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ANTIMICROBIAL ACTIVITY OF *NICOTIANA TABACUM* USING DIFFERENT SOLVENTS EXTRACTS

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Abstract

The present study investigates antibacterial activity of tobacco extracts from *Nicotiana tabacum* at different concentrations in different polar solvents. Six different extracts were prepared, using 5 different polar solvents viz., Ethanol, Ethyl acetate, n-Hexane, Acetone, Butanol and water. Four different concentrations (6, 12, 18 and 24 mg of sample disc^{-1}) of each extract were subjected for preliminary antibacterial screening against seven pathogenic bacteria by Kirby-Bauer Disk Diffusion method. The result of *in vitro* antibacterial screening showed that 6 extracts from *Nicotiana tabacum* revealed different ranges of antibacterial activities. Ethyl acetate extracted samples were more effective to control *Bacillus cereus* and *Erwinia carotovora* followed by butanol extracted samples against *Staphlococcus aureus* and *Agrobacterium tumefaciens*, while no significant inhibitory effects were observed in ethanol and hexane extracts. When tobacco extracts were studied for their antibacterial potential against gram-positive and gram-negative bacteria, the ethyl acetate extracts showed a good range of inhibitory effects against *Bacillus cereus* and *Erwinia carotovora* at highest concentration (24 mg sample disc^{-1}). Hexane, ethanol and aqueous extracts did not show a significant range of inhibitory effect but acetone extracts indicated non significant inhibitory effects against *S typhae, Staphlococcus aureus* and *Erwinia carotovora*.

Introduction

Plants for years have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies. The traditional medicine is used in all parts of the world and has rapidly growing economic importance (Ates & Erdogrul, 2003; Rehman *et al.,* 2004; Dash *et al.,* 2005; Agra *et al.,* 2007; Ushimaru *et al.,* 2007). Globally researchers are using extracts of plants for their antibacterial, antifungal and antiviral activities (Bakht *et al.,* 2011 a, b, c and d). It is reported that more than 400, 000 species of tropical flowering plants have medicinal properties (Yildirim *et al.,* 2000; Kumar *et al.,* 2005; Odugbemi, 2006). The characteristics of the plants that inhibit the growth of micoorganisms have been investigated in different laboratores around the world since 1926 (Erdogrul *et al.,* 2001; Ates & Erdogrul, 2003). Development of microbial resistance to available antibiotic and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants (Dash *et al.,* 2005; Naz *et al.,* 2010).

Tobacco (*Nicotiana tabacum*) belongs to the family Solanaceae which also includes some other important crop species such as tomatoes, potatoes peppers etc. Tobacco nicotine inhibits the growth of pathogens which is dose dependent (Maria *et al.,* 2007; Wang *et al.,* 2008; Suresh *et al.,* 2008). It is equally affective against gram- positive and gram-negative bacteria, along with the acid-fast *Mycobacterium phlei* and the opportunist fungi *Candida albicans* and *Cryptococcus neoformans*. Levels of inhibition ≥ 50% occurred when most of the affected organisms were cultured with nicotine at 100-250 µg/ml. The above mentioned concentrations of nicotine can be found *In vivo* (Russel *et al.,* 1981), especially in the oral cavity of smokeless tobacco users, making these findings physiologically relevant. Yildirim *et al.,* (2001) reported that the ether extracts of both the leaves and seeds and ethanol extract of leaves had shown antimicrobial activities on *Staphylococcus.* Wang *et al.,* (2008) reported inhibition of the activities of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* by Crude polyphenols extracted from tobacco leaf by 80% ethanol solution. Strong antimicrobial activities against *Klebsiella pneumoniae*, *Escherichia coli*, *Streptococcus faecalis*, *Mycobacterium phlei*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and the human pathogenic yeast, *Candida albicans* were detected in methanolic extracts of 24 plants used medicinally in the treatment of skin infections in four different regions of Colombia. Twenty-two extracts displayed activity against Gram-positive bacteria whereas none was active against the Gram-negative species (Lopez *et al.,* 2001). The present study investigates the antimicrobial activities (gram +ive and –ve) of different solvent extracts from *Nicotiana tabacum*.

Materials and Methods

Plant material: Plants of tobacco specie *Nicotiana tabacum* used in the present research work was collected from the farmer's tobacco fields at Jamal Garhi Mardan, Khyber Pukhtun Khwa Province. Plants were then washed with distilled water to remove dirt and soil particles. The plants were cut into small pieces and then dried in shaded area at room temperature for a period of one week. The dried plants leaves were grinded with ordinary grinder and then sieved through sever.

Procedure for plant extracts: Six hundred grams of dried powdered plant material of both tobacco species were taken into separate round bottom flasks and filled with 95% ethanol until dipped and then fixed with the condenser. This assembly was adjusted in heating mental and connected to the tape water supply. The material was boiled at 50ºC for 24 hrs and then filtered with the help of vacuum pump using Buchner funnel. Ethanol was isolated from the mixture of the extract through rotary evaporator at 60ºC under reduce pressure. Ethanol extract was then collected from the flask and dried through water bath at

60ºC. After drying, the extract was weighed and stored into a vile. Extract from the same plant material was collected exhaustively and this procedure was repeated thrice for the same plant material.

Part of the crude extract was used for further fractionation. The extract for fractionation was suspended into 100 ml distilled water having methanol (water: methanol at the ratio of 8:2) and then made the total volume up to 200 ml and poured it into a separating funnel, defatted it with 200 ml n-Hexane. The compounds soluble in n-Hexane separated in the upper phase were collected and the lower aqueous phase was extracted thrice with n-Hexane for maximum recovery. Extract was then dried through water bath and weighed and stored into a vile. The same procedure was adopted for Ethyl acetate, acetone and butanol.

Antibacterial activity bioassay: Antibacterial activities of the different extracts against various microorganisms (Table 1) were determined by Kirby Baur Disc Diffusion method. For gram +ve organisms**,** Azithromycin (30 µg disc^{-1}) was used as positive control while solvent media as negative control. In case of gram –ive organisms, Ciprofloxacin (30 µg disc^{-1}) was used as positive and solvent media as negative controls.

Table 1. Different microorganisms used in the present study.

Species	Number
Escherichia coli	ACCT#25922
Pseudomonas aeroginosa	ACCT#9721
Staphylococcus aureus	ACCT#6538
Salmonella typhi	Clinical Isolate obtained from microbiology laboratory Quaid-E-Azma University Islamabad
Agrobacterium tumefaciens	Recombinant DNA Technology V of IBGE, Agric. Univ. Peshawar KPK
Bacillus cereus	Clinical Isolate obtained from microbiology laboratory Quaid-E-Azma University Islamabad
Erwinia carotovora	Plant pathology Department, Agric. Univ. Peshawar KPK

Results and Discussion

The data revealed that ethanol extracted samples did not inhibit the activity of *Bacillus cereus* (0% ZI) at any concentration when compared with their respective controls. In case of *Staphlococcus aureus*, ethanol extracted sample inhibited the growth by 30.85% at highest concentration (i.e. 24 mg of sample disc^{-1}) when compared with other concentrations of the same solvent (Table 2). These results agree with those reported by Yildirim *et al.,* (2001), Wang *et al.,* (2007) and Wang *et al.,* (2008). It is clear from the results that ethyl acetate extracted samples were more effective to control *B cereus* (79% ZI) than *S. aureus* (56.77% ZI) at highest concentrations (Table 2). Acetone extracted samples had no inhibiting effect on *B cereus* and recorded zero percent inhibition. In case of *S. aureus*, acetone extracted samples were effective, maximum inhibition (45.66% ZI) being achieved at highest concentration when compared with other concentrations of the same extract (Table 2). Butanol extracted samples significantly inhibited the growth of *S. aureus* more (74.07% ZI) than *B cereus* (56.77% ZI) at maximum concentration of 24 mg of sample disc^{-1} when compared with other treatments of the same extract (Table 2). Water extracted samples did not inhibit the growth of both *S. aureus* and *B cereus* and recorded zero percent ZI (Table 2).

Table 2. Antibacterial activity of ethanol, ethyl acetate, acetone, butanol and water extracted sample from *Nicotiana tabacum* (NT) against *B. cereus* and *S. aureus* (gram +ive).

Plant extract	Conc. mg disc^{-1}	Zone of inhibition (mm)	Zone of inhibition (%)	*B cereus* Positive control 30 µg disc^{-1}	Negative control 6 µl disc^{-1}	Zone of inhibition (mm)	Zone of inhibition (%)	*S aureus* Positive control 30 µg disc^{-1}	Negative control 6 µl disc^{-1}
Ethanol									
	6	0	0	27	-	0	0	27	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	0	0			8.33	30.85		
Ethyl acetate									
	6	15.33	56.77	27	-	0	0	27	-
	12	17.66	65.40			7.66	28.37		
	18	20.00	74.07			12.66	46.88		
	24	22.33	79.00			15.33	56.77		
Acetone								27	-
	6	0	0	27	-	0	0		
	12	0	0			7.33	27.14		
	18	0	0			9.00	33.33		
	24	0	0			12.33	45.66		
Butanol									
	6	8.33	30.85	27	-	7.33	27.14	27	-
	12	9.66	35.77			15.33	56.77		
	18	12.66	46.88			16.66	61.70		
	24	15.33	56.77			20.00	74.07		
Water									
	6	0	0	27	-	0	0	27	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	0	0			0	0		

Our results also showed that ethanol extracted samples were effective to control *E. carotovora* (27.14% ZI) at highest concentration (24 mg of sample disc^{-1}). Ethanol and n-hexane extracted samples were ineffective to control *E. coli* at any concentration and showed zero percent ZI (Table 3). Similar results were also reported by Zaidi and Gul (2005) and Suresh *et al.,* (2008). The data further suggested that ethyl acetate extracted samples were more effective against *E carotovora* (82.70% ZI) than *E. coli* (77% ZI) at highest concentration of 24 mg of sample disc^{-1} when compared with other concentrations of the same treatment (Table 3). Acetone extracted samples recorded zero percent inhibition against *E. coli* at any concentration when compared with their positive control. Analysis of the data also indicated that acetone extracted inhibited the growth of *E. carotovora* by 35.77% at highest concentration of 24 mg of sample disc^{-1} when compared with other concentrations of the same solvent (Table 3). Butanol extracted samples were equally effective to control *E. carotovora* and *E. coli recording* 57.57% inhibition in their growth (Table 3). Our data further revealed that water extracted samples were effective against *E. coli* showing 30.45% inhibition in their growth at 24 mg sample/disc concentration when compared with other treatments (Table 3).

Table 3. Antibacterial activity of ethanol, n-hexane, ethyle acetate, acetone, butanol and water extracted sample from *Nicotiana tobaccum* (NT) against *E. carotovora* and *E. coli* (gram -ive).

Plant extract	Conc. mg disc^{-1}	Zone of inhibition (mm)	Zone of inhibition (%)	*E carotovora* Positive control 30 µg disc^{-1}	Negative control 6 µl disc^{-1}	Zone of inhibition (mm)	Zone of inhibition (%)	*E. coli* Positive control 30 µg disc^{-1}	Negative control 6 µl disc^{-1}
Ethanol									
	6	0	0	27	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	7.33	27.14			0	0		
n-Hexane									
	6	0	0	27	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	0	0			0	0		
Ethyl acetate									
	6	12.66	46.88	27	-	14.00	40.00	35	-
	12	15.33	56.77			20.33	58.05		
	18	19.66	72.81			23.66	67.60		
	24	22.33	82.70			26.66	76.17		
Acetone									
	6	0	0	27	-	0	0	35	-
	12	5.33	19.74			0	0		
	18	8.00	29.62			0	0		
	24	9.66	35.77			0	0		
Butanol									
	6	9.66	35.77	27	-	7.33	20.94	35	-
	12	11.33	41.96			10.33	29.51		
	18	13.33	49.37			15.33	43.80		
	24	15.66	58.00			20.00	57.14		
Water									
	6	0	0	27	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			7.33	20.94		
	24	0	0			10.66	30.45		

Table 4 presents data regarding ethanol extracted samples against *Agrobacterium tumefacien* and *Pseudomonas aeruginosa*. Our results revealed that ethanol extracted samples were ineffective to control *Agrobacterium tumefacien* at any concentrations while it inhibited the growth of *Pseudomonas aeruginosa* by 29.51% (ZI) at 24 mg sample disc^{-1} concentration. Similar results were also reported by David & Abuotor (2000). It is also clear from the results that n-hexane extracted samples did not inhibit the growth of *Agrobacterium tumefacien* and *Pseudomonas aeruginosa* at any concentration showing zero percent ZI (Table 4). Our results also suggested that ethyl acetate extracted samples were more effective to control *Pseudomonas aeruginosa* (70.45% ZI) than *Agrobacterium tumefacien* (56.66% ZI) at highest concentrations (24 mg sample disc^{-1}) when compared with their respective control (Table 4). It can be seen from the data that acetone extracted samples against *Agrobacterium tumefacien* were ineffective showing zero percent inhibition. In case of *Pseudomonas aeruginosa*, acetone extracted samples controlled the growth of *Pseudomonas aeruginosa* by 29.51% at highest concentration (Table 4). Butanol extracted samples were equally effective to control the growth of *Agrobacterium tumefacien* (52.20% ZI) than *Pseudomonas aeruginosa* (52.37% ZI) at highest concentration when compared with other treatments of the same solvent (Table 4). Water extracted samples were more effective to control *Pseudomonas aeruginosa* (35.22% ZI) at highest concentration (24 mg of sample disc^{-1}) when compared with other concentrations of the same extract (Table 4). While the same extract (water) did not inhibit the growth of *Agrobacterium tumefacien* (Zero percent inhibition) at any concentration.

Our data also suggested that n-hexane and water extracted samples did not control the growth of *S. typhae* (0% inhibition) at any concentration. The data further indicated that ethyl acetate extracted sample were more effective to control the growth of *S. typhae* (45.83%) at highest concentrations when compared with other solvents extracted samples (i.e. butanol (41.66%); acetone (38.33%) and ethanol (35.83%) (Table 5). These results agree with those reported by Yildirim *et al.,* (2001), Zaidi *et al.,* (2005) and Wang *et al.,* (2007).

Table 4. Antibacterial activity of ethanol, n-hexane, ethyle acetate, acetone, butanol and water extracted sample from *Nicotiana tobacco* (NT) against *A. tumefaciens* and *P. aeruginosa* (gram -ive).

Plant extract	Conc. mg disc^{-1}	Zone of Inhibition (mm)	Zone of Inhibition (%)	*A tumefacien* Positive control 30 µg disc^{-1}	Negative control 6 µl disc^{-1}	Zone of Inhibition (mm)	Zone of Inhibition (%)	*P aeruginosa* Positive control 30 µg disc^{-1}	Negative control 6 µl disc^{-1}
Ethanol									
	6	0	0	30	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	0	0			10.33	29.51		
n-Hexane									
	6	0	0	30	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	0	0			0	0		
Ethyl acetate									
	6	12.66	42.20	30	-	15.00	42.85	35	-
	12	14.00	46.66			20.33	58.08		
	18	15.66	52.20			23.33	66.65		
	24	17.00	56.66			24.66	70.45		
Acetone									
	6	0	0	30	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			0	0		
	24	0	0			10.33	29.51		
Butanol									
	6	5.33	17.76	30	-	10.00	28.57	35	-
	12	10.00	33.33			13.33	38.08		
	18	13.33	44.43			15.33	43.80		
	24	15.66	52.20			18.33	52.37		
Water									
	6	0	0	30	-	0	0	35	-
	12	0	0			0	0		
	18	0	0			10.33	29.51		
	24	0	0			12.33	35.22		

Table 5. Antibacterial activity of ethanol, ethyl acetate, acetone, butanol, n-hexane and water extracted sample from *Nicotiana tobaccum* (NT) against *S. typhae*.

Plant extract	Conc. mg disc^{-1}	Zone of Inhibition (mm)	*S typhae* Zone of Inhibition (%)	Positive control 30 µg disc^{-1}	Negative control 30 µg disc^{-1}
Ethanol					
	6	7.33	18.33	40	-
	12	10.33	25.83		
	18	12.33	30.83		
	24	14.33	35.83		
Ethyl acetate					
	6	7.33	18.33	40	-
	12	12.66	31.66		
	18	15.33	38.33		
	24	18.33	45.83		
Acetone					
	6	8.33	20.83	40	-
	12	10.33	25.83		
	18	12.33	30.83		
	24	15.33	38.33		
Butanol					
	6	8.33	20.83	40	-
	12	10.00	25.00		
	18	13.33	33.33		
	24	16.66	41.66		
n-Hexane					
	6	0	0	40	-
	12	0	0		
	18	0	0		
	24	0	0		
Water					
	6	0	0	40	-
	12	0	0		
	18	0	0		
	24	0	0		

References

Agra, M.F., P.F. Freitas and J.M. Barbosa-Filh. 2007. Synopsis of the plants known as medicinal and poisonous in Northwest of Brazil. *Rev. Bras. Farmacogn*., 17: 114-140.

Ates, D.A. and O.T. Erdogrul. 2003. Antimicrobial activities of various medicinal and commercial plant extracts. *Turk. J. Biol.,* 27: 157-162.

Bakht, J., M. Tayyab, H. Ali, A. Islam and M. Shafi 2011a. Effect of different solvent extracted samples of *Allium sativum* on bacteria and fungi. *Afri. J. Biotechnol.,* 10: 5910-5915.

Bakht, J., A. Islam, M. Tayyub, H. Ali and M. Shafi 2011b. Antimicrobial potentials of *Eclipta alba* by disc diffusion method. *Afri. J. Biotechnol.,* 10: 7668-7674.

Bakht, J., H. Ali, M.A. Khan, A. Khan, M. Saeed, M. Shafi, A. Islam and M. Tayyab. 2011 c. Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion. *Afri. J. Biotechnol.,* 10: 19825-19835.

Bakht, J., A. Islam and M. Shafi. 2011d. Antimicrobial potential of *Eclipta alba* by well diffusion method. *Pak. J. Bot.,* 43 (SI): 161-166.

Dash, S., L.K. Nath, S. Bhise and N. Bhuyan. 2005. Antioxidant and antimicrobial activities of *Heracleum nepalense* D Don root. *Trop. J. Pharmace. Res.,* 4: 341-347.

David, A.A. and E.M. Abuotor. 2000. Antibacterial activity of N*icotaina tabacum* leaves. *Fitoter.,* 71: 199-200.

Erdogrul, O.T., E. Cakiroglu and S. Karaman. 2001. Antibacterial activities of *Helichrysum plicatum subsp. Plicatum* extracts. *The Sci.,* 13: 176-178.

Kumar, K.S., T. Sivakumar, R.S. Sunderam, M. Gupta, U.K. Mazumdar, P. Gomathi, Y. Rajeshwar, S. Saravanan, M.S. Kumar, K. Murugesh and K.A. Kumar. 2005. Antioxidant and antimicrobial activities of *Bauhinia racemosa* L. stem bark. Braz. *J. Med. Biol. Res*., 38: 1015-1024.

Lopez, A., J.B. Hudson and G.H.N. Towers. 2001. Antiviral and antimicrobial activities of Colombian medicinal plants. *J. Ethnopharmacol*., 77: 189-196.

Maria, C.S., M. Souza, A. Pinheiro, M. Ferreira, R. Goncalves, T. Cristin and M. Peralta. 2007. Evaluation of anti-tubercular activity of nicotinic and isoniazid analogues. *Arkivoc,* 14: 181-191.

Naz, S., S. Jabeen, S. Ilyas, F. Manzoor, F. Aslam and Aamir Ali. 2010. Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. *Pak. J. Bot.,* 42: 455-462.

Odugbemi, T. 2006. Medicnal plants as antimicrobials In: *Outlines and pictures of Medicinal plants from Nigeria*. University of Logos press, 53-64.

Rehman, M.A. J.S. Mossa, M.S. Al-Said and M.A. Al-Yahya. 2004. Medicinal plant diversity in the flora of Saudi Arabia 1: a report on seven plant families. *Fitoterpia,* 75: 149-161.

Russel, M., M. Jarvis, G. Davitt and C. Feyerbend. 1981. Nicotine intake by snuff users. *British Med. J.,* 283: 814-817.

Suresh, K., S. Saravana Babu and R. Harisaranraj. 2008. Studies on *In Vitro* antimicrobial activity of ethanol extract of *Rauvolfia tetraphylla*. *Ethnobot. Leaflets*, 12: 586-90.

Ushimaru, P.I., T.N. Mariama, C. Luiz, B. Di Luciano and F.J. Ary. 2007. Antibacterial activity of medicinal plant extract. *Braz. J. Microbial.,* 38: 717-719.

Wang, H., M. Zhao, B. Yang, Y. Jiang and G. Rao. 2008. Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. *Food Chemist.,* 107: 1399-1406.

Yildirim, A., A. Mavi, M. Oktay, A.A. Kara, O.F. Algur and V. Bilaloglu. 2000. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea desf ex dc*), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. J. Agric and Food Chemist., 48: 5030-5034.

Yildirim, A., A. Mavi and A.A. Kara. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extract. *J. Agric. and Food Chemist.,* 49: 4083–4089.

Zaidi, M.I. and A. Gul. 2005. Antibacterial activity of nicotine and its cobalt complex. *Sarhad J. Agric.,* 21: 287-291.

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