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Original article

Phytoconstituents screening and antimicrobial activity of the invasive species Nicotiana glauca collected from Al-Baha region of Saudi Arabia

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ABSTRACT

Nicotiana glauca belonging to the family Solanaceae is an exotic perinnial bushy species that recently has invaded the mountainous areas of Al-Baha region at the south-west of Saudi Arabia, causing degredation to soil and vegetation composition of those mountains. Accordingly, this plant must be either removed or exploited as a source of useful products such as medicines as it has been proven to contain many effective compounds with therapeutic properties. Thus, the current study aimd to screening N. glauca preliminary phytoconsitiuents and its anticmicrobial activity. Results showed that varying amounts of alkaloids, steroid, tannins, flavonoid, were present in the extracts of N. glauca leaves, stems and flowers. Saponins were present only in the flowers extract. However, all previuos bioactive compounds were absent in the roots except alkaloids, therefore, roots posses lesser amount of the phytoconstituents compared to the other parts of the plants. Leaves extracts inhibited the growth of E. coli (16.3 ± 0.71 mm) and also inhibited the growth of S. aureus (11.0 ± 0.23 mm), while, the flower extracts also inhibited the growth of E. coli (6.7 ± 0.65 mm), and also inhibited the growth of S. aureus (15.8 ± 0.52 mm). This result coincide with the previous results of the screening of N, glauca phytochemicals where the highest amounts found in leaves and flowers extracts. The inhibition zone of the antibiotic gentamycin (reference control) were 32 mm in S. aureus and 38 mm in E. coli. The inhibition zone of gentamycin (reference control) were 32 mm in S. aureus and 38 mm in E. coli. The minimum inhibitory concentration (MIC) of ethyl acetate was ranged (1.5–2 mg/ml). The lower MIC is an indication of high effectiveness of extract. Therefore, such bioactive property would support the idea that removing N. glauca from the mountainous areas of Al-Baha, Saudi Arabia, is not the only solution, but the plant can be exploited as a beneficial source of medicinal and economic purposes such as; antibacterial, antifungal and insecticidal, however, further comprehensive exploration is recommended to confirm such propreties.

1. Introduction

The tree tobacco Nicotiana glauca is a fast-growing evergreen perinnial shrub, belongs to the Solanaceae family of which Nicotiana tabacum and Nicotiana rustica are best known for their use as tobacco (Mizrachi, et al. 2000). This plant however occurs mostly in warm areas because of its sensitivity to frost (Henderson, 2001), and it invades wetland areas along roadsides and along river banks, reaches altitudes of 3000 m above sea level. It has been regarded as a global invader that pose a threat to indigenous plants, since it has conquered Central America (Mexico, California, and the Channel Islands), Africa (Morocco, South Africa, and Namibia), Palestine and Australia. It has also adapted widely in many parts of the European Mediterranean region (Spain, Portugal, France, Italy, Sicily, Corsica, Sardinia, Greece and Crete) (Bogdanovic et al, 2006). The plant has recently invaded the mountainous areas of the Southwestern part of Saudi Arabia, causing degredation to soil and the plant composition (Alshahrani, 2008).

N. glauca is well known as a toxic plant (Panter, et al. 1999; Panter, et al. 2000), however, it has been used traditionally in medicine where warmed leaves are applied to the head to relieve headache, on the throat to relieve sore throat and put in shoes for painful feet, moreover, the plant has been used as an insecticide (Steenkamp et al, 2002). It was recorded through gas chromatographic separation analysis that N. glauca's tissues contain the fol-

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lowing compounds: Anabasine, Anatabine and Nicotine. Anabasine is an alkaloid compound similar to nicotine, but it is more toxic, and if it is consumed in concentrated quantities it leads to a rapid disruption in the functioning of the respiratory, nervous and cardiac systems, which may lead to death (Soberon et al, 2007) and (Furer et al, 2011). In addition, (Rinez et al., 2012) conducted a studty on the aqueous and organic extracts of various leaf and flower parts of N. glauca. They concloded these extracts may be used as biofungicides against some pathogenic fungi. Accordingly, since N. glauca is a harmful weed to the flora and soil of the natural habitats, it must be removed or exploited as a source of useful products such as medicines as it has been proven to contain many effective compounds with therapeutic properties. Thus, the current study aimd to screening N. glauca preliminary phytochemical consitiuents and its ptential anticmicrobial activity.

2. Materials and methods

Fresh speciemens of N. glauca were collected from Baha region in the Southwest of Saudi Arabia where this plant is widely distributed at high altitude more than 1000 m above the sea level. Green speciemens including leaves, stems, flowers and roots were collected, then chopped, and spread on traps in order to air dry. The dry, coarsely chopped plantmaterial was further ground to pass through a 2.4 mmesh screen using a Gehl Mix-All model 55 (Gehl Company, West Bend, WI, USA). The groundplant material was stored in plastic bags away from directlight at ambient temperature in an enclosed shed until use.

2.1. Phytochemical screening

The qualitative phytochemical screening for N. glauca. leaves, stem and roots aqueous extract, was conducted based on standard methods of analysis described by Kehail et al. (2017)

Test for the presence of alkaloids: In a test tube, to about 3 ml of each extract, 2 drops of Dragendoff's reagent was added. The formation of orange red precipitation proved the presence of alkaloids.

Test for the presence of flavonoids: In a test tube, to about 4 ml of each extract, a piece of magnesium ribbon and a drop of concentrated HCl were added. The red to crimson color detected the presence of flavonoids.

Test for the presence of saponins: In a test-tube, 5 ml of each extract was shaken vigorously for 2 min. The presence of saponins was indicated by the formation of froth that lasted for some minutes.

Test for the presence of tannins: In a test tube, to 2 ml of each extract, 3 drops of 5% ferric chloride ($FeCl_3$) solution was added. The presence of tannins was detected by the appearance of green, black or blue colour.

Test for the presence of terpenoids: In a test tube, to 5 ml of each chloroform extract, 5 ml of concentrated sulphuric acid was carefully added. The formation of a red-brown layer indicated the presence of terpenoids.

Test microorganism and antibacterial activity of plant extracts

Pure culture of Staphylococcus aureus and Escherichia coli were prepared in the University of Ha"il microbiology lab. The antibacterial activity of the plant extracts was determined by agar diffusion method. For this, fresh (overnight) isolated colony of S. aureus and E.coli were suspended in sterile saline to get turbidity of 0.5 McFarland standards. Then 0.1 ml. of this suspension was spread aseptically on sterile Muller Hinton agar medium (Hi media). Wells of 6 mm diameter were bored by sterile cork borer and 0.2 ml. of each extract (100 mg /ml in 10% DMSO) was added to them, then it was allowed to diffuse by keeping in freeze for 20 min. 10% DMSO in one of the wells was used as negative control. After extracts were diffused the plates were incubated at 37°c for 24 h. Zones of inhibition were then measured in mm. For each extract three replicates were maintained.

2.2. Determination of the minimum inhibitory concentration (MIC)

Tube dilution method was done to determine the minimum inhibitory concentration of the extracts (MIC). A series of two fold dilutions of each extracts ranging from 10 m S. aureus and E.coli g / ml to 0.3 mg/ml were made in Muller Hinton broth. Then, 0.1 ml of the suspension of S. aureus and E.coli was matched to 0.5 McFarland standard was seeded into each dilution. Two controls were maintained for each test batch including tube containing extract and growth medium without inoculum and organism control (i.e. tube containing the growth medium and inoculum). The tubes were incubated at 37°c for 24 h and checked for turbidity. Minimum inhibitory concentration was determined as highest dilution of the extract that showed no visible growth.

2.3. Data analysis

The qualitative phytochemical screening results were presented as detected in moderate concentration as (+) and detected in more concentration as (++), while the inhibition zones diameters (mm) were represented in (Mean ± SE) and the least significant difference (LSD) was applied on these values to determine the significant level as letters.

3. Results

The tree tobacco N. glauca is considered as undesirable weed which must be removed or used as a source useful products such as medicines as it has been proven to contain many effective compounds with therapeutic properties. Thus, the current study aimd to screening N. glauca phytochemical consitiuents and its anticmicrobial activity.

3.1. Phytochemical analysis

Table 1. shows the preliminary phytochemical analysis of extracts of Nicotiana glauca. Results showed that varying amounts of alkaloids, steroids, tannins, flavonoid, and saponins were present in the extracts of the leaves, stems, flowers and roots. Higher precipitation of steroids was present (+++) in leaves, followed by stems (++) and moderately present (+) in leaves, while no trace of steroids was found in roots. Flavonoids higher precipitation (+++) was noticed in leaves extract followed by flowers (++) and then moderately noticed in stems and roots extracts (+). Saponins' precipitation was noticed in all part extracts in moderate amount (+). Tannins higher precipitation (+++) was noticed in leaves extract followed by flowers (++) and stems, roots extract. (+). Finally, higher precipitation (+++) of alkaloids was noticed in both flowers and stems extract, while it was noticed in medium amounts (++) in both leaves and roots extract. In addition, the study showed that most of the phytocompounds were present in flowers, leaves and stmes of N. glauca compared to its roots.

3.2. Antibacterial activity

Table 2 shows the results of antibacterial activity of ethyl acetate, extracts of N. glauca. Also, Figs. 1 & 2 represent the antibacterial activity of extracts of N. glauca parts. In general, leves extracts revealed antibacterial activity more than the other parts of the plant. Leaves extracts inhibited the growth of E.coli (16.3 ± 0.71

Table 1
Preliminary phytochemical analysis of different parts of Nicotiana glauca.

Alkaloids	Tannins	Saponins	Flavonoids	Steroids	Plant parts
++	+++	+	+++	+	Leaves
+++	++	+	++	+++	Flowers
+++	+	+	+	++	Stems
++	+	+	+	–	Roots

Table 2
Antibacterial activity of Nicotiana glauca extracts.

Microorganism	Zone in mm ± SD 50 µl E.coli	Zone in mm ± SD 50 µl S. aureus
Leaves	16.3 ± 0.71	11 ± 0.23
Roots	0	0
Flowers	6.7 ± 0.65	15.8 ± 0.52
Stems	0	0
DMSO	0	0
Gentamycin	38	32

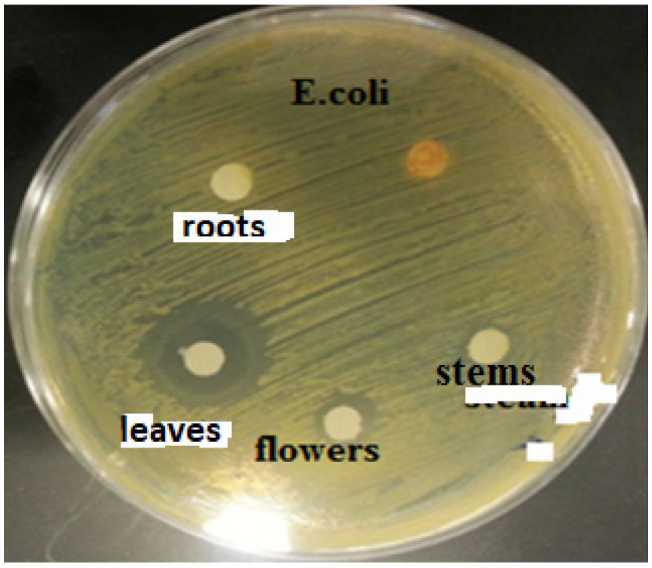

Fig. 1. Antibacterial activity of extracts of Nicotiana glaucaa gainsts E.coli.

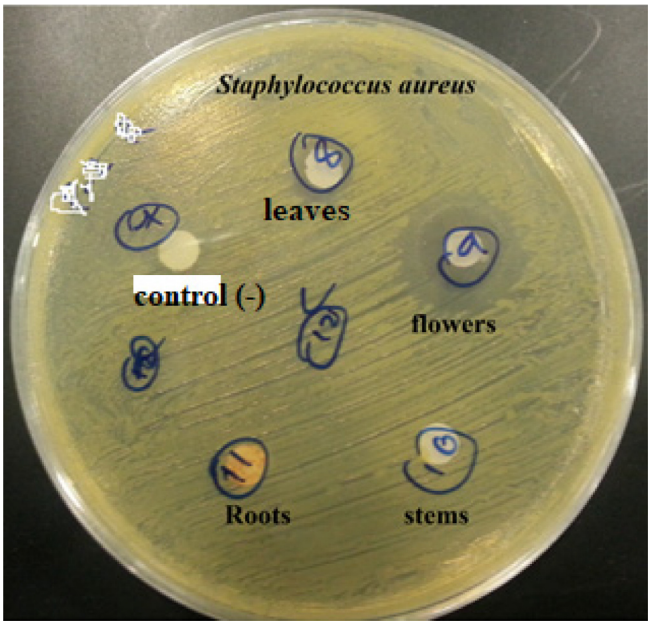

Fig. 2. Antibacterial activity of extracts of Nicotiana glauca againsts S. aureus.

Table 3
Minimum inhibitory concentration of Nicotiana glauca extracts (MIC) against Microorganism.

Microorganism	MIC (mg/ml)
S. aureus	2
E. coli	1.5

mm) and also inhibited the growth of S. aureus (11.0 ± 0.23 mm), while, the flower extracts also inhibited the growth of E.coli (6.7 ± 0.65 mm), and also inhibited the growth of S. aureus (15.8 ± 0. 52 mm). Also, the inhibition zone of the antibiotic gentamycin (reference control) were 32 mm in S. aureus and 38 mm in E.coli. Table 3 showed that the MIC of, ethyl acetate was ranged (1.5–2 mg/ml). The lower MIC is an indication of high effectiveness of extract.

4. Discussion

The results of the current study indicate that N. glauca is rich in its content of the secondary metabolites that have been reported previously to exhibit various biological activities such as; anti-inflammatory, antibacterial, antiviral, antiallergic, antiasthma, antimalarial, cytotoxic antitumor and treatment of neurodegenerative diseases (Williams, et al. 2004, Rijke, et al. 2006, Chebil, et al. 2006; Tsuchiya, 2010, Russo, et al. 2013, Kittakoop, et al. 2014, Cushnie, et al. 2014; Qiu, et al. 2014). In general, the study showed that most of the phytocompounds were present in flowers, leaves and stmes of N. glauca compared to its roots. Such distribution of concentrations of phytochemical constituents in plants could be attibuted due to diseases and environmental stresses such as; drought, heat/cold, mineral deficiencies (Dixon, 2001; Hassan, et al, 2014).

The antimicrobial outcomes in the current study coincide with previous results of some studies of the screening of N, glauca phytochemicals, where the highest amounts found in leaves and flowers extracts. For example, David, (2016) found that the most active consitituent against E. coli was total ethanolic extract of leaves (16.5 mm) and against P. aeruginosa was total ethanolic extract of flowers (14 mm).

Variation in the toxicity of different extracts could be attributed to the presence of the active compounds that have been extracted which may be influenced by several factors such as method of extraction (Rinez, et al 2012). Another consistent result with the current study suggested that N. glauca as antimicrobial agents and allopathic potential for biological control of weeds (Aldesuquy et al 2018). In another study, hexane and methanol extracts of fresh aerial parts of some wild plants including N. glauca were screened in vitro for antimicrobial activity against 15 Gram positive and negative pathogenic bacteria. Both methanol and hexane plant extracts showed strong antibacterial activity

against at least two pathogenic microorganisms tested. However, hexane extracts generally showed lower activity against microorganisms compared to methanol extracts (Rahman et al 2011). In addition, results of the previous study support the medical usage of the studied plants and suggest that some of these plants including N. glauca possess antimicrobial properties that can be used to cure infectious diseases.

5. Conclusion

The preliminary phytoconstituents screening concluded that Nicotiana glauca posses varying amounts of alkaloids, steroid, tannins, flavonoid, and saponins which were present more in the extracts of the leaves, stems, flowers than in roots. The study also concluded that leaves and flowers extracts could be used to treat bacterial infection. Therefore, such bioactive property should support the idea that removing N. glauca from the mountainous areas of Al-Baha, Saudi Arabia, is not the only solution, but the plant can be exploited as a beneficial source of medicnal and economic purposes such as; antibacterial, antifungal and insecticidal, however, further comprehensive exploration is recommended to confirm these properties.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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