

ANTIBACTERIAL AND ANTIOXIDANT EFFECTS OF CHEWABLE TOBACCO LEAF PRODUCT (PATTI) AND BETEL NUT ON ENVIRONMENTAL PATHOGENS

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ABSTRACT

This paper discusses antibacterial and antioxidant effect of tobacco (*Nicotiana tabacum*) and betel nut on different strains of bacteria which include (*Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Shigell dysenteriae* and *Bacillus subtilis*). The antibacterial activity of different tobacco leaf extracts (patti) namely as Saadapatti, Najma 800, Zafar Zafrani, Zafar Raja Rani and betel nut show high antimicrobial activity against *Bacillus subtilis*. The results of scavenging DPPH radical activity show that only betel nut has significant activity.

KEYWORDS: Antioxidant, Betel nuts, Disc diffusion, Disease preceding tobacco products

INTRODUCTION

The characteristics of the plants that restrain the growth of microorganisms have been explored in many laboratories around the world since 1926 (Erdogru *et al.*, 2001; Ates and Erdogru, 2003). Tobacco (*Nicotiana tabacum*) is a vital economic crop which belongs to the family of Solanaceae. In Commercial level of production tobacco can also attain from its other sister species such as *Nicotiana rustica*, which is smaller in height with fewer leaves than *Nicotiana tabacum*. On the dependence of dose the nicotine in tobacco restrains the pathogen (Bakht *et al.*, 2012, 2013, Maria *et al.*, 2007; Wang *et al.*, 2008; Suresh *et al.*, 2008). Tobacco is consumed orally in a variety of forms such as smoking (Beedi, Cigarette, Cigar, Cheroots, Chuttas, Pipe, Chillum, Dhumti, Hookah, Hookli) and Smokeless tobacco are used orally or nasally (Paan, Khaini, Mainpuri tobacco, Mawa, Mishri, Snuff, Zarda, Zafar raja rani, Zafar Zafrani, Najma 800 and saadapatti) or nasally in nasal use, a small quantity of very fine tobacco powder mixed with aromatic substances called dry snuff is inhaled (Naswar, Nas). In Asia, especially in Pakistan commercially chewing tobacco products are available with scents and flavoring agents. Smokeless tobacco is synthesized in industries at larger scale as well as at home in small scale.

Tobacco leaf has many polyphenols, phenolic, and other bioactive substances. Polyphenols alter the color and quality of tobacco leaf (Ruiz *et al.*, 1998; Bazinet *et al.*, 2005). In cigarette smoking polyphenols considered as precursor of bioactive phenolic compound (Bazinet *et al.*, 2005). Extensive series of pharmaceutical characteristics are found in Polyphenols such as, anti-carcinogenic, anti-oxidative and anti-arteriosclerotic (Wang *et al.*, 2001, Atoui *et al.*, 2005, Duangsri *et al.*, 2012). The influence of tobacco on a person's health depends on different aspects such as their age, weight and immune status. Most important is that the person is an enthusiast of the tobacco product. The consequence of tobacco as with any drug also relies on the dose taken (Gambia *et al.*, 2013). Betel quid chewing is frequently referred to as betel nut chewing. The tobacco has psychotropic and anthelmintic action because it contains Areca alkaloids, arecoline and lauric acid in huge quantity. These alkaloids have dominant parasympathetic activity, create euphoria, and counteract fatigue (Saini *et al.*, 2009, Shah, 2007).

From the literature investigation, no scientific assessment of antimicrobial activities of the chewing, loss tobacco on environmental pathogen has yet not been done so that's why the object of this paper is to determine the antibacterial and antioxidant potential of the different tobacco leaf product. Plants which have antioxidant action are capable to stabilize the free radicals in the body. Polyphenols and flavonoids are the compound of antioxidant phytochemicals which have been reported to reduce the circulation of free radical reactions, to protect the human body from disease (Kinsella *et al.*, 1993; Terao & Piskula, 1997).

MATERIALS AND METHODS

Collection of materials: The study was done at the Institute of Environmental studies, University of Karachi, Sindh Pakistan. Different tobacco plant products (Patti) namely (Zafar raja rani, Zafar Zafrani, Najma 800 and saadapatti) and betel nuts were collected from the local market of Karachi.

Preparation of crude aqueous extracts: All the materials used were taken from the new sealed container, then 1gm of each ground sample was soaked in 10 ml sterile distilled water and left undistributed for 24 hours. After 24 hour period mixture of different products were boiled for 30 minutes. The extracts were left to cool down and preserved at room temperature ($25\pm 2^{\circ}\text{C}$) till the use.

Antibacterial assay: Antibacterial activity of the aqueous extract of tobacco was tested by the disc diffusion method. The microorganism strains used in this study were collected from Institute of environmental studies and Department of Microbiology, University of Karachi. *Staphylococcus aureus*, *Escherichai coli*, *Salmonella typhi*, *Shigell dysenteriae* and *Bacillus subtilis* were used for antibacterial susceptibility testing. The strains of bacteria were cultured on nutrient agar and incubated at 37°C overnight for 24 hours. A zone of inhibition in mm of each bacterial strain was measured for interpretation of results.

Antioxidant activity: Antioxidant action of the tobacco extract sample was carried out by using the method described by (Lee *et al.*, 1998).

Preparation of sample: The ethanolic (300uM) of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was prepared. 10 µL of test sample and 90 µL solutions of stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was put in 96 well micro titer plates and incubated at 37°C for 30 minutes. Absorbance was evaluated at 515 nm by using a spectrophotometer. Percent inhibition of radicals by treatment of test sample was determined by comparison with DMSO (Dimethyl sulfoxide).

$$\text{Percent inhibition} = \frac{(\text{absorbance of the control} - \text{absorbance of the test sample})}{\text{absorbance of the control}} \times 100$$

For the standard control Ascorbic acid was used. The EC 50 values denotes the concentration (in ug/ml) of sample required to scavenge 50% of DPPH.

RESULTS AND DISCUSSION

Antibacterial activity: The aqueous extracts of above tobacco ingredients individually were prepared and tested for its antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigell dysenteriae* and *Bacillus subtilis* by disc diffusion method. The results which were obtained from Disc method are shown in Fig. 1. It is observed that *Bacillus subtilis* shows high inhibition activity among the entire tested organisms from all the products Zafar raja rani, Zafar Zafrani, Najma 800, Betel nut, except Saada Patti which has no effect on *Bacillus subtilis*. *S. type* has no effect on Najma 800 and betel nut. However, *E. coli*, *Shigell dysenteriae* and *S. aureus* show fair inhibition against all tobacco products.

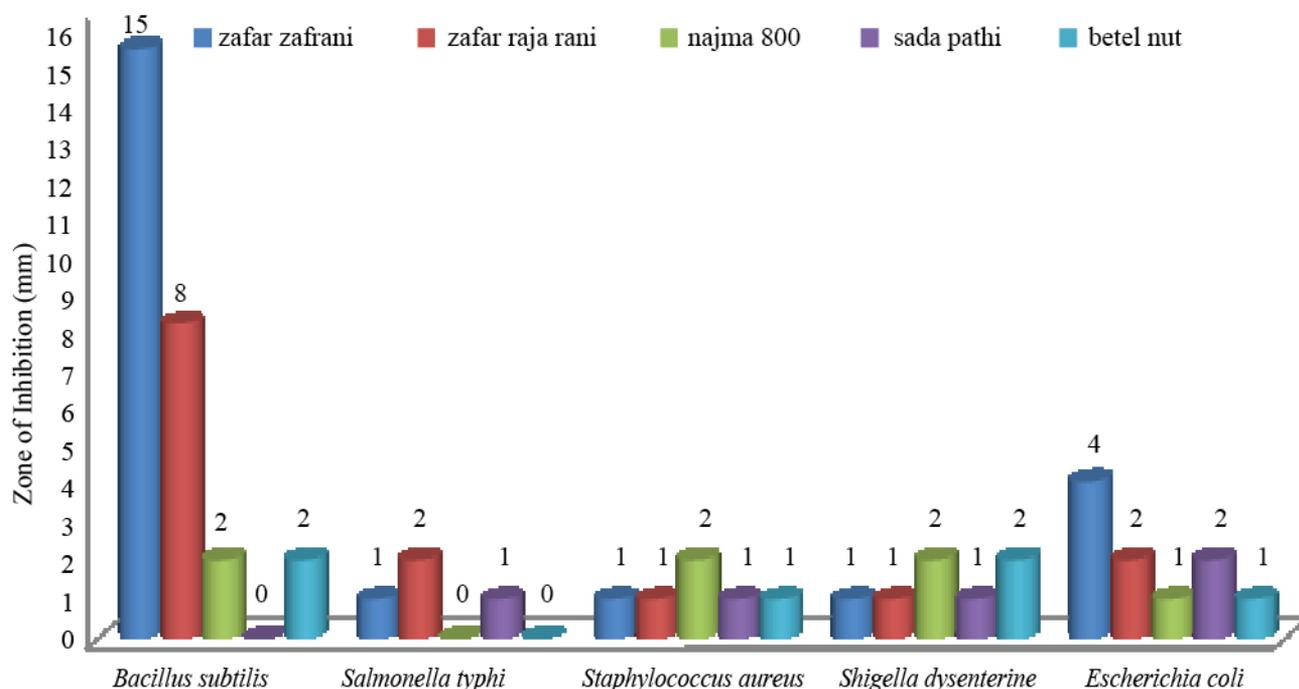


Fig. 1. Effect of different tobacco aqueous extracts on bacterial strain.

Antioxidant activity: The tobacco leaf extracts with higher antioxidant activity also had a high polyphenol compound (Duangsri *et al.*, 2012). The different product of commercially available tobacco (Zafar raja rani, Zafar Zafrani, Najma 800 and Betel nut) was tested for antioxidant potential. The Betel nuts show significant antioxidant activity (Table 1) but it is very low as compared to the ascorbic acid which is 2.27ug/ml.

Table 1. Results of antioxidant activity.

S. No.	Samples	Percentage inhibition \pm SE	EC50 ug / ml
01	Zafar Raja Rani	39 \pm 0.02	--
02	Zafar Zafrani	17 \pm 0.01	--
03	Najma 800	46 \pm 0.01	--
04	Betel Nut	70 \pm 0.01	937.5

SE = Standard error

Tobacco is the greatest disease-producing product. The use of smoking and chewing forms of tobacco negatively effect on oral health (Mubeen *et al.*, 2013). This paper, however, confirms that beside all of these health concerns the products of tobacco tested show antibacterial and antioxidant activities also.

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