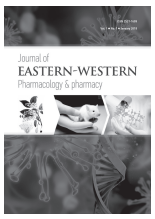

Original Article

https://doi.org/10.64269/jewpp.v6i1.4213

Journal of
EASTERN-WESTERN
Pharmacology & pharmacy

A STUDY ON SOME BIOLOGICAL ACTIVITIES OF *CACALIA HASTATA* L. EXTRACTS

Otgontsagaan Bolorchimeg, Tsevegjav Jaal, Duger Badral*

Department of General Science, Mongolian University of Pharmaceutical Sciences, Ulaanbaatar, Mongolia

KEYWORDS

Cacalia hastata L; *phenolic compound; antioxidant activity; antibacterial activity.*

Received: 2024/05/29 Accepted: 2024/12/30
Revised: 2024/10/20 Published: 2025/01/30

ABSTRACT

Background: *Cacalia hastata* L. is used in Mongolian traditional medicine to treat wounds, reduce fever, treat gastric ulcers, and expel bile. In addition, *Cacalia hastata* L. is determined to have antiinflammation and antibacterial and antiviral activities. In this study, we determined the antioxidant and antibacterial activities of *Cacalia hastata* L. extracts. Methods: The aerial parts of *Cacalia hastata* L. had been studied. These parts were extracted with 40%, 70%, 95% ethanol (1:10) by the remaceration method. Thin layer chromatography (TLC) was used for the identification of constituents as phenolic compounds and flavonoids, their quantities were determined by spectrophotometric methods, precipitation reactions were used for the qualitative analysis of the tannin, while its quantitative determination was performed by the direct titration with potassium permanganate using indigo sulfonic acid as an indicator. The antioxidant activity of extracts was evaluated by the DPPH assay and the antibacterial activity by the Agar diffusion method, respectively. Results: Quantities of phenolic compounds in 40%, 70% and 95% extracts were determined as 4.87%, 4.92%, and 4.17%, respectively. Whereas, total flavonoids were as 4.61%, 5.1%, and 4.13%, respectively. The reaction with ferric ammonium slag presented dark green color, which indicated the presence of a condensable agent. Total tannins were 7.4% in the 40% extract, 5.1% in the 70% extract, and 3.04% in the 95% extract, respectively, by titration with the potassium permanganate. In addition, all 40%, 70% and 95% extracts showed moderate DPPH scavenging activity as IC_{50}=48.5μg/mL, IC_{50}=74.8μg/mL, IC_{50}=51.2 μg/mL, respectively, comparad to rutin. The 40% and 70% extracts exhibited antibacterial activity against *Bacillus subtillis* with 3mm and 4mm inhibition zones, respectively. Conclusion: According to the results, the 70% ethanol extract of *Cacalia hastata* L. was more rich in biologically active products such as phenolic compounds, and flavonoids, and its extracts demonstrated antioxidant and antibacterial activities.

**Corresponding author: Department of General Science, Mongolian University of Pharmaceutical Sciences, Sonsgolon's road 4/A, Songinokhairkhan District, 20th khoroo, Ulaanbaatar, Mongolia; E-mail address: badral @monos.mn; Tel.99099947* (Duger Badral)

INTRODUCTION

Plant-based medications, often derived from natural sources like herbs and botanicals, are gaining popularity due to their perceived efficacy and minimal side effects. In traditional medicine, *Cacalia hastata* L has been used to heal wounds, gastric ulcers, poisoning fever, oral cavity, and gynecological diseases[1]. It is known as Yu-gu-shining and was used for purulent wounds and ulcers as a wound-healing, anti-exudative, and hemostatic agent as well as for bronchitis[2]. owadays, the extract of *Cacalia hastata* L. has been elucidated anti-inflammatory, antibacterial, spasmolytic, antipyretic, gastroprotector, and antihemorrhagic[3,4] and anti-viral properties[5]. *Cacalia hastata* L belongs to the family Asteraceae and grows in botanical, geographical provinces of Mongolia such as Khangai, Khentii, Khuvsgul, Mongol Daguur, and Dornod Mongol[6-9]**.** Moreover, this plant species widespread in the Arctic and the European parts of the country to Western and Eastern Siberia and the Far East in Russia, Eastern Europe, Korea, northeastern China, and northern Japan[6]. The chemical composition of C.*hastata* has been studied extensively and indicated the presence of various groups of biologically active substances, namely, alkaloids, sesquiterpenes, triterpenes, carotenoids, carbohydrates, pectin, phenolic compounds as well as coumarins and tannins, flavonoids and chlorogenic acid[2]. Therefore, our research aims to perform a quality analysis of plant raw materials according to standard criteria, the total amount of phenolic compounds and flavonoids, and determining antioxidant and antibacterial activities.

MATERIALS AND METHOD

2.1. Materials and chemicals

In this study, aerial parts of *Cacalia hastata* L. were used as a raw material and the study was evaluated in Mongolian University of Pharmaceutical Sciences and Institute of Biology, Mongolian Academy of Sciences. The raw materials were extracted with 40%, 70%, and 95% ethanol (1:10) by the remaceration method, and their qualitative and quantitative analyses of biologically active compounds were evaluated. Moreover, the 40%, 70%, and 95% ethanolic thick extracts were obtained by the same remaceration method following evaporation under 40°C and the thick extracts were used for antioxidant and antibacterial activities assay.

2.2. Qualitative analysis of flavonoids, phenolic compounds, and tannins of the extracts

The qualitative analysis of flavonoids was conducted by thin-layer chromatography. Two pieces of chromatography plates were prepared. Firstly, 5 µl (70% ethanol extracts) of each solution of the sample, and standard rutin were dropped on a silica gel plate (Silica gel 60 F254, Germany) and then placed in a solvent system of ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26) and was sprayed with 3% aluminum chloride[10]. The second plate with gallic acid was sprayed with a 2% ferric chloride (III)[10].

The qualitative reaction of the tannin was determined by precipitation method. The addition of 3-5 drops of 1% ferric ammonium sulfate to the extract revealed a dark green color, indicating condensable tannins[11.]

2.3. Quantitative analysis methods of total flavonoids, phenolics, and tannins in extracts

Total flavonoid content was measured spectrophotometrically at 401 nm wavelength and total phenolics were measured at 760 nm, respectively. The total tannins were analyzed using the titration of permanganometry method[10,11,12.]

2.4. Antioxidant activity

All prepared extracts from C.*hastata* aerial parts were evaluated for their antioxidant activity by the DPPH radical-scavenging method. Each dried 0.1 g of extract was dissolved in 10 mL of methanol, and diluted at concentrations up to 400, 200, 100, and 50 µg/mL. 1.5 mL methanol was added to each solution. After that, 1.5 mL of 0.1 mM DPPH solution, prepared by dissolving 4 mg of DPPH in 100 ml of methanol, was added and gently mixed for 30 min at 20° C.

The inhibition activity was determined by measuring the optical density at 517 (UV/Vis-1700 Macy, China)

and expressed as the sample concentration required to scavenge 50% of the DPPH free radicals (IC50). All samples were analyzed in triplicate, with rutin (40, 20, 10, and 5 μg/mL) used as a positive control[13].

2.5. Antibacterial activity

The antibacterial activity was determined by the agar diffusion method against four test organisms: Gram-positive *(Bacillus subtillis)*, Gram-negative (*Escherichia coli*) bacteria, and reference organisms (*fungi Penicillium and Aspergillus niger*). DMSO 0.1% was used as a control to test antibacterial activity. Test solutions were prepared by dissolving the extracts of C.*hastata* in 0.1% DMSO at a concentration of 100μg/ml. Pure bacterial cultures were inoculated into four wells of a Petri dish and incubated at 37o C for 48 hours. Then, the 40%, 70%, and 95% extracts were dissolved in DMSO, and each 75 μL samples were added to each well and incubated for another 24 hours. After incubation, zones of the growth inhibition are measured. The presence of antimicrobial activity is indicated by the absence of bacterial growth directly below the test sample[14].

2.6. Statistical analysis

Statistical analysis was performed using the SPSS25 program. Each measurement was performed with 3 repetitions and the results were obtained as mean ± standard deviation (SD).

RESULTS

3.1 Qualitative analysis of flavonoids, phenolic compounds, and tannins in *Cacalia hastata* L.

In the thin layer chromatograms of *Cacalia hastata* L. extracts, a brown-yellow spot was detected at the same level as the standard substance rutin (Rf=0.41), and a dark blue spot was detected at the same level as the standard substance gallic acid (Rf=0.86).

A tannins identification reaction was carried out on extracts, gelatinate was formed with 10% gelatin solution, and a dark green color with ferric ammonium slag revealed the presence of a condensable tannin.

3.2. Quantitative analysis of total flavonoids, phenolic content, and tannins in plant extracts

The total flavonoid content of the aerial parts of *Cacalia hastata* L. was measured spectrophotometrically at 401 nm and rutin was used as a standard for the calibration curve. The total phenolic content was determined by measuring the absorbance of the sample solution at 760 nm and comparing it with a calibration curve using gallic acid as a standard. The total flavonoid, phenolic content, and tannins of each extract are shown in Table 1.

Table 1. Results of total flavonoids, phenols and tannins of extracts from *Cacalia hastata* L.

Extracts	Total flavonoids (%)	Total phenols (%)	Total tannins (%)
40% ethanol extract	4.61±6.1	4.17±0.7	7.4±0.25
70% ethanol extract	5.1±6.43	4.92 ±1.5	5.1±1.1
95% ethanol extract	4.13±2.0	3.87±0.3	3.04±0.5

3.3. Antioxidant activity of *Cacalia hastata* L.

The antioxidant activity of extracts of Cacalia hastata L. was screened by the DPPH radical scavenging method with rutin as a positive control ($IC_{50,}$ 17±0.27) (Table 2).

Table 2. DPPH radical scavenging activities of extracts of *Cacalia hastata L.*

Extracts	Dilution	Inhibition (%)	IC_{50} (μg/mL)
40% ethanol extract	50	50.47±0.01	48.5±0.27
	100	61.24±0.43	
	200	87.67±0.04	
	400	89.53±0.006	
70% ethanol extract	50	46.32±0.01	74.8±0.02
	100	50.36±0.43	
	200	71.92±0.9	
	400	89.33±0.14	
95% ethanol extract	50	49.84±0.71	51.2±0.37
	100	68.81±0.02	
	200	86.22±0.35	
	400	90.36±0.04	

As a result, extracts of the aerial parts of *Cacalia hastata* L. were tested for their DPPH radical scavenging activity, and all ethanol extracts displayed antioxidant activity. Of them, the 40% ethanol extract exhibited good activity (IC_{50} 48.5±0.27μg/ml) compared to rutin as a positive control (IC_{50} 17 ±0.27 μg/ml).

3.5. Antibacterial activity of *Cacalia hastata* L.

The antibacterial activity of the extracts of *Cacalia hastata* L. was determined by the agar diffusion method, and bacterial cultures were prepared from standard strains of Gram-positive Bacillus subtillis, fungi Penicillium, Aspergillus niger, and Gram-negative *Escherichia coli*. As a result of the study, sample extracts were not active against fungi and Gram-negative bacteria (Figure 1. A, B, D). In contrast, in Gram-positive bacteria, the 70% extract inhibited bacterial growth in 4 mm, while the 40% extract in 3 mm diameter inhibition zone (Figure 1).

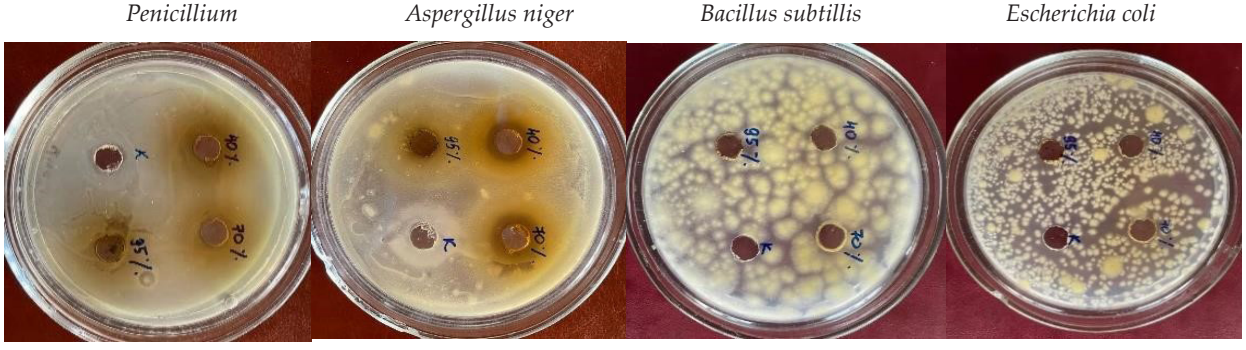

Figure 1. Antibacterial activity of *Cacalia hastata* L. extracts

According to these results, the 70% and 40% extracts were effective against Gram-positive, fast-growing, aerobic bacteria *Bacillus subtillis*, indicating that the extract of *Cacalia hastata* L. has antibacterial activity. Rarely, *B. subtillis* cause infections in humans, mainly being seen in patients with epticemia, pneumonia, endocarditis, wound infection, and intraocular inflammation, as well as meningitis diseases or an immunocompromised state[15].

DISCUSSION

According to Tankhaeva's study, the phenolic compound content in the 70% ethanol extract of *Cacalia hastata* L., leaves was found to be 3.34 ± 1.34%2. In our study, the total phenolic content for the 70%, 40%, and 95% ethanol extracts was determined as 4.92 ± 1.5%, 4.17 ± 0.7%, and 3.87 ± 0.3%, respectively. which indicated that the results were previous study.

The study showed that the total flavonoids in the 70% ethanol extract were 5.1 ± 6.43%, which was noticeably higher than the Lobanova I. et al. study, which determined the total amount of flavonoids in the same extract as 2.32 ± 0.08%5. This difference may be attributed to variations in extraction methods, plant material, geographical distribution, harvesting year, time of harvest, or environmental conditions.

Phenolics exhibit a wide range of biological activities, including antioxidant, antimutagenic, and anticancer effects, and the ability to modify gene expression16. Botoeva et al. studied the antioxidant activity of the chloroform extract of *Cacalia hastata* L., which showed an IC50 of 37.84 ± 1.11 μg/ml, and the *n*-butanol extract had an IC50 of 15.9 μg/ml17. In contrast, our study found that the IC50 values for 40%, 70%, and 95% ethanol extracts ranged from 48.5±0.27, 74.8±0.02 and 51.2 ± 0.37 μg/ml.

Phenolic compounds have been reported to inhibit the biosynthesis of nucleic acids and other metabolic processes in microorganisms18. Another reason for the strong antibacterial activity of flavonoids is their effect on the permeability of biological membranes[19].

Conflicts of Interest Statement: The authors declare that they have no conflicts of interest.

CONCLUSION

In this study, the 70% ethanol extract of *Cacalia hastata* L. contained higher levels of phenolic compounds compared to the other extracts. The different ethanol extracts exhibited the highest DPPH radical scavenging activity, with IC50 values ranging from 48.5±0.27 to 74.8±0.02. Research on the aerial parts of *Cacalia hastata* L. revealed that both the 70% and 40% ethanol extracts were effective against *Bacillus subtilis*, demonstrating the plant's antibacterial activity. These significant antioxidant and antibacterial effects may be attributed to the total phenolic and flavonoid contents of the extract. The presence of these compounds in *Cacalia hastata* L. suggests that the plant could have beneficial effects, warranting further investigation in future studies.

REFERENCES

1. Jambaninj D, Sulaiman S. A. S, Gillani S. W, et al. Technological study of preparing gel from the semi-solid extract of *Cacalia hastata* L. *J* Adv Pharm Technol *Res.* 2012; 3(1): 25–29.
2. Оленников Д.Н, Танхаева Л.М. Фенольные соединения листьев *Cacalia Hastata* L их количественный анализ. Химия растительного сырья. 2011; (3):143-148.
3. Ayushieva S. R, Razuvaevala G, Olennikov D.N, Lonshakova K.S. Phytofilm Khastaplen use in case of experimental parodontitismodelling. Stomatologiia. 2009; 88(1):14-16.
4. Volodiya Ts. Gastroprotector activity of herbal preparations. Thesis of dissertation for Sc.D in veterinary medicine. Ulaanbaatar: Mongolia; 2002:10-21
5. Lobanova I, Filippova E, Kotsupiy O, Protsenko M, Kharina T, Mazurkova N.*Cacalia hastata* L. anti-influenza activity and phytochemical characteristics of the extract: Northern Asia Plant Diversity; BIO Web of Conferences. 2021; (38):00073
6. Gubanov I.A. Conspectus on Mongolian Flora (vascular plants). Moscow:Valang Press: 1996:100.
7. Sanchir Ch, Batkhuu J, Boldsaikhan B, Komatsu K. Illustrated Guide of Mongolian Useful Plants. Ulaanbaatar: Admon Printing; 2003; (1):36.
8. Ligaa U, Davaasuren B, Ninjil N. Medicinal Plants of Mongolia Used in Western and Eastern Medicine. Ulaanbaatar: JCK Printing; 2005:349.
9. Medicinal plants in Mongolia. Switzerland: WHO Press; 2013:26-27.
10. Pharmacopoeia of the People's Republic of Chine. Vol 1. Chine medical Sciense Press; 2010:234-235.
11. National pharmacopoeia of Mongolia first edition. Ulaanbaatar; 2011:520-525,568,598.
12. Skinner W.W. Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists. Minneapolis, 1936:196.
13. Mensor L. L, Menezes F. S, Leitao G. G., Reis A. S, Dos Santos T. C, Coube C. S, Leitao S. G. Phytother. R*es.* 2001;(15):127−130.
14. Balouiri M, Sadiki M, Ibnsouda S.K. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;(6): 71-79.
15. Tokano M,Tarumoto N,mai K,Sakai ,Maeda T,Kawamura T, Seo K, Takahashi K, Yamamoto T, Maesaki S. Bacterial Meningitis Caused by Bacillus subtilis var.natto. Intern Med. 2022;62(13):1989–1993.
16. Jin Dai, Russell J. Mumper. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 2010;(15):7313-7352.
17. Ботоева Е.А, Оленников Д.Н, Танхаева Л.М, Убеева И.П.Антиоксидантная активность сухих экстрактов *Cacalia hastata*, Orthilia secunda и Panzerina lanata. Вестник Бурятского госуниверситета. 2010;(12): 53.
18. Havsteen B. Flavonoids. A class of natural products of high pharmacological potency. Biochem Pharmacol. *1983;32(7):1141–1148.*
19. Baba SA, Malik SA. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. J Taibah Univ Sci. 2015;9(4):449–454.