Studies on Chemical Constituents, Phytochemical Profile and Pharmacological Action of Datura alba

Ghias Uddin, Abdur Rauf and Samina Akhtar

Institute of Chemical Sciences, University of Peshawar, Peshawar-25120, KPK, Pakistan

Abstract: Phytochemical screening of various fractions of Datura alba showed bioactive secondary metabolites based on microbial and antioxidant activities. n-Hexane fraction showed a significant activity against three selected bacterial strains; Bacillus subtilis, Klebsiella pneumoniae and Staphylococcus aureus while other isolated fraction showed moderate activity. The ethyl acetate (88.89 %) and methanolic (76.94 %) fraction exhibited significant antioxidant properties at 100 µg/ml against quercetin. β-Sitosterol (1) and stigmasterol (2) were isolated hither unreported from the methanolic extract of plant.

Key words: Datura alba • Phytochemical profiling • β-Sitosterol • Stigmasterol • Antioxidant • Antibacterial activity

INTRODUCTION

Medicinal plants are an important source of producing valuable bioactive compounds which is great importance for the health of individuals and communities. The medicinal values of the plants are due to the chemical substances that produce a definite physiological action on human body and are called phytochemicals [1]. Infectious diseases are the leading cause of death in all over the world. Plants constitute of various naturals products that are important form medicinal point of view. As plants are abundance of these products and the expanding areas of health and medicine demands to evaluate the presence of these products, it is reported that over 50% of all modern clinical drugs are of natural product origin. They therefore, play an important role in development in the pharmaceutical industry. All these factors demand for more vast study of natural products and biologically active compounds [2]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either pure compounds or as standardized plant extract, provide unlimited opportunities for new drug leads because of unmatched availability of chemical diversity. There is a continuous and urgent need to discover new compounds with diverse chemical structures and novel mechanisms of action for new and reemerging infectious diseases [3].

Datura alba belong to family Solanaceae which is commonly known as is also known as angel’s devils trumpet. D. alba is an annual herb grown up to 3 ft in height [4]. It belongs to genus Datura, which consists of fifteen species. All parts of the Thorn apple have medicinal value, but only the leaves and seeds are officially used. D. alba is a very well-known plant for its various medicinal activities in various parts of the world. It is widely used for the treatment of asthma, healing potential of burn wounds, muscle spasm, whooping cough, hemorrhoids and skin ulcers etc. This plant is used by the Curve’s practitioners for all types of wounds [1]. The leaf extracts of D. alba show very good toxicity against aphids and ants [5]. On the basis of such interesting activity it was considered to carry out the phytochemical screening, antibacterial activity and radical scavenging activity of the plant to evaluate more important side of the plant. The plant appears in late winter/early spring season and bloom repeatedly. In current study, we have made an effort to identify potential bioactive secondary metabolite and their antioxidant activities.

Corresponding Author: Ghias Uddin, Institute of Chemical Sciences, University of Peshawar, Peshawar-25120, KPK, Pakistan.
MATERIALS AND METHODS

Plant Materials: The of D. alba were collected from the Ground of Institute of Chemical, Sciences, Peshawar, Peshawar, Khyber Pakhtunkhwa Pakistan. The plants were identified by Prof Dr Abdur Rashid Department, of Botany, University of Peshawar, Peshawar, Pakistan.

Extraction, Fractionation and Isolation: Shade dried of Datura alba were soaked in methanol for 5 days. The extract were concentrated using electric water bath at 50 °C. The solvent extract was concentrated under reduce pressure at 40 °C using rotavapor, the extracts obtained were suspended in water and successively partitioned with n-hexane, chloroform, ethyl acetate and methanolic fraction. The chloroform fraction was subjected to column chromatography which led to the isolation of two known compounds β-Sitosterol (1) and stigmasterol (2). The structure of these compounds was elucidated by comparing their physical and spectra data with already reported data in literature [6].

Phytochemical Profiling: The chemical tests were performed on the hexane, chloroform, ethyl acetate and methanolic extracts of Datura alba using standard procedure [7-10] to identify the bioactive secondary metabolite.

Test for Alkaloids: 0.2g of each fraction was warmed with 2%H₂SO₄ for 2 min. The reaction mixture was filtered and added a few drops of Dragendorff reagent to each filtrate. Orange red precipitate indicates presence of alkaloids.

Test for Tannins: A small quantity of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added. A dark green color indicates tannins.

Test for Glycosides: Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling solution A and B were added. Red color indicates presence of Glycosides.

Test for Reducing Sugars: Each extract were shaken with distilled water and filtered. The filtrate was boiled with fewer drops of Fehling solution A and B. An orange red precipitate indicates the presence of sugars.

Test for Saponins: 0.2 g of each extract was shaken with 5ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows presence of saponins.

Test for Flavonoids: 0.2g of each extract was dissolved in diluted NaOH and few drops of HCL were added. Yellow solutions that turn colorless indicate the presence of flavonoids.

Test for Phlobatanins: 0.2 g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2%HCL solution. Red precipitate indicates the presence of phlobatanins.

Test for Steroids: 2ml of acetic anhydride was added to the mixture of 0.5 g of each extract and H₂SO₄ (2ml). The color from violet to green in some samples indicates the presence of steroids.

Test for Terpenoids: 0.2 g of each extract was mixed with 2ml of chloroform and concentrated (3ml) H₂SO₄ was carefully added to form a layer. The formation of reddish
brown coloration at the interface indicates the presence of terpenoids.

**Test for Cardiac Glycoside:** To 2 ml of plant extract, 1 ml of glacial acetic acid and 5% ferric chloride was added. Then few drops of concentrated H2SO4 were added. Presence of greenish blue colour indicates the presence of cardiac glycosides.

**Test for Anthraquinones:** 0.5 g of each extract was boiled with 10% hcl for few min. The reaction mixture were filtered and allowed to cool. Equal volume of chloroform was added to each filtrate. Few drops of 10% ammonia was added to each mixture and heated. Rose-pink color indicates the presence of anthraquinones.

**DPPH Radical Assay:** The hydrogen atom or electron donation abilities of the corresponding extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of 2, 2-diphenyl-1-picyrylhydrazyl (DPPH.) Experiments were carried out according to the standard procedure [11]. Briefly, a 1 mM solution of DPPH radical solution in methanol was prepared and 1 ml of this solution was mixed with 3 ml of sample solutions in methanol (containing 20-100 ug) and control (without sample). The solution was stand for 30 min, in dark the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follows.

\[
\% \text{ DPPH} = \frac{\text{Control absorbance} - \text{extract absorbance}}{\text{Control absorbance}} \times 100
\]

The results obtained of phytochemical screening of *Dature alba* is presented in Table 1. The *n*-hexane fraction exhibited the presence of terpenoids, tanins and saponins while rest of phytochemical was not detected. The chloroform fraction showed the accumulation of terpenoids, tanins and saponins, glycosides, reducing sugar and amino acid. When ethyl acetate was tested for various phytochemical tests, it confirmed the presence of terpenoids, tanins and saponins, reducing sugar, beta cyanin and amino acids.

The antibacterial effect of various solvent fraction of *D. alaba* is presented in Figure 1. The medicinal value of the plant can be correlated to the presence of various bioactive secondary metabolites. The *n*-hexane and ethyl acetate fractions and methanolic extract of the *D. alaba* showed the presence of terpenoids which exhibits antiviral and antibacterial activities.

### RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Chemical components</th>
<th><em>n</em>-hexane</th>
<th>chloroform</th>
<th>EtOAc</th>
<th>MeOH</th>
<th>Crude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phlobatanins</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Key words: present: +, absent: -
Fig. 1: Anti-bacterial activities of crude extract and various solvent extracted fractions D. alba
Key: Well size: 6 mm, B.C = Bacillus subtilis, K.P = Klebsiella pneumoniae, S.A = Staphylococcus aureus

Fig. 2: DPPH radical scavenging activity of the crude extract and fractions of D. alba

Our findings correlate with the observations of previous screened medicinal plants for antimicrobial activity, where most of the active plants showed activity against selected bacterial strains. The pharmacological activity of D. alba was confirmed by the antimicrobial assay of various fractions and methanolic extract. Among the tested bacteria Klebsiella pneumoniae and Staphylococcus aureus exhibited complete resistance against all the tested solvent fractions except n-hexane. The chloroform fraction exhibited 16, 12, 10 and 10 mm zone of inhibition against Klebsiella pneumonia, Staphylococcus aureus, S. Typhimurium and Bacillus subtilis respectively. While the ethyl acetate was tested against these bacterial strains, it caused activity only against Staphylococcus aureus 10 mm zone on inhibition. Methanol fraction demonstrated moderate antibacterial effect against Klebsiella pneumonia, S. Typhimurium and Staphylococcus aureus and Bacillus subtilis with zone of inhabation ranging from 10-12.

The antioxidant effect of various solvent fractions Datura alba of is presented in Figure 2. The maximum (88.3%) free radical scavenging effect was observed with ethyl acetate fraction at higher concentration (100 µg/ml), while the effect was weak (22.4%) at 10 µg/ml. the antioxidant effect of n-hexane was higher (73.40%, at 100 µg/ml) but the effect at lowest concentration (10 µg/ml) was 5.01%. the free radical scavenging effect of chloroform and chloroform fraction was moderate (29.89 %) at highest concentration. Our results suggest that further work is needed to locate the active principles from the various extracts or fractions and such efforts could result in the discovery of new compounds possessing a wide range of bioactivity for the treatment of infectious disease.
REFERENCES


