemulsion showed the potent antimicrobial and antifungal activity. The development of microemulsion was confirmed by Transmission Electron Microscopic (TEM) analysis.

Conclusion: 5% (w/w) microemulsion from the ethanolic extract of the *Capparis decidua* was successfully prepared. The microemulsion was found to possess potent antibacterial and antifungal activities.

Keywords: *Capparis Decidua*, Formulation, Microemulsion, Antimicrobial and Antifungal Activity

INTRODUCTION

Capparis decidua belonging to the family Capparidaceae, is a dominating xerophytic shrub found in the desert region of Rajasthan, India. The plant is densely branched reaching a height of up to 5 meters and shows strong climatic adaptations which makes the plant unique [1]. *C. decidua* contains generous quantities of alkaloids, terpenoids, glycosides, fatty acids, vitamins, fiber and oils which have been reported to show high medicinal and nutritive value [2]. The plant possesses wider utility in the traditional folk medicine and is used in ailments to cure toothache, arthritis, asthma, cough, inflammation, intermittent fevers, malaria, rheumatism and swelling. There is numerous literature documented which has shown that different parts of the plants exhibit pharmacological properties like antimicrobial [3, 4], hepatoprotective activity [5], anti-atherosclerotic [6], antidiabetic [7, 8], anti-hypertensive [9], anti-hyperlipidemic and antioxidant properties [10-11].

Despite several recognized health benefits of the plant, not much work has been explored on development of formulations from this plant and its use as an antibacterial and antifungal agent. Water based formulations such as Microemulsion (ME) is becoming an increasing focus of attention today as it is known to improve the solubility and poor oral bioavailability of insoluble drugs [12-13]. It is defined as the thermodynamically stable, transparent, single optically isotropic liquid system of water, oil and surfactants frequently in combination with suitable co surfactants [14, 15]. In the present study, we have described the development of water based microemulsion from *C. decidua* plant extracts and its antibacterial and antifungal properties.

MATERIALS AND METHODS

Capparis decidua plants (500 g) were collected from District - Etawah (Uttar Pradesh, India). *C. decidua* stem was first wiped to remove the adhering dust particles, air dried and then grinded into fine powder. Di-acetone alcohol alcohol (DDA) and triton X 100.

Preparation of the plant extract

A round bottom flask containing 400 ml of ethanol was fitted to the sauxhlet apparatus containing 50 gram of dried powder of *Capparis decidua* and extraction was done for 48 hours. After the completion of

the extraction, crude extract was filtered using Whatman no.1 filter paper and dried using rotary evaporator to remove the ethanol.

Formulation of microemulsion

Microemulsion was prepared by mixing 0.05 gm of *C. decidua* extract with 0.35 gms of DAA and 0.20 gms of Triton X-100. Distilled water (0.40 gm) was then precisely added to the above mixture drop by drop with constant stirring. Water was added to make up the volume up to 100 %. Finally *C. decidua* 5% (w/w) microemulsion was ready for further studies [16].

Droplet size determination

The analysis of droplet size of the microemulsion formulations were determined using zetasizer (Malvern Instruments Ltd., U. K) which is based on the principle of dynamic light scattering (DLS). Droplet size of the microemulsion was found to be in nano range. The polydispersive index (PDI) which is a measure of the particle homogeneity was also determined by the same instrument. The value of PDI varies in the range of 0.0 to 1.0. Value of PDI closer to 0.0 is indicative of narrow size distribution of the formulation. PDI of the developed formulation was found to be 0.53 which signifies that the prepared microemulsion is monodispersive in nature, stable and the particles will not coalesce to form macroemulsion.

Stability studies

Visual examination showed that the microemulsion system was stable after being subjected to the higher temperature of 54°C and lower temperature of 5°C. No phase separation was observed at extremes of temperature.

pH

The pH of the microemulsion formulation was measured by a digital pH meter (SDFCL, microanalytica, India). The pH of the microemulsion was found to be 5.8 suggesting its use as a topical drug bearing no risk to skin irritation in humans as the pH of the human skin lies in the range of 5.5-6.4.

Viscosity

The viscosity of the microemulsion was determined by the zetasizer (Malvern Instruments Ltd., India) using the microrheology option.

The viscosity of the developed microemulsion was found to be 0.8872 cP.

Transmission Electron Microscope (TEM) Study

In order to determine the shape and the size distribution of the silver nanoparticles, Transmission electron microscope (make-JEOL LTD) analysis was also carried out. A drop of the microemulsion solution was placed on a carbon-coated standard copper grid to obtain the TEM images.

Antibacterial and Antifungal Activity

We have used the Broth Dilution Method to evaluate the antibacterial activity. *E. Coli* MTCC 443, *Kl. pneumoniae* MTCC 7028, *S. Aureus* MTCC 96, *S. pneumoniae* MTCC 1936 and *C. Albicans* MTCC 227 and *A. Niger* MTCC 282, strains were used for screening of antibacterial and antifungal activities: The strains were procured from an Institute of Microbial Technology, Chandigarh, India. DMSO was used as diluents / vehicle to get the desired concentration of drugs to test upon standard bacterial strains. Serial dilutions were prepared in primary and secondary screening. The control tube

containing no antibiotic was immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of a plate of medium suitable for the growth of the test organism and put for incubation at 37°C overnight. The tubes were then incubated overnight. The MIC of the control organism was observed to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism was recorded as the MIC. The amount of growth from the control tube before incubation [which represents the original inoculum] was compared.

In primary screening, 1000 microgram/ml, 500 microgram/ml, and 250 microgram/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in a second set of dilution against all microorganisms. The drugs found active in primary screening were similarly diluted to obtain 200 micro/ml 100 microgram /ml, 50 microgram/ml, 25 microgram/ml, 12.5 microgram /ml, 6.250 microgram/ml, and concentrations. The highest dilution showing at least 99 % inhibition zone was taken as MIC. The result of this is much affected by the size of the inoculum. The test mixture should contain 10⁸organism/ml [17-19].

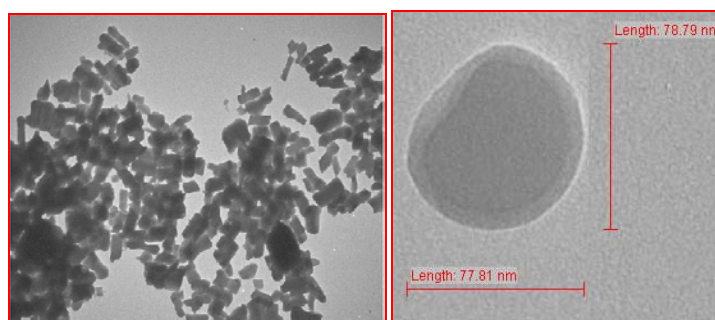


Fig. 1: Transmission Electron Microscope (TEM) of the Microemulsion

RESULTS AND DISCUSSION

It is well known that plant has a wide range of applications due to the presence of alkaloids, terpenoids, glycosides, fatty acids and vitamins in good quantity. Recent studies on this plant show that it may be used as antibacterial, antidiabetic, antihypertensive and antioxidant agent [3, 7-11]. Keeping above facts in mind, we have chosen this plant and decided to prepare various formulations of its ethanolic extract. Obviously, the uses of formulated materials have much more advantage over pure materials due to several reasons. Now a days, almost all medicines and drugs in the market are the formulated form of their active ingredients. Various formulations such as EC (Emulsifiable concentrate), ME (microemulsion), SC (Suspension concentrate), CS (Capsule suspensions), WG (Water dispersible granules) and WP (Wettable powder) are being used for a number of household and agricultural purposes. Microemulsion (ME) is a water-based formulation, which even after dilution with water remains clear. It is thermodynamically stable system containing a water insoluble component. Minimal agitation is required for the making microemulsion. Once ME (Microemulsion) is formed, it remains clear for years. There is neither phase separation nor turbidity in the formulation. Due to its smaller droplet size and lower viscosity, efficacy of the formulation is far better than conventional formulations like EC (Emulsifiable concentrate).

In the present study, we have prepared 5% (w/w) microemulsion from the ethanolic extract of the Capparis decidua. TEM analysis of microemulsion (ME) reveals that the size of the particle in above microemulsion is in the range of 75-85 nm, which clearly indicates that formulation, is microemulsion. The viscosity of the ME is in the range of centipoise (0.88 cp) which also confirms development of microemulsion. The prepared microemulsion was tested for its antibacterial and antifungal activities by the Broth dilution method for MIC determination. The MIC as determined by the Broth dilution method is a quantitative assay, which gives the minimum amount of the antibiotics, which is required to inhibit the growth of the microorganisms. The antibacterial and antifungal activity of microemulsion was compared with four standard drugs viz. Gentimycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Griseofulvin. The results clearly indicate that the microemulsion prepared by the *C. decidua* extracts exhibits a promising antibacterial and antifungal activity. The MIC of the microemulsion as well as the standard drugs against different strains of bacteria and fungi are summarized in table 1 and table 2 respectively. The main advantage of this formulation over pure extract can be explained on the basis of that the plant extract in crude form cannot be used directly in medicine but in form of a formulation. Microemulsion is a good choice as it is water based environment and user friendly.

Table 1: MIC of *C. decidua* Microemulsion against different strains of bacteria

| S. No. | Antibacterial agent used | MIC (µg/ml) | | | |
|--------|--------------------------|---------------------------|-----------------------------------|----------------------------|----------------------------------|
| | | <i>E. coli</i> (MTCC 443) | <i>Kl. pneumoniae</i> (MTCC 7028) | <i>S. aureus</i> (MTCC 96) | <i>S. pneumoniae</i> (MTCC 1936) |
| 1 | Microemulsion | 125 | 200 | 625 | 100 |
| 2 | Gentamycin | 0.05 | 1.0 | 0.25 | 0.5 |
| 3 | Ampicillin | 100 | 100 | 250 | 100 |
| 4 | Chloramphenicol | 50 | 100 | 50 | 50 |
| 5 | Ciprofloxacin | 25 | 25 | 50 | 50 |
| 6 | Norfloxacin | 10 | 10 | 10 | 10 |

Table 2: MIC of *C. decidua* Microemulsion against different strains of fungi

| S. No. | Antifungal agent used | MIC ($\mu\text{g/ml}$) | |
|--------|-----------------------|-------------------------------|----------------------------|
| | | <i>C. Albicans</i> (MTCC 227) | <i>A. Niger</i> (MTCC 282) |
| 1 | Microemulsion | 250 | 1000 |
| 2 | Nystatin | 100 | 100 |
| 3 | Greseofulvin | 500 | 100 |

CONCLUSION

The present study describes the formulation of microemulsion (5% w/w) of ethanolic extract of *C. decidua*. The microemulsion showed comparable antibacterial activity (100 $\mu\text{g/ml}$) with standard drug ampicillin against the bacterial strain of *S. pneumoniae*. However, the microemulsion was found more potent than the standard drug griseofulvin for its antifungal activity against *C. Albicans*. This study reveals that the plant based formulations may have a good biological activity however more work in this direction is required.

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CONFLICT OF INTEREST

The authors declare that we have no conflict of interest.

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