EVALUATION OF ANTIOXIDATION AND RADICAL SCAVENGING ACTIVITIES IN PYROLIGNEOUS ACID SAMPLES

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Abstract: Wood vinegar or pyroligneous acid (PA) samples were tested for their total phenolic compounds and antioxidative and radical scavenging activities. Total phenolic content of samples was determined by using Folin Ciocalteau agent. PA samples from Maihongson and Chiang Mai were contained total phenolic content of, expressed as gallic acid equivalents/mL of sample, 5.25±0.37 mg GAE/ml and 4.38±0.37 mg GAE/ml, respectively. Their IC_{50} was calculated as the amount of concentrated pyroligneous acid (PA) causing a 50% inhibition of the DPPH radical. There were 276.2 ppm and 248.8 ppm, for PAs from Maehongson and Chiang Mai, respectively. IC_{50} values for ABTS [2,2 azobis (3-ethylbenzo thiozoline-6-sulphonic acid) diammonium salt] assays were found 224.2 ppm and 229.4 ppm for samples from Maehongson and Chiang Mai, respectively.

Introduction

Pyroligneous acid, wood liquids, liquid smoke, liquid wood, bio-crude oil and wood distillates or wood vinegar are synonyms. The crude condensate is produced from the distillation of smoke generated in the process of making charcoal [1]. The composition of smoke has been extensively studied in recent years and more than 2000 compounds were identified [1-5]. These compounds belong to many different chemical classes: aldehydes; ketones; alcohols; acids; esters; furan and pyran derivatives; phenolic derivatives; hydrocarbons; nitrogen compounds.

Pyroligneous acid (PA) has been traditionally used as fungicide, sterilizing agent, deodorizer, antimicrobial, fertilizer and growth promoting agent [3, 6-10]. Smoke flavors are considered to be Generally Regarded As Safe (GRAS), so they can be used in foods as an additional barrier to prevent microbial growth at levels which comply with good manufacturing practice [11-13].

Nowadays, antioxidants have gained more importance because of their positive involvement as health promoters in conditions such as cardiovascular problems, atherosclerosis, treatment of many forms of cancer, and the aging process [14]. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical scavengers. Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups. The phenolic content may contribute directly to the antioxidative action. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [15-16].

The objective of this study was to evaluate the antioxidant activity and estimate the phenolic content of the pyroligneous acid. Samples collected from Maehongson and Chiang Mai provinces were evaluated of free radical scavenging assays such as 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 1,1′-diphenyl-2-picrylhydrazyl (DPPH). The radical scavenging potentials were compared to gallic acids. Its total phenolic content was determined by Folin–Ciocalteu's reagent (FCR).

Materials and Methods

Chemicals: Potassium persulfate was purchased from Ajax Finechem, Australia. 1, 1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), gallic acid, and sodium carbonate (Na_2CO_3) were purchased from Fluka, Germany. Folin-Ciocalteu's phenol reagent was purchased from Merck, Germany. HPLC grade methanol was purchased from Fisher Scientific UK Limited**,** UK. Gallic acid (GA) was purchased from Sigma–Aldrich (Steinem, Germany). Ultra-pure water (18 MΩ.cm), Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA), were used to prepare all solutions. All other chemicals and solvents used were of analytical grade available commercially. The UV–VIS measurements were performed using Hitachi U-2900 spectrophotometer.

Preparation of concentrated pyroligneous acid: The raw wood vinegar or pyroligneous acid (PA) samples collected from Maehongson and Chiang Mai, Thailand during October 2008 were produced from mixed biomass such as wood and bamboo. The raw pyroligneous acid has clear reddish brown color which is similar to the pleasing hue of black tea, beer or wine. The sample was stored in the dark at room temperature before analysis. Sample of 200 ml raw pyroligneous acid was filtered through Whatman No.1 filter paper to eliminate solid particles and dust. The filtrate obtained was then evaporated using a Buchi RE 111 rotary evaporator at 80°C until dryness. Crude extract were weighed and dissolved and made up to 10 ml with methanol.

DPPH radical scavenging activity: The free radical scavenging activity was measured by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method proposed by Brand Williams [17] and Gaulejac et al [18]. A 100 µl of sample solution was added to 300 µl of $6x10^{-5}$ mol/L methanolic solution of DPPH. The absorbance at 515 nm was measured using an ultraviolet-visible spectrophotometer after the solution has been allowed to stand in the dark for 30 min at room temperature. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, was calculated. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\%\text{DPPH radical scavenging activity} = 100\left(\frac{A_{control} - A_{sample}}{A_{control}}\right) \quad (1)$$

A = Absorbance

IC_{50} value, the concentration of sample required for 50% inhibition of DPPH free radical, was determined from the plot between %inhibition and concentration.

Cation radical scavenging activity: The total antioxidant activity of the samples was measured by ABTS cation radical decolorization assay according to the method of Re et al. [19] with some modification. $ABTS^{\bullet+}$ was produced by reacting 7 mmol/L ABTS aqueous solution with 2.45 mmol/L potassium persulfate in darkness for 12-16 hours at room temperature. Prior to assay, this solution was diluted in aqueous and equilibrated at 30°C. An aliquot of each sample of 100 µl was mixed with 300 µl of diluted ABTS cation radical solution. The absorbance at 734 nm was measured using an ultraviolet-visible spectrophotometer after the solution has been allowed to stand in the dark for 30 min at room temperature. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using equation 1 and IC_{50} are also calculated.

Total phenolic content assay: The Total phenolic content of the (PA) concentrated was determined according to the Folin-Ciocalteu spectrophotometric method described by Singleton et al. [20] with some modifications. Briefly, 1 mL of pyroligneous acid was mixed with 2.5 mL of 10 %(v/v) Folin-Ciocalteu's phenol reagent and allowed to react for 8 min. Then, 1 mL of 7.5%(w/v) Na_2CO_3 solution was added. After incubation for 15 min at 50°C the final volume was made up to 25 mL with ultra-pure water. The absorbance at 760 nm was determined. The measurement was compared to a standard curve of gallic acid (GA) solution and the total phenolic content was expressed as milligrams gallic acid equivalents/g of sample. Each sample was done in triplicate.

Results and Discussion

Scavenging effect of the concentrated pyroligneous acid on DPPH radicals and ABTS cation radicals: Pyroligneous acids were examined for their physical properties and the results are shown in Table 1. The moderately acidic and smoke odors of brown liquid were found for both samples. A comparison has been made between their total antioxidant capacity of concentrated PA samples from Maehongson and Chiang Mai provinces. Methods employed to evaluate antioxidative capacity were DPPH and ABTS. Plots between %inhibition of DPPH and ABTS cation radicals and concentration of PAs are shown in Figure 1. IC_{50} values were determined from the plot between %inhibition of DPPH and ABTS cation radicals and concentration extracts. An example of IC_{50} determination can be seen in Figure 1. Percentage of inhibition of DPPH radical from concentrated PA samples from Maehongson and Chiang Mai provinces were ranged from 92.03 ±0.36 to 96.62±0.49 and 80.86±0.51 to 88.15±0.96, respectively**.** IC_{50} values of both samples were comparable means similar antioxidant powers. The results are shown in Table 1.

Determination of total phenolic content of pyroligneous acid: the total phenolic content of concentrated (PA) both from Maehongson and Chiang Mai provinces were 5.25±0.3 และ 4.38±0.37 mg, gallic acid equivalents/mL of the sample, respectively. In short, the results suggest that both PAs from Maehongson and Chiang Mai provinces were rich source of polyphenolic compounds.

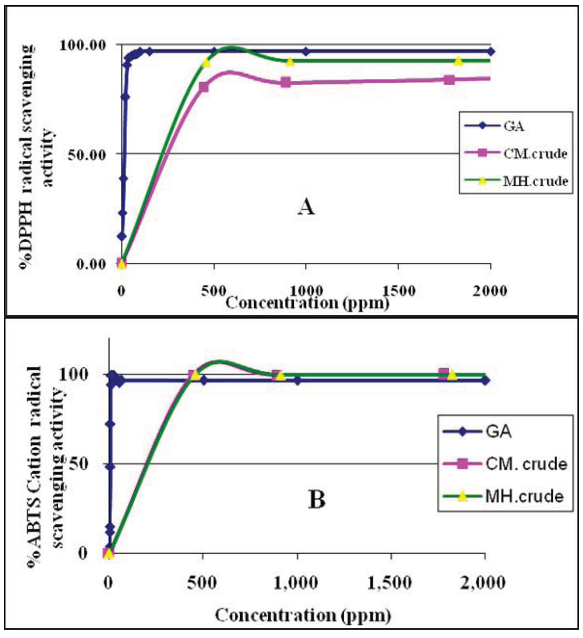

Figure 1. Scavenging effect of concentrated PA samples on DPPH radical (A) and ABTS cation radical assays (**B**) (CM : Chiang Mai**, MH:** Maehongson).

Table 1: Physical characteristic, IC_{50} and total phenolic content of PA samples.

Sample No.*	Color	Odor	pH	IC_{50}		TPC
				DPPH mg/L	ABTS mg/L	FCR mg GAE/mL
1.	Brown	Smoke	4.3	276.2	224.2	4.38±0.37
2.	Brown	Smoke	4.0	248.8	229.4	5.25±0.37

*****1 PA from Chiang Mai and 2 PA from Maehongson province

4. Conclusions

The concentrated of pyroligneous acid (PA) from Maehonsorn provinces, evidently showed higher total phenolics content when compared to same standards such as gallic acid. Comparable effective antioxidative activities when using DPPH and ABTS radical scavenging activity were obtained. In the present work, however, the components responsible for the antioxidant activities are unclear. Therefore, further work is in progress for the isolation and identification of the antioxidant components in pyroligneous acid.

6. References

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