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Solanum nigrum roots as an antibacterial agent

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Abstract : This study was carried out to evaluate the anti-bacterial activity of the ethanolic extract of *Solanum nigrum* roots tissues. The antibacterial activity was detected against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. The study revealed that the maximum inhibition activity was against the Gram positive bacteria *Staphylococcus aureus* and the minimum inhibition was against the Gram negative bacteria *Pseudomonas aeruginosa*. The results suggest the root tissues extract of *S. nigrum* as a potential antimicrobial agent.
Key Words : *Solanum nigrum*, antibacterial activity, roots tissues, *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*.

Introduction:

Antibiotics brought about a revolution to control pathogenic diseases and infections, however, these drugs are out of reach to millions of people. In addition, there is a problem represented by the increased microbial resistance to these natural or even the synthetic antibiotics. These problems evoked an increasing demand for the discovery and development of new antimicrobial agents. The use of plant extract as alternative form of medical treatment is gaining great popularity since the late 1990. Earlier in the 1990 approximately one third of people, surveyed in the United State, used at least one unconventional therapy during the previous year[1] .The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants [2]

People who live in remote places depend on traditional healers, which they know and trust[3]. About three quarters of world's population are estimated to be dependent mainly on plants and plant extracts for the care of their health[4]. Several medical plants have been used as dietary supplements and in the treatment of numerous diseases without proper knowledge of their mode of action. However, only a few herbs and bio active chemical substances have attracted the interest of scientists and have been put forward for investigations. Therefore, there is a need for more well documented clinical trials and more investigation to justify their pharmacological actions and toxicity for safe and effective treatment [5,6].

The aim of this study is to investigate the antibacterial activity if *S. nigrum* roots tissues has and establish a possibility for its use in pharmaceutical industry.

Phytochemical components in plants have been important agents in developing modern medicines and the bases for many drugs in today's world. Their effects on treating many diseases for many years have been well documented[7,8]. *Solanum nigrum* is commonly known as Black Nightshade, Makoy and Deadly Nightshade. The plant has been extensively used in traditional medicine in India and other parts of the world to cure liver disorders and chronic skin ailments (Psoriasis and Ringworm). *S. nigrum* belongs to the family Solanacea. This family consists of 90 genera and around 3000 species. In this family *S. nigrum* constitutes the largest and the

most complex genus consists of more than 1500 species[9] .Many analytical reports showed that *Solanum* plants are important source of large number of phytochemical compounds with substantial curative application against human pathogens [10].

Genus *Solanum* (Solanaceae) is rich in steroidal glycoalkloids, an important group of plant secondary metabolites. These compounds are used as starting material for the synthesis of steroidal drugs. More than 100 different types of glycoalkasloids have been isolated from more than 350 Solanum species [11] .Steroidal glycoalkaloids have antimicrobial, insecticidal and fungicidal properties which provide resistance against several insect pests and herbivores[12] .Tissue culture of *S.nigrum* has considerable attention for its important medicinal properties. A series of *in vitro* and *in vivo* plants were successfully produced and chemical analysis revealed contents of glycoalkaloids higher than those reported for intact field plants [13]

Experimental:

Sample collection:

The roots tissues of *S. nigrum* collected from the Tissue Culture Unit in Genetic Engineering and Molecular Biology Research Center, Assuit, Egypt.

Roots culture medium:

The plants of *S. nigrum* as source of explants tissue culture were collected from Alwasta, Assuit, Egypt. Three segments of leaves (explants) were cultured on Murashige - Skoog (MS) medium 4.4g/L, 30 g/L sucrose and 3 g/L gel right and supplemented with different concentrations of IAA & NAA (0, 0.5, 1, 1.5, 2, 2.5 and 3) mg/L alone. The pH of medium was adjusted to 5.7 using 1N HCl or 1N NaOH before adding 3g/L gel right. The medium was dispensed into the culture Petri dish and was subsequently autoclaved under 105 kPa at a temperature at 121°C for 15-20 min. Three segments of explants (leaves) were transferred into petri dishes of culture media. Three segments of explants? were transferred into the petri dishes containing the culture media. The cultures were incubated in the growth room for 16 hours under a fluorescent light (2000-3000 lux) for photo period followed by 8 hours in the dark. The temperature was maintained at 25 ± 2°C with 50 - 80% relative humidity.

Ethyl alcohol extract of roots tissues:

100 g of the roots tissues of *S. nigrum* were continuously extracted by soxhlet using 200ml of the polar solvent ethyl alcohol 95% for 24 hours at 40^{o}C. The extract dried by rotary evaporator at approximately 50^{o}C and kept at -20^{o}C until time of use.[18]

Antibacterial Activity Determination:

1. Test microorganisms:

Clinical isolates of *Staphylococcus aureus*, *Pseudomonas aerogenosa* and *Escherichia coli* used in this study were obtained from Central Laboratory in Al- Thora hospital in Taiz city-Yemen.

2. Identification of bacteria :

The clinical isolates of the test microorganisms were cultured in the enriched medium blood agar. After incubation at 37°C for 24 hr the samples were sub cultured in the selective media; Manitol Salt Agar to isolate *Staphylococcus aureus*, King A (*Pseudomonas* Agar P medium) to isolate *Pseudomonas aerogenosa* and Violet Red Bile Glucose Agar to isolate *Escherichia coli* [14,15]. The bacteria were identified by biochemical tests according to Bergey's Manual® of Systematic Bacteriology [16,17]. Stocks of the isolates were reserved in agar slant nutrient agar at 4°C[18].

3. Determination of antibacterial activity:

The microorganisms (bacteria) cultured on to Muller-Hinton broth (MH) (Oxoid, Hampshire, UK). The plates were then incubated overnight at 37°C for 24 hr to allow maximum growth of the microorganisms. The antibacterial activity was done by utilizing the hole -in- plate bio assay procedure[19] **.**The suspension was used to

streak of the surface of (MH) agar plates with sterile swab. Using a sterile cork-borer of 7mm diameter, five holes were made in to the set agar in Petri-dishes containing the bacterial culture. Concentrations (40, 80, 120, 160, 200) mg/ml of roots tissues extracts and control were poured in to the wells. The plates then incubated at 37c^{o} for 18-24h. Antibacterial activity of the test samples was determined by measuring the diameter of zone of inhibition expressed in millimeter. The assay was repeated twice and the mean of the two experiments was recorded.

Statistical Analysis:

The significance of the difference anti-bacterial activity of the extract concentrations were tested by one way analysis of variance (ANOVA) SPSS.22 program. A probability value of difference (p value ≤ 0.05) was considered to denote a statistically significant. All data were presented as mean values ± standard deviation (SD). Each experiment was performed with three replicates and repeated twice.

Results:

Roots tissues were initiated after four weeks of inoculation on MS Medium supplemented with IAA and NAA alone, in IAA from (1.5 - 3) mg/L but in NAA from (1- 3) mg/L. The best roots was observed and the highest rooting percentage was obtained in (2.5) mg/L NAA (Figure 1).

Figure 1: Roots tissues of *Solanum nigrum* on MS Medium supplemented with (2.5) mg/L NAA

a) Staphylococcus aureus *b) Escherichia coli* c) *Pseudomonas aeruginosa*

Figure 2: The bacteria used in the study and the areas of inhibition zones obtained using concentrations of the root extract.
1 = 200 mg/ml , 2 = 160 mg/ml , 3 = 120 mg/ml , 4 = 80 mg/ml , 5 = 40 mg/ml

Concentration (mg/ml)	Inhibition zone (mm) Mean ± SD		
	Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeruginosa*
Control	0 ± 0.00 e	0 ± 0.00 c	0 ± 0.00 d
40	10.5 ± 0.707 d	0 ± 0.00 c	0 ± 0.00 d
80	12.5 ± 0.707 c	11.5 ± 0.707 b	9 ± 0.00 c
120	14 ± 0.00 b	11.5 ± 0.707 b	10.5 ± 0.707 b
160	14.5 ± 0.707 b	11.5 ± 0.707 b	10.5 ± 0.707 b
200	16 ± 0.00 a	13 ± 0.00 a	12 ± 0.00 a

Table 1: The effect of *Solanum nigrum* roots tissues extract on bacteria.
Mean values followed by the same letter under different treatment within a column are not significantly different from each other at $p < 0.05$ (Duncan's multiple range test).

The concentrations 40, 80, 120, 160 and 200mg/ml of extract showed varying degrees of inhibitory effect on the bacterial species under investigation (Figure 2, a, b and c).

Our results showed that the zones of inhibition are directly proportional to the concentration of root extract used in all the three bacterial strains (Figure 2 and table 1). The maximum activity of the extract was noticed against the gram positive bacteria *Staphylococcus aureus* (16mm) in 200mg/ml. This concentration showed an effect on the gram negative bacteria but to lower extents (13mm and 12 mm) on *Escherichia coli* and *pseudomonas aeruginosa*, respectively. This trend of effect was maintained in all the concentrations except that the lower concentration (40 mg/ml) showed no effect on the gram negative bacteria *Escherichia coli* and *pseudomonas aeruginosa* (table 1). Interestingly, the effect of the extract did not change on *Escherichia coli* over a wide range (from 80 to 160 mg/ml).

Discussion:

This study revealed that the Gram positive bacteria *S. aureus* were more susceptible to the extracts than the gram negative bacteria *E.coli and P. Aeruginosa*. Plants are important sources of potentially useful compoundes for the development of new chemotherapeutic agents. Many reports are available regarding anti-viral, anti-bacterial, anti-fungal, anti-helminthic and anti-inflammatory properties of plants.[20] Plants contain alkaloids, glycosides, lignins, tannins, and terpenoid compounds like monoterpenes, sesquiterpenes, diterpenes or triterpenes. These compounds probably get through the bacterial cell wall/membrane and suppress their growth or if these compounds deeply penetrated, might kill them completely.

Our results are similar to the previous findings by other workers, who explored the antibacterial potential of natural products, against wide ranges of microorganisms, particularly from various members of family Solanaceae [21,22] . Further investigation will be interesting for determining the actual agent in *S. nigrum* root extract responsible for the differential effect on the bacteria species under investigation and its way of action.

Conclusion:

This investigation has shed light on the possibility of the use the roots tissues of *S. nigrum* in producing effective antibiotics against some human pathogens especially the gram positive bacteria *Staphylococcus aureus*. However, the effectiveness of the plant extract for treating the bacterial infections in human need more extensive investigation to address the from, dosage and probable toxicity of the drug for the human body. The effectiveness of the root extract against other bacterial groups and species to find a probable mode of action and understand the mechanism of action will be very interesting.

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