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ORIGNAL ARTICLE

Phytochemical Analysis and Antibacterial Activity of *Nicotiana tabacum* and *Nicotiana rustica*

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ABSTRACT

Background: All over the world, natural products containing different secondary metabolites have been used for antibacterial purposes, and as folk medicines with significant effects. Amongst many different plants, Tobacco plants are cultivated all over the world, but natively belong to America. These plants contain variety of secondary metabolites and possess significant antibacterial activity.

Objectives: To conduct phytochemical analysis and measure the antibacterial potential of Tobacco plants *Nicotiana tabacum* and *Nicotiana rustica* using their extracts.

Methodology: Multiple chemical tests such as Wagner's test and Fehling's test were used to determine the presence of different types of secondary metabolites. Both plant species were also screened for their antibacterial activity using agar well diffusion method.

Results: Phytochemical analysis of the extracts from both plant species i.e. *Nicotiana tabacum* and *Nicotiana rustica* indicated the presence of secondary metabolites including tannins, alkaloids, terpenoids, saponins, steroids, and flavonoids. Significant antibacterial activity of both plant extracts was observed against *Staphylococcus aureus*, but not against *Escherichia coli*.

Conclusion: It can be concluded that both plant extracts showed the presence of secondary metabolites, with significant inhibitory effect observed against *Staphylococcus aureus*, and no effect against *Escherichia coli*.

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INTRODUCTION

Medicinal plants have been used for treatment of various diseases in past and around 5000 species have been identified[1] so far; that are used in cosmetics and pharma industries for the development of new products[2-3]. Various kinds of organic compounds can be produced by plants, in which some compounds do not contribute to their own functions and are referred as secondary metabolites[4]. Natural products contain diverse bioactive properties and can lead to the drug discovery[5]. Most of them are categorized as Generally recognized as safe, (GRAS) family of plants that are prominently used as folk medicines[6-14] which led to the discovery of new drugs[6].

Medicinal plants are the source of natural products having the properties to cure the disease. The 50% available drugs; including pharmaceutical and cosmetics products in the market[7] belongs to the natural products isolated from

the medicinal plants[8-9]. In Chinese and Egyptian cultures, these natural products are still used as medicine and more than 75% population believes and relies on traditional natural products as reported by WHO[10-11].

Plants are rich source of antioxidant, anti-fungal, anti-viral and anti-parasitic agents[12-14]. Among the plant family Solanaceae, *Nicotiana tabacum* plant is cultivated all over the world but natively belongs to America[15].It has been reported to be sensitive to environmental factors including humidity, nature of land and temperature. Some species of *Nicotiana tabacum* have also been used for ornamental purposes[16]. Similarly, another plant *Nicotiana rustica* belongs to the family Solanaceae, contains nicotine in greater amount that is used for the production of pesticides products. These plants contain variety of secondary metabolites and possess significant antibacterial activity[12-14].

MATERIAL & METHODS

Distilled water and all the necessary glassware were obtained from Department of Chemistry, University of Swabi, KPK. All the chemicals used in these experiments were purchased from the Sigma Aldrich.

Instrumentation

The plant leaves were grinded to fine powder by using grinder. Similarly, the extract was concentrated using water bath (HH-S_6-China). Filter paper discs (Oxoid USA) were used for the antibacterial assessment while, sterilization was done by using autoclave.

Collection of Plants

The whole plants of *Nicotiana tabacum* and *Nicotiana rustica* were collected from the district Swabi, KPK. Plants were then washed with water in order to remove dust and impurities. After washing, *Nicotiana tabacum* and *Nicotiana rustica* plants were shade dried for one week in order to avoid any phytochemical reaction. After that, the dried plants were grinded into small pieces with the help of electric grinder. The grinded powder then subjected to the extraction procedure.

Extraction Procedure

Grinded powder from both plants was soaked separately into a mixture of methanol and distilled water in a ratio of 1:2 in conical flask (Erlenmeyer flasks), caped with the aluminum foil. These flasks were kept in shelf for 4 days at room temperature. After 4 days, the mixture was subjected to extraction techniques; evaporated in water bath at 70°C and concentrated samples were collected. The procedure was repeated thrice and the extract was stored in clean sterile bottle for further use.

SYNTHESIS OF REAGENTS

Fehling's Solution A

For the preparation of Fehling solution, 7g of $CuSO_4.5H_2O$ was added to 100ml of distilled water and then 2 drops of dilute H_2SO_4 were added to the solution, that turned the solution blue suggesting the Fehling solution A is ready.[20]

Fehling's Solution B

For the preparation of Fehling solution B, 35g potassium tartrate and 12g NaOH was mixed with distilled water, as a result a colorless solution was formed confirming the formation of Fehling solution B.[20]

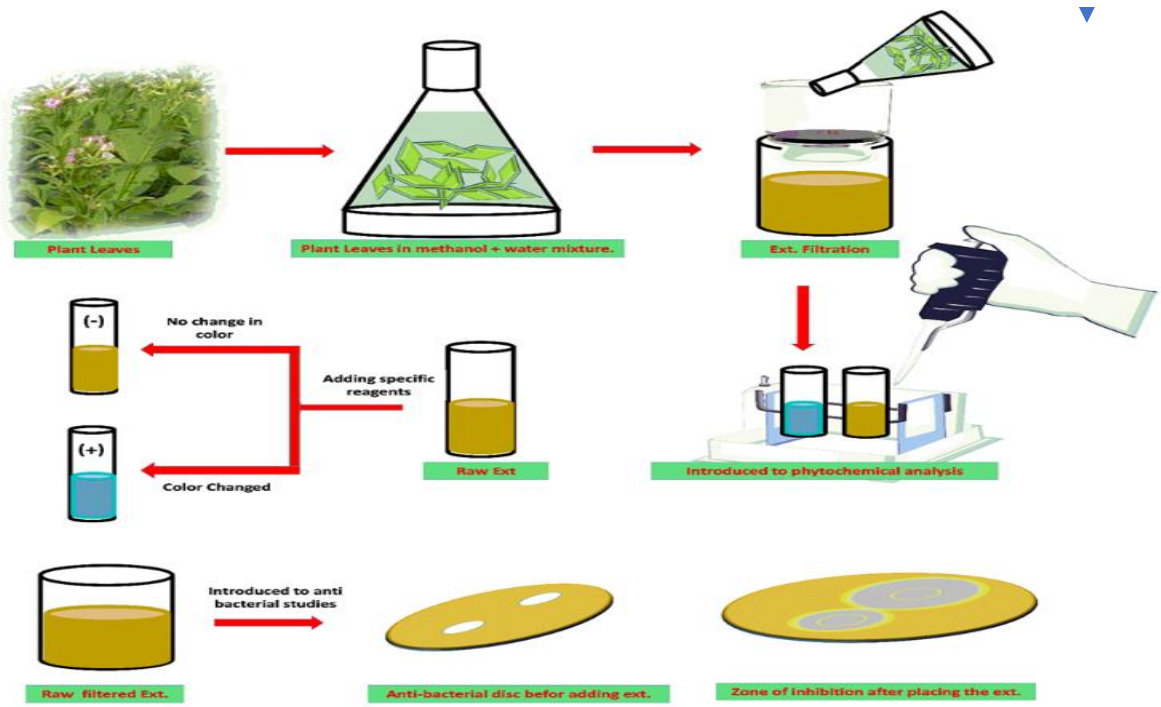
Wagner's Solution

A total of 2g of iodine and 6g of KI (potassium iodide) were dissolved in 100L of distilled water to prepare Wagner solution.[21]

BIOLOGICAL ACTIVITY

Agar Well Diffusion Method

The biological activity was checked by agar well diffusion method following procedure from Murray *et al*., well diffusion protocol[17]. Antibacterial activity was done using Mueller–Hinton agar (MHA). For the preparation of media, 1.9g MHA was dissolved in 50ml of deionized water, the prepared media was autoclaved for 15min. Media (15ml) each was poured into sterilized petri dishes to make MHA sensitivity testing plates. Later, the agar surface was inoculated by spreading 200µl of the microbial inoculum of *S. aureus* and *E. coli*, followed by making wells (~8mm) in agar with a sterile cork borer. 100µl *Nicotiana tabacum* and *Nicotiana rustica* extracts were transferred to their respective wells in each plate. The plates were incubated at 37°C for 24h. After 24h, the zones of inhibition were observed surrounding wells, which indicates that extract from both the species possess significant antibacterial activity.



Scheme-1. Extraction procedure, phytochemical analysis and anti-bacterial activity of *Nicotiana tabacum*and *Nicotiana rustica.*

RESULTS AND DISCUSSION

Phytochemical analysis of the *Nicotiana tabacum* (Fig. **1**a) and *Nicotiana rustica* (Fig. **1**b) extracts was performed using different reagents. The following test has been performed for the identification of different secondary metabolites in the extract of *Nicotiana tabacum* and *Nicotiana rustica* species. The entire procedure is depicted in scheme-**1**.

Tannins Identification

For the identification of tannins in the extract of both plant species, specific amount of the selected plant extract was taken and mixed with distilled water. The filtrate was then collected from the reaction mixture after heating. After this, the filtrate was poured in the test tube and ferric chloride drops were added to that filtrate resulting in the formation of new color i.e. dark greenish. The appearance of new color confirms the presence of tannins in both plants as shown in the inset of Fig. **1**(c)[17].

Alkaloid Identification

For the identification of alkaloids, 0.3g of each extract was dissolved in 2ml of distilled water separately and warmed up with 2% sulphuric acid for 3min, and then filtered. After that, few drops of Mayer's solution was individually added to each plant extract in the test tube. The creamy white precipitates were observed in both extracts which indicates the presence of alkaloids in Fig. **1**(d) [18].

Amidine Identification

Both extracts (0.5g) were separately mixed in 3ml of ammonium hydroxide. Benzene was added to the reaction medium in test tubes. Lack of reddish color indicated the absence of amidine in the both extracts.[19]

Phlobatannins Identification

For the detection of phlobatannins, 0.5g of both extracts was mixed with distilled water in the separate test tubes, followed by filtration. After this, 3% HCl was added and boiled. Absence of reddish color suggests the absence of phlobutannins in both plants.[19]

Terpenoid Identification

For this purpose, 0.3g from both extracts was added to 2ml chloroform and filtered into the test tube. After that, 3ml of sulphuric acid was introduced resulting in the layer formation followed by the appearance of reddish brown color, which shows the positive result for terpenoids. We have observed that each plant extract possesses terpenoids see Fig. **1**(c).[19]

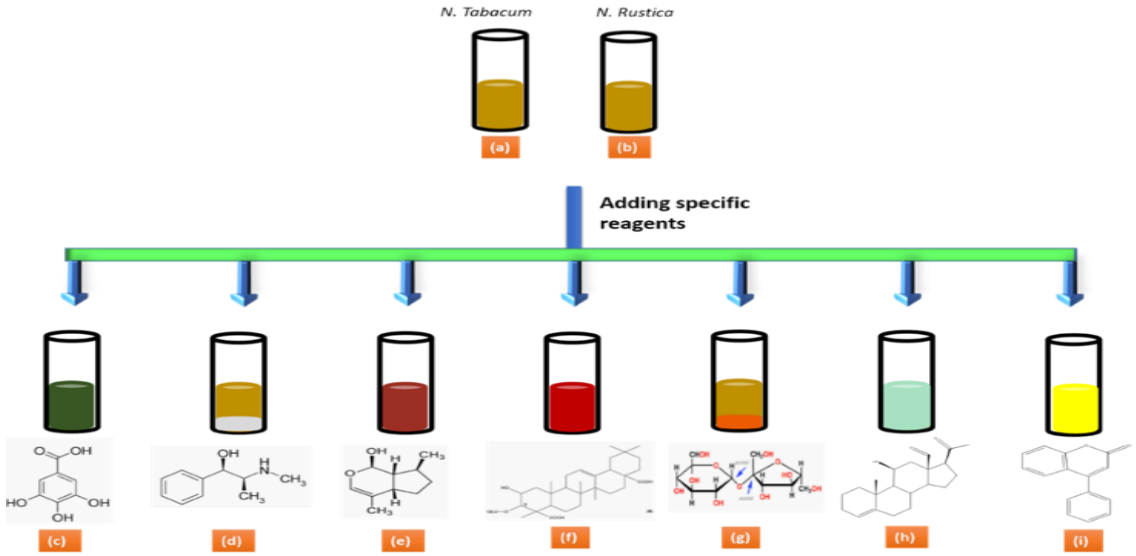


Fig 1. Raw extracts of *Nicotiana tabacum*(a) and *Nicotiana rustica* (b). After the addition of specific reagents, change in color indicates the presence of tannins (c), alkaloides (d), terpenoides (e), saponins (f), reducing sugars (g), steroids (h) and flavonoids (i).

Saponins Identification

For the identification of saponins, 0.4g of both extracts was mixed with distilled water separately. After 10min, the formation of froth and change in color in test tube B indicated the presence of saponins as manifested in Fig. **1**(f).[19]

Reducing Sugar Identification

Each plant extract (0.4g) was mixed with 2ml of distilled water in test tube and then filtered. After that, Fehling solution A & B were introduced and boiled for 8min. According to the literature, the presence of precipitates in orange-red color is the indication of reducing sugars which was absent in these species see Fig. **1**(g).[19]

Steroid Identification

For the confirmation of steroids in the selected plant species, the acetic anhydride (3ml) was mixed with both plant extracts separately and then filtered into the test tubes, followed by the addition of 2ml of conc. sulphuric acid. The blue green color was observed in both species, which confirmed the presence of steroids; see Fig. **1(h)**. [19]

Flavonoids Identification

About 0.3g from each extract was mixed with dilute NaOH, and then HCl was added in test tubes. Appearance of yellow color confirmed the presence of flavonoids in the plant extracts. It was observed that both plant species have flavonoids; see Fig. **1**(i).[19]

Antibacterial Activity

Both the species were subjected to antibacterial activity using Agar Well diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Results showed that the inhibitory effect against *Staphylococcus aureus* was found in the extract of both *Nicotiana tabacum* and *Nicotiana rustica* see Fig. **2**(b), while no inhibitory effect was observed against *E. coli* species; see Fig. **2**(a).[19]

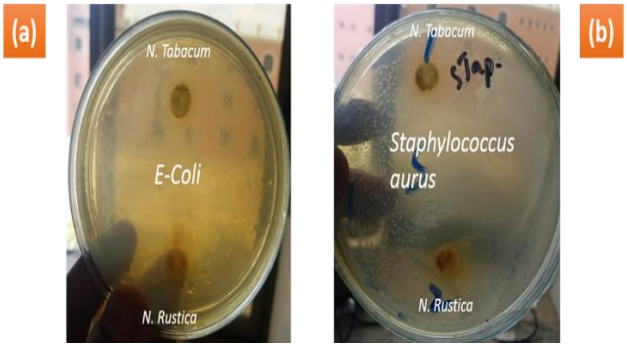


Fig 2. Antibacterial activity of *Nicotiana tabacum* and *Nicotiana rustica* against *Escherichiacoli* (a) and *Staphylococcus aureus* (b).

CONCLUSION

Keeping in view the findings of present research work, it can be concluded that like many different plants, quantitative assessment of *Nicotiana tabacum* and *Nicotiana*

rustica plant extracts also indicates the presence of bioactive phytochemicals like flavonoids, alkaloids, steroids, saponins, terpenoids, alkaloids and tannins. Interestingly, both plant extracts also confirmed significant antibacterial effect toward *Staphylococcus aureus*, while no significant effect have been observed against *Escherichia coli*.

CONFLICTS OF INTEREST

None.

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None.

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LIST OF ABBREVIATIONS

GRAS	Generally Recognized as Safe
KI	Potassium Iodide
$CuSo_4$	Copper Sulphate
MHA	Mueller–Hinton agar
HCl	Hydrochloric Acid
H_2SO_4	Sulphuric Acid
NaOH	Sodium Hydroxide
WHO	World Health Organization

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