Antimicrobial, Antifungal and Antioxidant Activity Evaluation of Various Organic Solvent Extracts of *Cleome Turkmena* Bobrov

Mohammad Hossein Farjam, Manijeh Joukar, Fatemeh Ranjbar

**ABSTRACT**

This study was carried out to investigate the antioxidant potential and antibacterial activities of ethanol, methanol, ethyl acetate, n-hexan and chloroform extracts of *Cleome turkmena* Bobrov. Antioxidant effects were determined by DPPH assays while antibacterial activities were using the MIC method. The results showed that both the methanol and ethanol extracts of aerial part exhibited potentially good antioxidant and antibacterial activities compared to the chloroform extracts. The ethanol extract exhibited the best antioxidant and antibacterial activities. Chloroform and n-hexane extracts samples exhibited lower antioxidant and antibacterial activities. All the extracts showed good antioxidant activity.

**INTRODUCTION**

Present years have evident a rebirth of interest in traditional medicines and plant-derived drugs and a return to ‘nature cure’ all over the world. Perfumed and medicinal plants produce and preserve an array of biochemical products, many of which are extractable and useful as chemical feed stocks or as raw material for various scientific investigations. There is a continuous and vital need to find out new antimicrobial compounds with diverse chemical structures and new mechanisms of action for new and re-emerging infected illness [1]. Plants have always been a generic origin of medicaments, either in the form of traditional preparations or as pure active program. It is thus reasonable for decision makers to identify locally available plants or plant extracts that could usefully be added to the national list of drugs [2]. According to World Health Organization, medicinal plants would be the best source to obtain a diversity of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy [3]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in distinct parts of the universe [4].

The extension and deploy of antibiotic resistance and the improvement of new strains of illness creating pathogens, is of great concern to the global health community. The only alternative method of handling antimicrobial drug resistance is the use of conventional antimicrobials from natural products. Therefore, development of new antimicrobials from other sources such as bioactive plant metabolites will help in solving these problems. Plants are rich in a vast diversity of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [5,6]. In the present study, the aerial part extract of *Cleome turkmena* Bobrov was used to detect the antibacterial, antifungal and antioxidant activity.

*Cleome* is a genus of flowering plants in the family Cleomaceae and its different parts are used in medicine as well as home remedies for common ailments. This genus is distributed in the tropical and warm temperate regions of the world with about 170 species of herbaceous annual or perennial plants [7]. Sixteen species of the genus *Cleome* are known wild in moderate and subtropical areas of southern Iran [8]. Several species of *Cleome* have been target of phytochemical studies, e.g triterpenes and flavonoids have already been isolated [9]. Moreover, several pharmacological activities such as anti-inflammatory [10,11], antioxidant [12,13], antineoplastic [14], antioxidative stress [15], antimicrobial [16,17], antipyretic [18], diuretic [19] and analgesic
effects [20,21] have already been demonstrated for crude extracts or compounds obtained from different Cleome spp. On the other hand, some species of this genus are used as folk medicine in the treatment of scabies, rheumatic fever and inflammation [22,23], bronchitis and diarrhea [24], stomachache, headaches, neuralgia and other localized pains [25]. Meanwhile, the oil of just one species of genus Cleome (C. hirta Oliv.) is repellent to livestock tick and maize weevil [26].

C. turkmena Bobrov, is gramineous, yearly plant, with exalted and ramified stems, bulky and vertical roots, and endemic in Iran. The present work is carried out on Cleome turkmena Bobrov aerial part extract with the following objectives:

1. To screen and evaluate the antibacterial activity of ethanol, methanol, chloroform, ethyl acetate and N-hexan extracts against clinically isolated species of Staphylococcus epidermidis, Corynebacterium glutamicum and Staphylococcus aureus.
2. To evaluate the antioxidant activity of ethanol, methanol, chloroform, ethyl acetate and n-hexan extracts by DPPH method.

MATERIAL AND METHODS

Purveyance of Extracts:
The aerial part of Cleome turkmena Bobrov was collected from Khorasan Torbatejam between Mahmud Abad and Melo, and dried. The dried fruit powder of Cleome turkmena Bobrov was extracted with n-hexan, chloroform, ethyl acetate, methanol and ethanol for five days. It was then filtered to get the crude extracts which was then evaporated to dryness under vacuum and the dried extracts of n-hexan, chloroform, ethanol, ethyl acetate and methanol were used for antibacterial activity.

DPPH radical scavenging activity:
The radical scavenging activity of plant extracts against the stable free radical DPPH was measured as described previously [27], with some modifications. Briefly, 4 different concentrations of the plant extract dissolved in solvents were incubated with a methanolic solution of DPPH (100 µM) in 96-well microplates. Concentrations were carefully chosen according to the activity of this plant, in order to produce an appropriate dose-response curve. Plant extract concentrations used in this study ranged from 1.6 to 100 µg/mL. After 30 min of incubation at room temperature in the dark, the absorbance at 490 nm was measured by a microplate reader (Bio-Rad, model 680). The percentage inhibition (%I) for each concentration was calculated by using the absorbance (A) values according to the following formula:

\[ \%I = \frac{(A_{DPPH} - A_p)/A_{DPPH}}{x 100} \]

Where \( A_{DPPH} \) and \( A_p \) were the absorbance of the DPPH solutions containing solvent and plant extract, respectively. The dose response curve was plotted by using the software Sigma Plot for Windows version 8.0 and IC values for extract was calculated. These values were divided by the extraction yield (Y) to calculate the IC value for the dry plant.

Antimicrobial activity:
The antimicrobial effects were tested by the minimum inhibitory concentration (MIC) by using the macro dilution broth technique [28]. This activity of the plant extracts were screened against six bacterial strains and three fungal strains. The bacteria used were Escherichia coli (PTCC 1396), Klebsiella bacter (PTCC 1053), Escherichia albertii (PTCC 1399), Staphylococcus aureus (PTCC 1431), Staphylococcus epidermis (PTCC 1435), Corynebacterium glutamicum (PTCC 1532 and three fungal strain, Aspergillus niger (PTCC 5154) Fusarium solani (PTCC 5284), Alternaria alternaria (PTCC 5224), obtained from the Persian type culture collection (PTCC), Tehran, Iran. twice.

RESULT AND DISCUSSIONS

The different solvent extracts of aerial part of C. turkmena Bobrov tested were inhibitory to all the test organisms at various levels. The ethanol and methanol extracts showed high activity against bacteria. The methanol extract of this plant was inhibitory to all the test organisms of which, Staphylococcus epider areus and Staphylococcus epider midis as found to be highly sensitive. The ethanol extract also exhibited high activity against the growth of Corynebacterium glutamicum, Staphylococcus epider areus and Staphylococcus epider midis in comparison with other organisms. The results of the study showed that all the extracts had good inhibitory activity against Gram-positive test organism. Although all five extracts showed promising antibacterial activity against test bacterial species, yet maximum activity was observed in ethanol and methanol extract. The minimum inhibitory concentration in these solvents ranged in 8-128 µg/mL. The inhibitory activity of the various solvent extracts was dose-dependent, enhanced with increase in concentration. (Table 1)
In the continuance study it was determined different solvent extract of *C. turkmena* Bobrov antioxidant effects. Among the extracts prepared with various solvents, the strongest effect was supplied by methanol, also other extracts have been remarkable antioxidant property on inhibition of DPPH assay. Oil. The methenolic extract had the highest antioxidant activity measured with DPPH assays was 120.25 ± 0.90 μg/ml. Table 2) Also Ethanol extract (IC_{50}=108.1±0.3) and Crude extract (100.13±0.91) Had high antioxidant activity.

### Table 1: Antimicrobial activity of various extracts of *C. turkmena* Bobrov.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>PTTC</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Ethyl acetate</th>
<th>N-hexane</th>
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<tbody>
<tr>
<td><strong>Gram-negative</strong></td>
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<td><em>E. coli</em></td>
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<td>128</td>
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<td><em>Klebsiella</em></td>
<td>1053</td>
<td>64</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>256</td>
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<tr>
<td><em>E. alburti</em></td>
<td>1399</td>
<td>64</td>
<td>32</td>
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<td>128</td>
<td>256</td>
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<tr>
<td><strong>Gram-positive</strong></td>
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<td></td>
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<tr>
<td><em>Staphylococcus</em> epidermidis</td>
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<td>32</td>
<td>16</td>
<td>128</td>
<td>64</td>
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<td><em>Corynebacterium glutamicum</em></td>
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<td>16</td>
<td>32</td>
<td>64</td>
<td>256</td>
<td>128</td>
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<tr>
<td><em>Staphylococcus</em> aureus*</td>
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<td>8</td>
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<tr>
<td><em>Fusarium</em> solani</td>
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<td>256</td>
<td>256</td>
<td>128</td>
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<tr>
<td><em>Alternaria</em> alternaria*</td>
<td>5224</td>
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<td>128</td>
<td>256</td>
<td>128</td>
<td>512</td>
</tr>
</tbody>
</table>

### Table 2: % Inhibition of DPPH – Free – Radical Scavenging Activity of aerial part Extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH IC_{50} (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>100.13±0.91</td>
</tr>
<tr>
<td>Ethanol</td>
<td>108.1±0.3</td>
</tr>
<tr>
<td>Methanol</td>
<td>120.25 ± 0.90</td>
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<tr>
<td>Chloroform</td>
<td>76.22±1.31</td>
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<tr>
<td>Ethyl acetate</td>
<td>60.1±0.1</td>
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<tr>
<td>N-hexane</td>
<td>31.15±1.9</td>
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<tr>
<td>BHT</td>
<td>19.72 ± 0.8</td>
</tr>
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</table>

**KEY:** BHA = Butylated Hydroxyl Anisole

### REFERENCES


