Research Article

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Computational and experimental investigation of antibacterial and antifungal properties of Nicotiana tabacum extracts

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Abstract: The identification of novel anti-infective agents of synthetic and natural origin represents one of the main aims of contemporary drug discovery. In the current work, four different varieties of Nicotiana tabacum, namely, K399, SPG28, Swat No. 1, and Swat No. 2, were studied to assess the antibacterial and antifungal properties of their extracts. The extracts contain anthraquinones, alkaloids, saponins, terpenoids, tannins, resins, steroids, proteins, and carbohydrates, and the antibacterial and antifungal activities were evaluated toward four bacterial and four fungal strains. N. tabacum K399 showed the highest zone of inhibition against E. coli. Similarly, K399 showed the highest antifungal potential, as the highest zone of inhibition for the set was detected against C. albicans. Then, the underlying molecular mechanism was further investigated, and the extracts were tested for their inhibitory potential against urease, an enzyme which is conserved in bacteria and fungi. Additionally, computational tools were enrolled to assess the role of rutin and chlorogenic acid, which are among the main constituents of N. tabacum leaves, in interacting with urease through molecular docking. Combined together, the computational and experimental results support the antibacterial and antifungal potential of N. tabacum extracts, particularly, that obtained from K399 variety.

Keywords: Nicotiana tabacum, rutin, chlorogenic acid, antibacterial, antifungal

1 Introduction

The term “tobacco” refers to the processed leaves of diverse plant species from the genus Nicotiana belonging to the family of Solanaceae, which are abundantly cultivated in Asia, America, Australia, and southwestern Africa [1–3]. Several varieties of Nicotiana species are not only consumed primarily for recreational purposes but also for medicinal purposes [4]. More specifically, even though nearly 60–75 different species have been identified, N. tabacum L. and N. rustica L. are predominantly grown for human use [5].

Several studies aiming at determining the phytochemical constituents of Nicotiana species and their associated bioactivities were reported [6,7] as N. tabacum, in particular, is found to be a rich source of various bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant potential. In this connection, N. tabacum contains several active constituents besides the eponymous nicotine, including flavonoids, phenolic acids, alkaloids, terpenoids, etc., which are responsible for its different biological effects [6,8–10]. Besides, the phytochemical profile of N. tabacum leaves is widely affected by various factors like dehydration, treatment process, fermentation, and storage. Polyphenols account for nearly 7% of the dry weight of tobacco and their content varies depending upon variety, stage of harvesting, maturation stage, and other environmental parameters such
as temperature [11]. More specifically, the polyphenolic composition of tobacco includes rutin, chlorogenic acid, flavone, and scopolamine. Interestingly, among these species, rutin and chlorogenic acid account for nearly 80% of the total polyphenol content of N. tabacum leaves [12–14].

In contemporary drug discovery, great attention and efforts are pointed towards the world of natural products for the identification of novel antibacterial [15] and antifungal agents [16,17]. More specifically, recent contributions in the literature shed light on the potential of N. tabacum extracts to contrast the proliferation of bacteria and fungi [18,19]. In this context, the aim of the current study is to evaluate the potential of extracts from K399, SPG38, Swat No. 1 (SN1) and Swat No. 2 (SN2) varieties of N. tabacum as antifungal and antibacterial agents. The extracts were tested in vitro, and urease inhibition was also evaluated. Additionally, computational studies were carried out to support the interpretation of experimental data.

2 Methods

2.1 Samples collection and extraction

Four different indigenously available varieties of N. tabacum, i.e., SN1, SN2, SPG28, and K399, were collected from local farmers of Swabi District, Khyber Pakhtunkhwa (KPK), Pakistan. These samples were brought to the Department of Chemistry, University of Swabi, KPK, Pakistan for further processing, and the sample voucher NO.UOS-BOT/120 was deposited in the Department of Botany, University of Swabi.

Leaves from the four different species of N. tabacum were separated from stems, cleaned, washed, and dried for 2 weeks in the shade. After drying, plant material (2 kg) was grinded, and the resulting powder was collected and used for further analysis [20]. Then, powdered leaves (100 g) from the four different varieties were placed in a 1 L volumetric flask with 800 mL of methanol for 7 days. Afterwards, the mixture was filtered using grade 1 filter paper from Whatman (Maidstone, UK), and the filtrate was evaporated under reduced pressure to provide the crude extracts, which were stored at −20°C. All used chemicals, reagents, and solvents were of analytical grade and were obtained from Honeywell-Fluka (Charlotte, NC, USA), Sigma-Aldrich (St. Louis, MO, USA), and Merck (Darmstadt, Germany).

2.2 Preliminary phytochemical analysis

The preliminary screening for the detection of different phytochemical compounds like saponins, proteins, steroids, flavonoids, alkaloids, tannins, anthraquinones, terpenoids, and carbohydrates was performed using standard protocols based on qualitative assays [21–23]. All experiments were carried out in triplicate.

2.3 In vitro antibacterial and antifungal activity

Well diffusion method was adopted to evaluate the antibacterial potential of extracts prepared from four varieties of N. tabacum against S. typhi, E. coli, B. subtilis, and S. aureus [24]. Ampicillin was used as reference compound in this study, and the zone of inhibition (mm) was measured to assess the antibacterial properties. All the tests were performed in triplicate and mean value ± SD was calculated.

The broth dilution method was employed for the determination of antifungal properties of extracts prepared from four varieties of N. tabacum against T. harzianum, A. brasiliensis, C. albicans, and A. niger [24]. Mycostatin was used as reference compound in this study. The inoculum was added to each tube and incubated for 7 days. Following incubation, the antifungal properties of tubes containing standard drug and extracts were evaluated. The zone of inhibition (mm) was then calculated to assess the antifungal properties. All the tests were performed in triplicate and mean value ± SD was calculated.

2.4 Urease inhibition

Jack bean urease inhibitory activity of the extracts was determined by adopting previously reported procedures [24,25]. Briefly, each extract (25 µL) and enzyme (25 µL of 0.25 mg/mL solution) were incubated for 15 min at 30°C. After the initial incubation, urea (55 µL) was added to the mixture, which was again incubated in the same conditions. Then, 45 µL of phenol reagent was introduced in each well (0.005% w/v sodium nitroprusside and 1% w/v phenol) together with 70 µL of alkali reagent (0.1% w/v NaClO and 0.5% w/v NaOH). The absorbance (A) of this mixture was measured at 630 nm. The final mixtures were incubated for additional 50 min at 30°C, and the absorbance at 630 nm was then measured. Thiourea was used as a positive control in this analysis. Percent inhibition of urease was calculated by using the following formula:

$$\text{Percent inhibition} = \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \times 100$$

All the tests were performed in triplicate and the mean value ± SD was calculated.
2.5 Molecular modeling

The structure of the macromolecular target was retrieved from the RCSB Protein Data Bank (www.rcsb.org, PDB ID 3LA4) and the PDB file was selected in agreement with previous works [26,27]. 3D models of ligands were built using Avogadro 1.2.0, and geometry was optimized using the same software [28]. Target was prepared for the blind docking experiments, performed using AutoDock Vina, using default parameters [29]. Receptor search volume was set according to the grid parameters reported as follows: Grid center: $x = -52.260$, $y = -13.702$, $z = 77.095$, size: $75 \times 95 \times 80$ Å. The number of docking poses was set to 10, with other Vina parameters set as default. Residue numbering used in the PDB file was adopted. Output data, such as calculated binding energies and interaction patterns, were analyzed and scored using UCSF Chimera molecular viewer [30]. This software was also used to produce the artworks. Calculated binding energy values are expressed in kcal/mol and refer to the most favored predicted pose.

3 Results

The aim of this study is to evaluate the potential of the extracts of different varieties of *N. tabacum*, namely, K399, SPG28, SN1, and SN2 (Figure 1) as antibacterial and antifungal agents. The first step consists of the extraction of bioactive components. In particular, after processing, the methanolic extracts obtained from dried leaves of the four samples were subjected to qualitative assays to preliminarily determine their content in terms of classes of phytochemical constituents.

The qualitative phytochemical analysis was performed to assess the presence or absence of specific chemical classes of bioactive secondary metabolites in the different varieties [23], and the results are reported in Table S1 in the Supporting information.

More specifically, the K399 methanolic extract showed the presence of tannins, alkaloids, saponins, flavonoids, steroids, terpenoids, carbohydrates, polyphenols, and polypeptides, while anthraquinones, C-glycosides, and resins were not present. On the other hand, anthraquinones, alkaloids, saponins, terpenoids, flavonoids, steroids, carbohydrates, C-glycosides, and polyphenols were detected in the extract from the SPG38 variety, while tannins, resins, and polypeptides were not present. The SN1 methanolic extract showed the presence of tannins, alkaloids, steroids, saponins, flavonoids, resins, carbohydrates, and polypeptides, while C-glycosides and terpenoids were absent. Eventually, the SN2 methanolic extract contained anthraquinones, alkaloids, steroids, flavonoids, resins, carbohydrates, cardiac glycosides,
polyphenols, and polypeptides, while tannins, saponins, sterols, terpenoids, and polypeptides were not present.

Overall, phytochemical screening of extracts from all four different varieties of *N. tabacum* revealed the presence of alkaloids, flavonoids, polyphenols, and carbohydrates in all the tested varieties. It must also be noted that emodins, free reducing sugars, anthocyanins, coumarins, and betacyanins were not detected in any of the *N. tabacum* tested varieties.

Although it is widely accepted that the phytochemical composition of *N. tabacum* may vary depending upon processing conditions, temperature, maturation stage, time of harvesting, and storage [31,32], these findings are in agreement with a previous work by Shekins et al., who reported the presence of glycosides, alkaloids, tannins, phenols, and reducing sugars in methanolic extracts of *N. tabacum* [33].

Following the previously reported promising results concerning the antibiotic potential of *N. tabacum* extracts [18], the antibacterial activity of *N. tabacum* methanolic extracts against *B. subtilis*, *E. coli*, *S. aureus*, and *S. typhi* was tested using the well diffusion method. In this study, ampicillin was used as positive control, and the results are reported in Figure 2 and Table S2 in the Supporting information. The highest antibacterial activity in terms of zone of inhibition (17.0 ± 0.3 mm) was observed in the case of K399 extract against *E. coli*, while SN2 extract was the least effective against the same strain (7.5 ± 0.6 mm). Nevertheless, overall, all the extracts from the four different varieties of *N. tabacum* possessed significant antibacterial activity against the experimented strains.

The potential of *N. tabacum* constituents as antifungal agents was discussed in recent reports [19]. In this context, the extracts from the four varieties of *N. tabacum* were used to test the inhibitory zone towards fungi such as *T. harzianum*, *A. brasiliensis*, *C. albicans*, and *A. niger*. According to the results, reported in Figure 3 and Table S3 in the Supporting information, K399 again showed the highest zone of inhibition (16.5 ± 0.2 mm) against *C. albicans* but it was less effective against *A. niger* (7.0 ± 0.2 mm). These findings are in agreement with the observations by Jabeen et al., who showed that alcoholic extracts of *N. tabacum* possess highest antifungal property against *A. flavus* as compared to *A. niger* [34].

Then, we aimed at investigating a potential mechanism of action by which the extracts may exert their anti-infective activity. In this context, urease is a metalloenzyme that contributes to the regulation of nitrogen metabolism, as it hydrolyzes urea into ammonia and carbamate. Most importantly, it is conserved in many organisms among plants, bacteria, and fungi [35]. Additionally, urease is a target investigated, also by means of computational tools, in the field of drug discovery applied to anti-infective agents [36].

The calculated IC_{50} values of the extracts as jack bean urease inhibitors for K399, SPG28, SN1, and SN2 were 37.09 ± 2.76, 22.87 ± 1.54, 41.98 ± 2.01, and 10.11 ± 0.98 μg/mL, respectively. For thiourea, which was used as a positive control, an IC_{50} value of 21.54 ± 1.00 μg/mL was calculated. On the other hand, the urease inhibitory potential of SPG28 extract was the highest at 200 μg/mL (highest concentration tested). The results, listed in Table 1, demonstrate that the extract inhibit the enzyme, and that the lowest IC_{50} value was observed for SN2 variety.

Eventually, we carried out a preliminary computational study to investigate the binding mode to the same macromolecular target examined in vitro of two highly

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**Figure 2:** Antibacterial activity of *N. tabacum* SPG38, K399, SN1, and SN2 varieties against various bacterial strains.

**Figure 3:** Antifungal activity of *N. tabacum* SPG38, K399, SN1, and SN2 varieties against various fungal strains.
representative components of the polyphenolic fraction of *N. tabacum* leaves, namely, rutin and chlorogenic acid \([12–14]\). These compounds have been widely investigated for their multitarget activity \([37–40]\). Indeed, computational tools are nowadays widely used in the drug discovery workflow \([41,42]\). Docking studies showed that rutin and chlorogenic acid may interact with the active site of urease, in close proximity to the metal ions within the protein (Figure 4a). More specifically, a more promising calculated binding energy value was calculated for the first compound (\(-8.2\) kcal/mol) when compared to the latter (\(-7.1\) kcal/mol). A detailed view of the predicted interaction patterns with the enzyme is reported in Figure 4b and c.

4 Conclusion

In this study, the presence of bioactive components in *N. tabacum* K399, SPG28, SN1, and SN2 extracts was

<table>
<thead>
<tr>
<th>Extract/standard</th>
<th>% Inhibition at 200 µg/mL</th>
<th>IC₅₀ (µg/mL)</th>
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<tbody>
<tr>
<td>K399</td>
<td>75.32</td>
<td>37.09 ± 2.76</td>
</tr>
<tr>
<td>SPG28</td>
<td>89.43</td>
<td>22.87 ± 1.54</td>
</tr>
<tr>
<td>SN1</td>
<td>69.32</td>
<td>41.98 ± 2.01</td>
</tr>
<tr>
<td>SN2</td>
<td>91.54</td>
<td>10.11 ± 0.98</td>
</tr>
<tr>
<td>Thiourea</td>
<td>98.45</td>
<td>21.54 ± 1.00</td>
</tr>
</tbody>
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Figure 4: Computed binding mode for rutin (orange) and chlorogenic acid (green) with urease (PDB ID 3LA4) (a) A detailed view of the residues interacting with the natural compounds is depicted for rutin (b) and chlorogenic acid (c). The involved amino acids are highlighted in blue.
highlighted. The four varieties contain alkaloids, flavonoids, polyphenols, and carbohydrates, plus some classes of compounds that specifically characterize the single varieties.

In vitro studies demonstrated that N. tabacum extracts, and K399 in particular, possess significant antibacterial and antifungal activities. Besides, enzymatic inhibition and computational studies support the hypothesis that urease inhibition is involved in the observed biological effect.

Taken together, the computational and experimental studies support the role of N. tabacum extracts as anti-infective agents, in a field of research in which the identification of novel remedies is an urgent need [15,43].

Although further studies are needed to clarify the involvement of single compounds at the molecular level, these findings pave the way for the identification of novel classes of nature-inspired anti-infective agents from N. tabacum leaves.

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: All data generated or analyzed during this study are included in this published article.

References


