Antioxidant activity of extracts from *Uncaria scandens*

**Fulu Zhang**1,2, **Aimei Yang**1,2*, **Songyao Ma**3*, **Huilan Shang**1,2 and **Zhihui Wang**

1School of Life Science and Engineering, Lanzhou University of Technology, Lanzhou, 730050, P. R. China

2Key Laboratory of Herbal-Tebitan Drug Screening and Deep Processing of Gansu Province, Lanzhou University of Technology, Lanzhou 730050, China

3Institute of Natural Energy, Gansu Academy of Sciences, 730046, Lanzhou, P. R. China

*Corresponding author’s e-mail: aimeiyang@163.com

**Abstract:** The anti-oxidation activity of petroleum ether, ethyl acetate and n-butanol extracts from ethanol extract of *Uncaria scandens* were studied, using DPPH free radical scavenging, hydroxyl radical scavenging, and total reducing power determination. The results showed that the ethanol extract of *Uncaria scandens* has strong antioxidant activity, especially the DPPH free radical scavenging ability, which is similar to the positive control vitamin C.

1. Introduction

*Uncaria* is one of important genus of the *Rubiaceae* family. It consists of 34 species of plants and about 11 species grows in China, most of which have been used as traditional herbs [1-2]. The hook and stem of *Uncaria scandens* used as a Traditional Chinese Medicine (TCM). The stems of *Uncaria scandens* contain a variety of indole alkaloids, which are the main active ingredients [3-5].

In this paper we report the antioxidant activities of the ethanol extract of *Uncaria scandens*. DPPH free radical scavenging, hydroxyl radical scavenging and total reducing power were used to systematically evaluate the antioxidant activity of three different extracts of ethanol extract of *Uncaria scandens*. Providing reference information for further development and utilization of the plant [6-8].

2. Materials and Method

2.1. Plant material

The fresh air-dried plant material of *Uncaria scandens* was bought from Huang zhong Tibetan Hospital of Qinghai province, China, in January 2011 and was identified by Prof. Guo-Liang Zhang, College of Biology, Lanzhou University, Lanzhou, China. The five species of bacteria are *Escherichia coli*, *Staphylococcus aureus*, *Bacillus licheniformis*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae*, were provided by the laboratory of College of Life Science and Engineering, Lanzhou University of Technology, China.

2.2. Chemicals

vitamin C, DPPH, absolute ethanol, trichloroacetic acid, 30% hydrogen peroxide, ferrous sulfate, salicylic acid, Potassium ferricyanide, sodium dihydrogen phosphate, disodium hydrogen phosphate, and ferric chloride were all analytically pure.
2.3. Instrument
HH-S26 constant temperature water bath (Jintan Meixiang Instrument Co, Ltd.), 712G visible spectrophotometer (Shanghai Jingke Instrument Co.Ltd.), ALC-210.4 analytical balance (ACCULAB), pipetting gun, test tube, glass cuvette.

3. Extraction and Isolation
The air-dried powder of *Uncaria scandens* (4.5kg) was extracted with ethanol for 3 times at room temperature. The combined extracts was concentrated under reduced pressure to give a residue (248g), The total extract was suspended with distilled water and filtered to obtain a water-soluble partial extract and a water-insoluble partial extract. The water-soluble partial extracts were respectively extracted with ethyl acetate and n-butanol, and the solvent was recovered under reduced pressure to give ethyl acetate extract A and n-butanol extract A. The water-insoluble partial extract was suspended with water and extracted with petroleum ether, ethyl acetate and n-butanol respectively, and the extracts was concentrated under reduced pressure to obtain petroleum ether extract, ethyl acetate extract B and n-butanol extract B.

4. Determination of Antioxidant Activity of *Uncaria scandens*

4.1. DPPH radical scavenging activity
Accurately transfer 2mL of sample solution with a mass concentration of 0.01, 0.02, 0.03, 0.04, 0.05, 0.10, 0.15, 0.20, 0.25 mg/mL into each tube, add 2mL of 0.05 mg/mL DPPH ethanol solution, shake well. Leave at room temperature for 30 min in the dark. The zero was adjusted with absolute ethanol and the absorbance A1 was measured at 517nm. At the same time, 2mL of absolute ethanol was used instead of DPPH ethanol solution, and the absorbance value A2 was measured; 2mL of absolute ethanol was used instead of the sample, and the absorbance value A0 was measured. The clearance rate is calculated according to formula (1):

\[
\text{clearance rate} (\%) = \left[1 - \frac{(A1 - A2)}{A0}\right] \times 100\%
\]

The higher the clearance rate, the stronger the sample's DPPH radicals scavenging activity [9-10].

4.2. Hydroxyl radicals scavenging activity
Accurately transfer 2mL of sample solution with mass concentration of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 0.14, 0.16mg/mL into each tube, and add 1mL of 6mol/L ferrous sulfate solution, 1mL of 6mol/L salicylic acid-ethanol solution, 1mL 0.1% H₂O₂ solution. Finally, the volume was adjusted to 10mL, shaken, kept at 37℃ for 30 min, adjusted to zero with 20% ethanol, and the absorbance A1 was measured at 510nm. At the same time, 1mL of distilled water was used instead of 0.1% H₂O₂ solution, and the absorbance value A2 was measured; 2mL of absolute ethanol was used instead of the sample solution, and the absorbance A0 was measured. According to formula (2):

\[
\text{Clearance} (\%) = \left[1 - \frac{(A1 - A2)}{A0}\right] \times 100\%
\]

Calculate the clearance rate. The higher the clearance rate, the stronger the sample's ability to scavenge hydroxyl radicals [11].

4.3. Determination of total reducing power
Accurately transfer 2.5mL of the sample solution with a mass concentration of 0.02, 0.04, 0.06, 0.08, 0.1, 0.12, 0.14, 0.16 mg/mL in a stoppered test tube, and then add 2.5mL of 0.2mol/L phosphate buffer solution (pH=6.6) and 2.5mL of 1% potassium ferricyanide solution, shake well. 50 ℃water bath, kept for 20 min, rapid cooling. Add 2.5mL 10% trichloroacetic acid solution, mix, centrifuge at 5000r/min for 5min, take 5mL of supernatant, add 4mL distilled water and 1mL 0.1% ferric chloride solution, mix, let stand for 10min, measure at 700nm Absorbance values. The above experiment was repeated with vitamin C as a positive control [12].
5. Results and discussion

Figure 1. DPPH radicals scavenging ability

It can be seen from the results that the DPPH free radical scavenging ability of each solvent extract of *Uncaria scardens* increases with the increase of concentration, and there is a dose-effect relationship within a certain range. At low concentrations, the ethyl acetate fraction extract A has a slightly better ability to scavenge DPPH free radicals than Vc. When the concentration reached 0.25 mg/mL, the DPPH free radical scavenging rate of each sample did not increase substantially. The DPPH free radical scavenging ability of petroleum ether extract is much lower than other samples. The sample has the ability to scavenge hydroxyl radicals: vitamin C > ethyl acetate A ≈ ethyl acetate B ≈ n-butanol B > n-butanol A > petroleum ether.

Figure 2. *Uncaria scardens* extract scavenging ability of hydroxyl radicals

The *Uncaria scardens* extract has a certain scavenging effect on the hydroxyl radicals produced by the Fenton system, and the scavenging ability increases with the increase of the sample mass concentration. At low concentrations, the ethyl acetate extract A has a slightly better ability to scavenge hydroxyl radicals than Vc. The petroleum ether extract has a poor ability to scavenge hydroxyl radicals, and the removal rate at the maximum concentration is still less than 20%. The ability to scavenge hydroxyl radicals: vitamin C > ethyl acetate A ≈ ethyl acetate B ≈ n-butanol B > n-butanol A > petroleum ether.
Figure 3. Total reducing power of \textit{Uncaria scandens} extract

The total reducing power increases with increasing sample mass concentration and has a good linear relationship over the range of test concentrations. The total reducing power of ethyl acetate extract A is slightly higher than Vc.

6. Conclusion

The antioxidant actives of the \textit{Uncaria scandens} extracts are mainly concentrated in the ethyl acetate fraction.

Acknowledgement

This research was supported by the Foundation of Gansu province key research and development plan, Gansu, China (17YF1NA057), and the open fund of Key Laboratory of Herbal-Tebitan Drug Screening and Deep Processing of Gansu Province, Lanzhou University of Technology, Gansu, China (20180804).

References

[1] Heitzman M E, Neto C C, Winiarz E. (2005) Ethnobotany, phytotechnology and pharmacology of Uncaria (Rubiaceae) J. Phytochemistry, 66(1):5-29.
[2] YU Zai-bai, SHU Guang-ming, QIN Song-yun. (1999) Investigation and study on the traditional Chinese medicine resources of Uncaria sinensis J. Chinese Journal of Traditional Chinese Medicine, 24(4): 6-10.
[3] Gerhard laus. (2004) Advanced in Chemistry and Bioactivity of Genus Uncaria J. Phytother Res ,18: 259-274.
[4] Zhang J, Yang M, Peng W. (2016) Chemical Constituents of Uncaria Scandens(Smith) Hutchins J. Journal of Hainan Normal University, 1-2.
[5] Da-Yong M A, Wang Y, Yan C. (2008) Chemical Constituents from Uncaria scandens Smith J. Chinese Journal of Pharmaceuticals, 39(7): 507-509.
[6] Yin Wenqing, Duan Shaoqing, Zhang Yan. (2010) Antioxidant Activities of Different Solvent Extracts and Total Alkaloids from Uncaria J. Journal of Guangxi Normal University (Natural Science), 28(1): 31-34.
[7] Zhang H, Jiang L, Ye S. (2010) Systematic evaluation of antioxidant capacities of the ethanolic extract of different tissues of jujube (Ziziphus jujuba Mill.) from China J. Food and Chemical Toxicology, 48(6):0-1465.
[8] Ardestani A, Yazdanparast R. (2007) Antioxidant and free radical scavenging potential of Achillea santolina extracts J. Food Chemistry104(1):21-29.
[9] LI Yijun, CUI Shengyun. (2011) Mechanism of ascorbic acid scavenging DPPH free radicals J. Food Science ,32(1):86-90.
[10] Kosani M, Rankovi B, Vukojevi J. (2011) Antioxidant properties of some lichen species J. Journal of Food Science and Technology, 48(5):584-590.
[11] YAN Jun, YAN Xiao-jun, ZOU Quan-fu. (2009) Determination of hydroxyl radicals generated by
Fenton reaction by spectrophotometry J. Journal of Chengdu University (Natural Science Edition), 28(2):91-93.

[12] Wen En Z, Qian-Qian LI. (2011) Determination of Total Antioxidant Capacity of Red Pigments from Chinese Jujube Peel by the Ferric Reducing/Antioxidant Power Assay J. Journal of Zhengzhou University (Engineering Science).