THE TABACCO LEAF EXTRACT AND ANTIBACTERIAL ACTIVITY IN TEXTILE

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Abstract: Effect of the use of water and organic solvents such as acetone, N,N-dimethylformamide (DMF) and ethanol at various concentrations on the total polyphenol content and antioxidant activity was studied for the tobacco leaf. The fifty percent DMF extract showed the highest polyphenol content of 167.4 mg GAE/g and provided the greatest antioxidant activity. The results showed that solvent with different polarity had significant effect on polyphenol content and antioxidant activity. The inhibition activities on *Escherichia coli* and *Staphylococcus aureus* were also measured for evaluating the antimicrobial activity of cotton fabric treated with the tobacco leaf extract. The diameters of inhibition zones were 21.33 ± 0.86 and 18.50 ± 0.77 mm, respectively. The results showed that the polyphenol extracted from tobacco leaf had great potential as antioxidant and antimicrobial agent for textile application.
Keywords: Tobacco leaf, polyphenol, antioxidant, antibacterial activity

1. Introduction

Natural bioactive compounds with antimicrobial properties are gaining considerable attention as attractive eco-friendly alternative to synthetic antimicrobial agents for textile applications, especially in medical and health care textiles, as they are safe, non-toxic and skin-friendly [1]. These naturally active compounds such as, for example, the until now less employed polysaccharides and their derivatives, natural dyes, as well as some other extracts from roots, stem, leaves, flowers fruits and seed of diverse species of plants exhibiting antibacterial properties, have already been explored as textile finishing agents in their crude form or as microcapsules [2-4].

Tobacco (*Nicotiana tabacum L.*) belongs to the family Solanaceae and is a very important economic crop. However, over 20% of tobacco resources are discarded as processing waste, which pollute the environment and cause a big waste. In fact, the discarded tobacco leaves are economically valuable because of abundant bioactive compounds in them. Therefore, it is important to investigate and utilize the resource of tobacco leaf. Tobacco leaf is a good bioresource due to abundant phenolics and other bioactive substances. Tobacco leaf is rich in polyphenols which possess various bioactivities and affects the color and quality of tobacco leaf [5, 6]. Polyphenols have a wide range of pharmaceutical properties including antioxidative, anticarcinogenic and antiarteriosclerotic [7-10]. However, from the literature available, no scientific evaluation of the antimicrobial activities of the polyphenol from tobacco leaf in textile has yet been done. Thus, the objectives of the present study were focused on developing an eco-friendly natural antimicrobial finish from the polyphenol extracted from tobacco leaf for textile application. The effect of different extracting solvents on total polyphenol and antioxidant activity of tobacco leaf was also investigated. The tobacco leaf extracts applied to cotton fabrics were determined for their antimicrobial effectiveness by employing standard test methods and the findings are discussed in this paper.

2. Materials and Methods

2.1 Materials

The tobacco leaves (cultivar Burley 21) were donated from the local company in province of Sukhothai and ground into powder.

2.2 Microorganism strains

The microorganism stains used in this study were all purchased from Thailand Institute of Scientific Technology (TIST), Bangkok. *Escherichia coli* ATCC 25922as a representative of Gram-negative bacteria and *Staphylococcus aureus* ATCC 25923as a representative of Gram-positive bacteria were used for this study as both are reference strains used for antimicrobial susceptibility testing. The strains were cultured on nutrient agar and incubated aerobically at 37 $^{\circ}$C overnight.

2.3 Culture media

The nutrient agar was prepared by dissolving 5 g peptone, 1.5 g beef extract, 1.5 g yeast extract, 5 g NaCl and 20 g agar in 1000 ml distilled water, boiling the mixture and adjusting its pH value to between 6.4–6.8. The nutrient mixture was then sterilized by autoclaving at 15 psi pressure (121 °C) for 20 min. Nutrient agar was prepared by pouring the nutrient mixture to the same thickness on sterilized petri plates. The test bacteria were then grown overnight at 37 ^{o}C, 120 rpm in 10 ml nutrient broth. This broth was used for seeding the bacteria onto the agar plates. These bacteria suspensions (approximately10^5 cells/ml) were used for seeding the bacteria onto the agar plates.

2.4 Extraction and purification

For water extraction, ground tobacco leaf sample (0.2 g) was infused with 10 ml freshly boiled distilled water for 10 min in a thermos flask. The mixture was filtered through Whatman No. 1 and rapidly cooled under tap water. For organic solvent extraction, different concentrations of acetone, DMF and methanol were used. Ground tobacco leaf sample (0.2 g) was extracted with 2 ml of solvent for 1 h on a horizontal shaker. The mixture was centrifuged at 8500 g for 10 min and subsequently decanted. The residue was re-extracted twice more for 2 h and the extraction procedure was repeated twice more for 3 h as explained above. The five supernatants were combined and determined for polyphenol content and antioxidant activity. Each solvent extraction was carried out in triplicate. In order to solely obtain the extracts, the solvent was evaporated under controlled pressure in a rotary evaporator. The weight of solid remains after evaporation completed was recorded to calculate a yield of extraction.

2.5 Fabric treatment

The white cotton fabric was desized in a liquor containing 5 g of nonionic soap in a liter of water. The material to liquor ratio was taken as 1:40. The fabric was boiled at 95 $^{\circ}$C for 1 h and dried under shade. The desized cotton fabric was pretreated with alum at 80 ^{o}C and then treated with the extracts of tobacco leaf in 10 % DMSO for 45 min. The extracts (50 µg polyphenol per ml) were applied to the cotton fabric by dipping in bath at material to solution ratio of 1:10 at 60^{o}C and neutral pH. Samples were treated with 10 % alum after treated with the extracts. The fabric was then dried at 80^{o}C for ten minutes. Finally, the fabric samples were tested for antimicrobial activity.

2.6 Antimicrobial assay

Antimicrobial activity of the extracts was tested by the disc diffusion method. The treated and untreated fabrics were placed on top of the seeded media of the two bacterial strains. The antibacterial assay plates were incubated at 37°C for 24 h and the diameters of the zones of clearing were noted. For this study, the diameter of the zone of inhibition around each test fibers was taken as a measure of the antibacterial activity. In addition, the cotton fabrics treated with the standard antibiotics, kanamycin (10 µg) were also experimented which would serve as positive antibacterial controls. Untreated fabrics were served as negative antibacterial controls. Each experiment was carried out in triplicates and the mean diameter of the inhibition zones was recorded.

2.7. Total polyphenol content

Total polyphenol contents of the extracts were determined by the Folin–Ciocaulteu assay. One millilitre of the extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent (1 N) and the solution was kept at 25 °C. The absorbance of the blue color was measured using a spectrophotometer at 725 nm. Gallic acid was employed as a standard reference. The results were expressed as gallic acid equivalents (mg Gallic acid/g of tobacco leaves on dry weight basis).

2.8. Determination of antioxidant activity by the DPPH radical scavenging method

The antioxidant activity of the extract samples was measured by using the DPPH assay. Fifty microliter of the tobacco leaf extract diluted 15-fold with distilled water (directly, 5- and 10-fold dilution in additional assays) was mixed with an aliquot of 1950 µl of 6 x 10^{5} M DPPH radical in methanol. Distilled water was used as a control instead of extract. The reaction mixture was vortex-mixed and let to stand at 25°C in the dark for 60 min. Absorbance at 517 nm was measured using a spectrophotometer using methanol as a blank. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation [11].

$$AA\ (\%) = \frac{\text{Abscontrol} - \text{Ab s sample}}{\text{Ab scontrol}} \text{x100}$$

3. Result and Discussion

3.1. Polyphenol content

The polyphenol contents of the extracts from tobacco leaf were determined by the Folin–Ciocaulteu assay and presented in Table 1. Polyphenol content ranged from 2.7 to 167.4 mg GAE/g. Polyphenol contents of the extracts were strongly dependent on the solvents at different

concentrations used. All extracts prepared with 50% solvents contained highest level of polyphenol and followed by those with 80% and 100% solvents, respectively. Among the solvents tested, the highest level of polyphenol was achieved by using 50% DMF followed by 50% acetone, 80% DMF and 80% acetone, respectively. The lowest amounts of polyphenol were obtained with 100% ethanol. Our results clearly showed that higher content of polyphenols was obtained with an increase in polarity of the solvent used.

Table 1 *Effect of different solvents on polyphenol content of the tobacco leaf extracts*

Solvent	Polyphenol content [a](mg/g)	Antioxidant activity[b] (%)
Water	52.3 ± 1.22	45.6 ± 1.01
Acetone		
50%	159.3 ± 0.97	94.6 ± 0.73
80%	152.6 ± 1.24	91.7 ±1.61
100%	3.6 ± 0.41	1.8 ±1.38
Ethanol		
50%	112.6 ± 1.83	88.2 ± 0.81
80%	84.5 ± 0.63	53.3 ±1.35
100%	2.7 ± 0.85	1.04 ±0.71
DMF		
50%	167.4 ± 0.55	97.1 ± 0.86
80%	160.9 ± 0.93	93.0 ± 1.64
100%	63.17 ± 0.49	52. 1 ±1.08

[a] Data are expressed as means ±SE of triplicate experiments.
[b] Samples were diluted 15-fold for antioxidant activity determination.

3.2. Antioxidant activity

Solvents used for polyphenol extraction in tobacco leaf had significant effects on DPPH scavenging capacity determination (Table 1). The extracts with concentrations of 50% and 80% of organic solvents used exhibited considerably high DPPH radical scavenging activity. Among the extracts, the order of high antioxidant activity was 50% DMF > 50% acetone > 80% DMF > 80% acetone. The tobacco leaf extracts with higher antioxidant activity also had higher polyphenol content. The extracts obtained using high polarity solvents were considerably more effective radical scavengers than those using less polarity solvents. Therefore, change in solvent polarity causes its ability to dissolve a special group of antioxidant compounds. It also influences the antioxidant activity estimation.

3.3 Antibacterial activity of treated fabrics

The disc diffusion method was used to determine the antimicrobial activity of cotton fabrics treated with the extracts of tobacco leaf. The antimicrobial activity on pathogenic strains of Gram-positive (*S. aureus*), Gram-negative (*Escherichia col*) bacteria was evaluated in the present study. Cotton fabrics with kanamycin were used as a positive control because it has been commonly employed as the antibiotic for Gram-positive and Gram-negative bacteria. Inhibition zones of bacteria by the tobacco leaf extracts and kanamycin were measured. According to the results in Table 2, diameters of the inhibition zone of fabric with kanamycin were 23.46 ± 1.21 mm for *E. coli*, and 19.67 ± 1.09 mm for *S. aureus.* Cotton fabrics treated with the tobacco leaf

extract showed good antimicrobial activity on *E. coli* (21.33 ± 0.86 mm), and *S. aureus* (18.50 ± 0.77 mm). This result indicated that the polyphenol extracts from tobacco leaf was a good antimicrobial agent with strong antimicrobial activity for the textile.

Table 2. *Evaluation of antibacterial activity of treated fabrics*

Treatment	**Diameter of Inhibition zone (mm) ± S.D.[a]**	
	E. coli	***S. aureus***
Untreated	No	No
With kanamycin (10 μg)	23.46 ± 1.21	19.67 ± 1.09
With the tobacco extract (50 μg)	21.33 ± 0.86	18.50 ± 0.77

[a] Mean value of three determinations, each from a different plate

4. Conclusion

Extracting solvent significantly affected total polyphenol content and antioxidant activity of the tobacco leaf extracts. The polyphenol content of the extracts varied depending on the concentration of solvent and solvent type. The most efficient solvents for polyphenol extraction were 50% DMF. The 50% DMF extract also showed the highest antioxidant activity. The inhibition activity of cotton fabrics treated with the polyphenol of tobacco leaf on tested pathogenic bacterial strains were investigated in this study. The results confirmed that it had good antimicrobial activity against *E. coli* and *S. aureus*. From our results, it appears important to develop natural antioxidants and antibacterial agents from tobacco leaf for textile application. This may be one way for extensively utilizing the tobacco resource.

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