# Comparative Study on Antioxidant Properties of Various Solvent Extracts Relating to Indocalamus tessellatus Leaves of Agricultural Tourism Products

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Abstract: The rapid development of rural tourism in China has become an important force in China's tourism industry. Rural tourism is increasingly becoming an effective carrier to solve the problem of urban-rural integration and the construction of new countryside, and the production, development and marketing of agricultural products are gradually becoming a key selling point of rural tourism. As a tourist agricultural product, Indocalamus tessellatus leaves were extracted by ethanol, petroleum ether and water respectively. Three different extracts revealed different results of each experiment. The ethanol extract with the most total phenol content is the most effective antioxidant in all experiments. The effects of five different pH values on the antioxidant activities at two diverse temperatures were also studied. The antioxidant activity of the extract kept the temperature at 40 °C. It reached the maximum value at pH 7, and decreased gradually after hiding at 5 °C for 30 days ($p$<0.05). The results showed that Indocalamus leaf has significant antioxidant activity, so it can be used as a potential source of natural antioxidants in the food industry.

## 1 INTRODUCTION

China has the most abundant bamboo resources in the world, and the chemical components of bamboo leaves are widely used in the production of new medicines and foods. Indocalamus tessellatus leaf, as one of the types of columnar leaf, has been used for hundreds of years because of its good leaf use, long-lasting fragrance and health care function. Indocalamus tessellatus leaf is one of the agricultural tourism products (Yildirim 2000). With the deepening of people's understanding of Indocalamus tessellatus leaf and its medicinal value, the research and development of Indocalamus tessellatus leaf extract have been extensively investigated in recent years.Indocalamus tessellatus leaf has the medicinal characteristics of diuresis, hypoglycemia and hypotension (Blois 1958). However, until recently, it was found that their mechanism of action was related to antioxidant activity. Some papers reported the content of Cathaya argyrophylla, Paeonia, Cimicifuga nanchuanensis and other flavonoids in Indocalamus tessellatus leaf. Phenolic compound is the main phytochemical substance of the antioxidant activity of agricultural tourism products. On the basis of relevant experts and scholars' research on rural tourism and agricultural products, this paper will make a special study on the function improvement of agricultural products in rural tourism, and put forward the idea of increasing added value of agricultural products, so as to further adjust the rural industrial structure and provide theoretical support and policy suggestions for increasing farmers' income (Ohkawa 1979).

## 2 MATERIALS AND METHODS

### 2.1 Chemicals

The reference standard of rutin was purchased from National Institute for the Control of Pharmaceutical and Biological Products (purity ≥ 98%). 2-diphenyl-1-pyridyl hydrazide and 2-thiobarbituric acid were purchased from Nanjing Jiancheng Bioengineering Institute (China). Ethanol, sodiumnitrite,



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aluminum nitrate, sodium hydroxide were purchased from commercial sources.

## 2.2 Materials

Indocalamus tessellatus leaves (chequer-shaped tessellatus leaves, collection time: December, 2018 and July, 2019 respectively, place: Huangshan, China). The Indocalamus tessellatus leaves were grounded in a pulverizer and passed through an 60-mesh sieve, dried to constant mass in the oven at 60 ℃ for 6 h, cooled and then stored.

## 2.3 The Effective Components Extracted from Indocalamus Tessellatus Leaves

The Indocalamus tessellatus leaves (20 g) were extracted overnight with 100 ml each of ether, ethanol or water, respectively, in a mechanical shaker at room temperature. Each extract was filtered with filter paper. The filtrate obtained from ether and ethanol was evaporated to dryness at 40℃ in a rotary evaporator (Beijing Chemical-Regent Company, China) and the water extract was freeze-dried. The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 5℃ until use.

## 2.4 Reducing Power Assay

**Yildirim, Mavi and Kara (2000)** methods were used to evaluate the ability of extract to reduce iron (III). The dry extract (125–1000 μg) was placed in 1 ml of the corresponding solvent, mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10g $l^{-1}$), and the mixture was incubated at 50 ℃ for 30 minutes. After incubation, add 2.5ml trichloroacetic acid (100 g $l^{-1}$) and centrifugate the mixture to 1650g for 10min. Finally, 2.5ml supernatant was mixed with 2.5ml distilled water and 0.5ml $FeCl_3$ (1 g $l^{-1}$) (Siddhuraju 2002).

## 2.5 2-Diphenyl-1-Pyridyl Hydrazide Radical Scavenging Activity

Blois (1958) method was used to determine the DPPH free radical scavenging ability of the extract. Mix 1 ml of 1 mM DPPH ether solution with 3 ml of ether extract containing 50-400 μg of dry extract. Then rotate the mixture vigorously and leave it in darkness at room temperature for 30 minutes. The absorbance was measured at 517nm and the activity was expressed as a percentage of DPPH clearance relative to the control using the following equation:

$$\text{2-diphenyl-1-pyridyl hydrazide scavenging activity (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## 2.6 Antioxidant Activity

The antioxidant activity of BHT was estimated. The obtained reaction solution (1 ml) was used for 2-thiobarbituric acid determination (Yen 1993).

The oxidation degree of oil was determined by 2-thiobarbituric acid reported by Ohkawa, ohishi and Yagi (1979). The above reaction solution (1ml) was mingled with zero point two percent (w/V) 2-thiobarbituric acid solution (3 ml) and 0.05 M sulfuric acid (2.5 ml). Heat the mixed liquor in a 95 ℃ water bath for 30 minutes. Cooling the solution in ice for five minutes, 4.0 ml 1-butanol was used to extract the colored substance. The absorbance of n-butanol layer was measured at 532 nm. Taking malondialdehyde diethylacetal as the standard, the standard curve was established and expressed as malondialdehyde equivalent. The definition of antioxidant activity (AOA) can be expressed as the percentage of inhibition of lipid peroxidation relative to the control group selected in the experiment. The following formula can be used:

$$\text{AOA}(\%) = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

# 3 RESULTS AND DISCUSSION

## 3.1 Extract Yield and Total Phenolics

As shown in Table 1, the yield and total phenol data of different extracts of Indocalamus tessellatus leaf. Dry leaf weight ranges from 7.02% (water extraction) to 13.46% (ethanol extraction). According to the experimental results, the amount of total phenol (gallic acid equivalent) represented as a percentage by weight of dry extract is between 6.75% of water extract and 8.97% of ether extract. Ether is the most effective solvent for extraction of Indocalamus tessellatus leaf antioxidant.

## 3.2 Reducing Power

Many researches had shown that the electron donating ability of bioactive compounds is related to their antioxidant activity. In this paper, the ability of the extract to reduce iron (III) to iron (II) was determined and compared with vitamin C. All the three extracts showed a certain degree of electron delivery capacity, which was concentration dependent, but the electron delivery capacity was lower than that of vitamin C (Fig. 1, table 2). The ether extract with the most total phenol content is the most effective reducing agent, while the water extract with the lowest phenol content has the weakest property. A similar relationship between iron (III) reduction activity and total phenol content has been reviewed on some studies (Zhu 2002).

Table 1: Study on the content of total phenols and the extract extracted by different solvents from Indocalamus tessellatus leaves.

| Sample | Yield↑ | Total phenolics‡ |
|---|---|---|
| ethanol | 13.46±1.33[a] | 8.97±0.09[a] |
| Petroleum ether | 7.98±0.88[b] | 8.68±0.39[b] |
| Water | 7.02±0.97[c] | 6.75±0.42[c] |

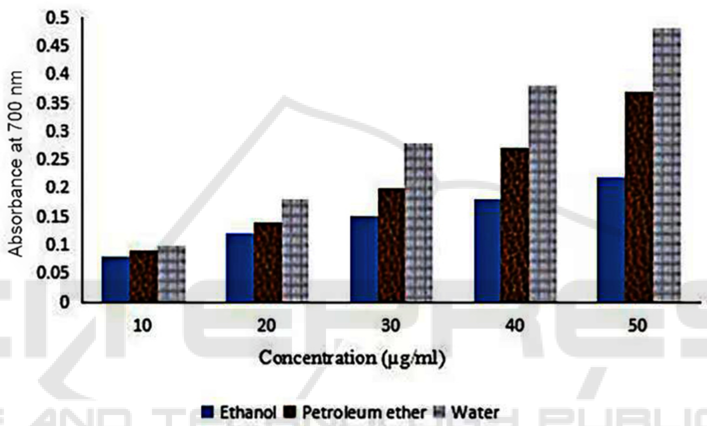


Figure 1: Reducing powers of different solvents extracts of Indocalamus tessellatus leaves.

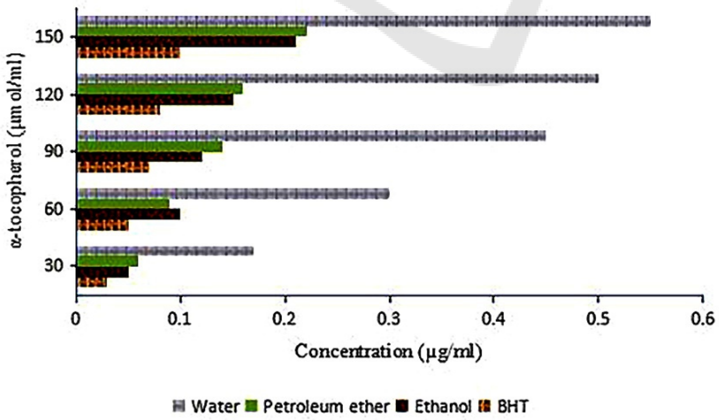


Figure 2: The results of different solvents extracts of Indocalamus tessellatus leaves.

Table 2: Comparison of antioxidant properties of Indocalamus tessellatus leaves extracts.

| Sample | Reducing power | TAOC | Antioxidant activity‡B | 2-diphenyl-1-pyridyl hydrazide EC50 |
|---|---|---|---|---|
| BHT | nd | 3.873±0.026[a] | 86.42±0.52[a] | 39.11±0.48[c] |
| Ascorbic acid | 2.667±0.002[a] | nd | nd | 58.86±0.28[b] |
| Ethanol | 0.244±0.001[b] | 1.385±0.004[b] | 79.79±0.48[b] | 80.49±0.79[a] |
| petroleum ether | 0.215±0.007[c] | 1.377±0.003[b] | 60.51±0.92[c] | nd |
| Water | 0.162±0.005[d] | 0.652±0.003[c] | 40.12±0.48[d] | nd |

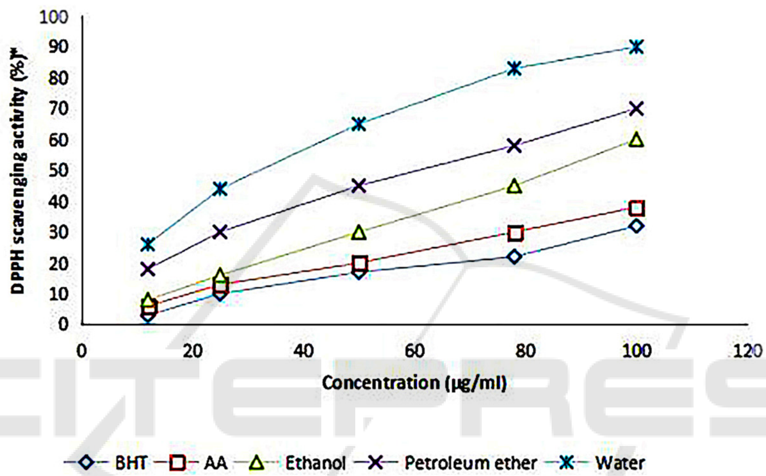


Figure 3: 2-diphenyl-1-pyridyl hydrazide radical scavenging activities of ethanol, petroleum ether and water extracts of Indocalamus tessellatus leaves.

### 3.3 2-Diphenyl-1-Pyridyl Hydrazide Radical Scavenging Activity

The effect of three extracts on 2-diphenyl-1-pyridyl hydrazide scavenging activity at different concentrations (Fig. 3). The ether extract with the highest total phenol content is the most active free radical scavenger, followed by ethanol and water extract. However, ether extract was not as effective as BHT and ascorbic acid in the positive control group because the amount of extract ($p<0.05$) required to scavenge 50% 2-diphenyl-1-pyridyl hydrazide radical in the reaction mixture (EC50) was significantly higher than BHT and ascorbic acid (Table 2). The results of 2-diphenyl-1-pyridyl hydrazide free radical scavenging experiments show that extracts donated by hydrogen and / or electron may prevent the active free radical species from reaching the biomolecules in susceptible organisms and food systems.

### 3.4 Antioxidant Activity of Indocalamus Tessellatus Leaves Extract

In this study, 2-thiobarbituric acid was used to determine the inhibitory effect of Indocalamus tessellatus leaves extract on the lipid peroxidation of flaxseed. By measuring the absorbance of Indocalamus tessellatus leaves extract at 532 nm, the content of thiobarbituric acid reactant produced by Indocalamus tessellatus leaves extract on the oxidation of flaxseed oil was determined. All three extracts can prevent the formation of thiobarbituric acid reactant produced by ferrous sulfate (Table 3). According to the evaluated percentage of antioxidant activity shown in Table 2, it is found that the extract with the highest activity is ether extract, with the activity of 81%, which is lower than BHT value (90%) ($p<0.05$). The active sequence was related to the total phenol content of each extract.

Table 3: Effect of Indocalamus tessellatus extracts on the production of malondialdehyde.

| Control | BHT | Ethanol | | | Petroleum ether | | | Water | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 12.5ppm | 25ppm | 50ppm | 12.5ppm | 25ppm | 50ppm | 12.5ppm | 25ppm | 50ppm |
| 6.3 | 0.3 | 2.8 | 1.9 | 0.7 | 3.9 | 3.3 | 2.1 | 5.8 | 5.2 | 3.7 |

malondialdehyde equivalents (mmol/kg oil)

## 3.5 The Influence Factors of Temperature, Ph Value and Stability

Table 4 shows the effect of temperature on the antioxidant stability of ethanol extract. At fifty degrees Celsius for sixty minutes, the antioxidant activity value of the extract was basically the same, and the change of malondialdehyde equivalent of oil oxidation was not significant ($p<0.05$). When heated at fifty degrees Celsius for 120 min, the antioxidant activity of the extract decreased by 3%. When heated at one hundred ℃ for sixty minutes, the antioxidant activity of the extract decreased by 9% ($p<0.05$), and decreased continuously with the prolongation of boiling time. However, even after heating at One hundred degrees Celsius for One hundred and twenty minutes, the remaining antioxidant activity was about sixty-eight percent. The decrease of antioxidant activity and subsequent heating at One hundred degrees Celsius may be related to the loss of natural antioxidants in the extract or the formation of new compounds with oxidation promoting activity.

The effect of pH value on the sturdiness of ether extract is demonstrated in Table 5. In the presence of the extraction solution, the malondialdehyde equivalent formed by the oxidation of the oil decreased gradually, the lowest one was at median pH, and increased continuously at alkaline pH ($p<0.05$), indicating that the antioxidant activity of the extraction solution had a strong dependence on the pH value of the system. The effect of storage on the stability of Indocalamus tessellatus leaf ether extract was also studied for more than three months with an interval of thirty days (Table 6). There was no change in antioxidant activity of the temperature of 5℃ for thirty days. At the end of storage, the residual activity of the extract was about 65%, indicating that it could still be used as a source of natural antioxidants.

Table 4: The results of ethanolic extract.

| Temperature | 40℃ | 80℃ |
|---|---|---|
| Non-treated | 0.48±0.02b | 0.48±0.02c* |
| 60min | 0.51±0.03b | 1.1+0.04b* |
| 120min | 0.73±0.03a | 1.42±0.03a* |

Table 5: Effect of Indocalamus tessellatus extracts on the production of malondialdehyde.

| Control | BHT | Ethanol | | | Petroleum ether | | | Water | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 12.5ppm | 25ppm | 50ppm | 12.5ppm | 25ppm | 50ppm | 12.5ppm | 25ppm | 50ppm |
| 6.3 | 0.3 | 2.8 | 1.9 | 0.7 | 3.9 | 3.3 | 2.1 | 5.8 | 5.2 | 3.7 |

malondialdehyde equivalents (mmol/kg oil)

Table 6: The results of ethanolic extract.

| | PH | | | | |
|---|---|---|---|---|---|
| Malondialdehyde equivalents (mmol/kg oil) | 3 | 5 | 7 | 9 | 11 |
| | 0.536±0.02b | 0.528±0.05b | 0.477±0.03c | 0.610±0.03b | 0.698±0.04a |

## 4 CONCLUSIONS

Based on the comprehensive development status and characteristics of rural tourism, this paper studies the current status and problems of rural tourism agricultural products processing, and takes Indocalamus tessellatus leaves as an example for analysis, proposes corresponding finishing methods, and puts forward countermeasures and suggestions. The possible innovation of this article is that the subject combines rural tourism and agricultural product processing, and proposes a new model that uses rural tourism as a platform to enhance the deep processing of agricultural products. It provides basic research data for the study of antioxidant activity of plant extracts. However, how to implement these strategies and achieve effective results in combination with the characteristics of agricultural tourism products still needs further research. The next step will be further research on other plants to improve the scientific research level of food processing industry.

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