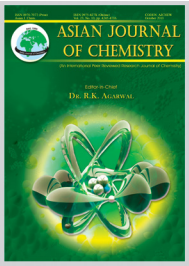
Asian Journal of Chemistry; Vol. 24, No. 7 (2012), 2961-2964

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



# Phytochemical Investigation and Antimicrobial Activity of *Agastache rugosa* Growing in Xinjiang, China

H.Y. GONG[1,2,3], J.B. DING[2], M. ZHU[2], H.B. LIU[3], S.G. TIAN[3] and X.M. MA[1,2,*]

[1]Traditional Chinese Medicine Hospital Affiliated to Xinjiang Medical University, Xinjiang State Research Center of Traditional Chinese Medicine, Urumqi 830000, Xinjiang, P.R. China
[2]College of Basic Medicine, Xinjiang Medical University, Urumqi-830011, Xinjiang, P.R. China
[3]Xinjiang Key Laboratory of Famous Prescription and Science of Formulas, Urumqi-830011, Xinjiang, P.R. China

*Corresponding author: Fax: +86 09914365192; Tel: +86 15981757393; E-mail:gonghaiyan1217@sina.com

(*Received*: 27 June 2011; *Accepted*: 6 February 2012) AJC-11042

It is reported that *Agastache rugosa* from Xinjiang of China has great biological/medicinal values. In this study, the phytochemistry and antimicrobial activity in different parts of *A. rugosa* growing in Xinjiang, China were investigated for the first time. Water, methanol, ethanol, chloroform and petroleum ether extracts of flower, stem, leaf from *A. rugosa* were prepared with successive extraction by the method of ultrasound. The detection of various phytochemicals of each extract was studied under standard methods. It was found that flower, stem, leaf parts have the same chemical constituents simultaneously, but have different chemical constituents. The essential oil of *A. rugosa* was extracted by the method of hydrodistillation. It showed strong inhibition against *Staphylococcus aureus, Escherichia coli* of essential oil from flower part; strong inhibition against *Escherichia coli, Blastomyces albicans* of essential oil from leaf part, and low activity against *Blastomyces albicans* from flower and leaf parts.

**Key Words: *Agastache rugosa*, Phytochemical investigation, Antimicrobial activity.**

## INTRODUCTION

*Agastache rugosa* (Fisch. et Mey), a Agastache genus and *A. rugosa* species perennial herb, which belongs to the Lamiaceae family and widely distributed in the fields of Xinjiang in China but cultivated as a medicinal plant[1]. Some of the medical uses for the plant include using it as a treatment for people suffering from anxiety, nausea, bacterial infections, or gas. The herb is known under many different names, such as Korean mint, purple giant hyssop, Indian mint and the wrinkled giant hyssop. The plant is used for ornamental as well as medicinal purposes. It is one of the 50 fundamental herbs in Chinese herbology. As one of the 50 fundamental herbs, *A. rugosa* is known as huò xiang. *A. rugosa* is reported to have antifungal activity, antibacterial activity, carminative and antipyretic properties[2]. Carminatives are substances that reduce the amount of gas produced in the gastrointestinal system and can also prevent flare-ups of acid reflux disease. Earlier studies indicated that components of *A. rugosa* have various pharmacological actions such as antioxidant activity[3] and anti-HIV integrase actions[4]. *A. rugosa* has been used as a wild vegetable and as herbal drug in traditional therapies. The plant has also been used a fungicide to prevent fungus from growing on potato crops.

Essential oils are natural compounds, which show great promise as a new prototype from which antifungal agents may be developed[5-8]. In a previous report, essential oil of *A. rugosa* has antifungal activity[9,10]. To our best of knowledge, phytochemical investigation and evaluation of antimicrobial activity for flower and leaf parts of *A. rugosa* (Fisch. et Mey) in Xinjiang of China has not been reported.

In the present study, we investigated whether there is antimicrobial activity diversity between in two different parts of *A. rugosa*. At the same time, we initiated this study to phytochemical characteristics of different parts of *A. rugosa* from Xinjiang and reported the results.

## EXPERIMENTAL

The aerial parts of *A. rugosa* were collected in October 2010, locally from the Liyu mountain of Xinjiang Province, China. The voucher specimen was identified by Yonghe Li, a chief apothecary of the Chinese Medicine Hospital of Xinjiang, is photographed, recorded and, after being displayed, is dried and accessioned into the herbarium of Traditional Chinese Medicine Ethnical Herbs Specimen Museum of Xinjiang Medical University. The voucher specimen number: 2010-352.

TABLE-1
PHYTOCHEMICAL TESTS OF THE SUCCESSIVE EXTRACTS OF *Agastache rugosa* STEM

| Chemical constituents | Aqueous extraction | Ethanol extraction | Methanol extraction | Chloroform extraction | Petroleum ether extraction |
|---|---|---|---|---|---|
| Proteins and amino acids | - | - | - | - | - |
| Saponins | + | - | - | + | + |
| Carbohydrates | - | - | + | + | - |
| Phenols and tannins | + | + | + | - | - |
| Flavonoids | + | - | + | + | - |
| Alkaloids | - | - | - | + | + |
| Organic acids | + | + | + | + | - |
| Steroids | + | - | + | + | - |
| Coumarins and lactones | - | - | - | + | + |
| Cardiac glycosides | - | - | - | - | - |
| Anthraquinones | - | - | - | - | - |
| Volatile oil | + | + | - | - | - |
| Fats | - | - | + | + | + |
| Glycosides or polysaccharide | + | - | - | + | + |

Solvents, namely, petroleum ether, chloroform, ethanol (95 %), methanol and reagents, namely, ammonia, iodine, ferric chloride, acetic acid, nitric acid, sulfuric acid, silicowolframic acid, hydrochloric acid, bromocresol green, α-naphthol, ninhydrin, gelatin and so on, were purchased from Tianjin Fu-Yu Meticulous Chemical Reagent Company, China.

**Test organisms:** Organisms such as *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) were used for study. The organisms were maintained by serial subculturing every month on nutrient agar slants and incubating at 37 ºC for 18-24 h. The cultures were stored under refrigerated condition. The antifungal activity of the oil was tested against *Blastomyces albicans* (ATCC 10231).

Penicillin (Zhongnuo Pharmaceutical Institute Company, H13021634) was served as positive control to determine the sensitivity of *Staphylococcus aureus* tested. Gentamycin Sulfate Injection (Zhenzhou Linrui Pharmaceutical Co. Ltd, H41020318) was served as positive control to determine the sensitivity of *Escherichia coli* tested. Fluconazole (Tianjin Pharmaceutical Group Xinzheng Co. Ltd, 100108) was served as positive control to determine the sensitivity of *Blastomyces albicans* tested.

**Preparation of the extracts:** The preliminary phytochemical screening was carried out on water, methanol, ethanol, chloroform and petroleum ether extracts of flower, stem, leaf parts from *A. rugosa* for the detection of various phytochemicals. Tests for common phytochemicals were carried out by standard methods[11].

**Extraction of essential oils:** Dried leaf and flower (200 g) were separately submitted to hydro-distillation in a Clevenger-type apparatus for 6 h[12]. At the end of each distillation the oils were collected, dried with anhydrous sodium sulfate prior to analyses, measured and transferred to glass flasks and stored at 4 ºC.

**Detection method:**

**Test for antibacterial activity:** Antibacterial activity of essential oils from leaf and flower parts were studied against two bacterial strains *viz*. *Staphylococcus aureus* and *Escherichia coli*. The inhibition effect of essential oils from leaf and flower parts on bacterial growth was determined, a macrodilution broth susceptibility assay was used, as recommended by NCCLS (1999)[13] and described in experiment technique of medical microbiology[14]. The oils were added aseptically to sterile melted Mueller Hinton Broth medium to produce the concentration range of 0.336-0.00525 mg/mL for flower essential oil, range of 0.302-0.00472 mg/mL for leaf essential oil. For the determination of MIC (Minimum Inhibitory Concentration), standard reference antibiotics (penicillin and gentamycin) were used as positive control.

All tests were performed in Mueller Hinton Broth and performed in triplicate.

**Test for antifungal activity:** The antifungal activity of the oils against fungal isolates (*Blastomyces albicans*) was evaluated using the broth dilution method. The oils were added aseptically to sterile melted Sabouraud's Broth medium to produce the concentration range of 0.336-0.00525 mg/mL for flower essential oil, range of 0.302-0.00472 mg/mL for leaf essential oil. Fluconazole was used as a reference antifungal drug. MIC values were determined as the lowest concentration of the essential oils were absence of growth was recorded. Each test in this study was repeated triplicate and performed in Sabouraud's Broth.

## RESULTS AND DISCUSSION

**Phytochemical investigation:** The successive extracts of water, methanol, ethanol, chloroform and petroleum ether extracts by the method of ultrasound were subject to various chemical tests for the identification of the phytoconsituents. The results are showed in Tables 1-3.

It was found that flower, stem, leaf parts have followed chemical constituents simultaneously: the water extract contained saponins, glycosides; the ethanol extract contained phenols, tannins, flavonoids, steroids; the chloroform extract contained saponins, carbohydrates, organic acids, steroids; the petroleum ether extract contained saponins, fats. It was found that flower,stem, leaf parts have the same chemical constituents simultaneously, while have different chemical constituents.

**Antimicrobial activity:** The hydrodistillition of essential oils from the dried flower and leaf gave oils in 0.29 % and 0.57 % (w/w) yields, based on the dry weight of the plant, respectively. The *in vitro* antibacterial activities of essential oils from leaf and flower parts of *A. rugosa* against the microorganisms were qualitatively and quantitatively assessed by the MIC values.

TABLE-2
PHYTOCHEMICAL TESTS OF THE SUCCESSIVE EXTRACTS OF *Agastache rugosa* FLOWER

| Chemical constituents | Aqueous extraction | Ethanol extraction | Methanol extraction | Chloroform extraction | Petroleum ether extraction |
|---|---|---|---|---|---|
| Proteins and amino acids | - | - | + | - | - |
| Saponins | + | + | + | + | + |
| Carbohydrates | - | - | - | + | - |
| Phenols and tannins | - | - | + | - | - |
| Flavonoids | - | - | + | - | - |
| Alkaloids | - | - | - | - | + |
| Organic acids | - | - | + | + | - |
| Steroids | - | + | + | + | - |
| Coumarins and lactones | - | + | + | - | - |
| Cardiac glycosides | - | - | - | - | - |
| Anthraquinones | - | - | - | - | - |
| Volatile oil | + | - | - | - | - |
| Fats | - | + | + | + | + |
| Glycosides or polysaccharide | + | + | + | - | - |

TABLE-3
PHYTOCHEMICAL TESTS OF THE SUCCESSIVE EXTRACTS OF *Agastache rugosa* LEAF

| Chemical constituents | Aqueous extraction | Ethanol extraction | Methanol extraction | Chloroform extraction | Petroleum ether extraction |
|---|---|---|---|---|---|
| Proteins and amino acids | + | - | - | - | - |
| Saponins | + | + | + | + | + |
| Carbohydrates | + | + | + | + | - |
| Phenols and tannins | - | + | + | - | - |
| Flavonoids | - | + | + | + | - |
| Alkaloids | + | + | - | - | - |
| Organic acids | + | - | - | + | + |
| Steroids | + | + | + | + | + |
| Coumarins and lactones | + | + | + | - | - |
| Cardiac glycosides | - | - | - | - | - |
| Anthraquinones | - | - | - | - | - |
| Volatile oil | - | - | + | + | - |
| Fats | + | + | - | - | + |
| Glycosides or polysaccharide | + | - | - | - | + |

According to the results given in Table-4, the MIC values for antimicrobial activity that were sensitive to the essential oils of leaf and flower parts were in the range of 0.0094-0.0378 mg $mL^{-1}$, 0.021-0.042 mg $mL^{-1}$. As can be seen in Table-4, the essential oils from leaf and flower parts of *A. rugosa* were found to have moderate to high antimicrobial activity. It showed strong inhibition against *Staphylococcus aureus* and *Escherichia coli* of flower part; strong inhibition against *Escherichia coli* and *Blastomyces albicans* of leaf part. And low activity against *Blastomyces albicans* from flower and leaf part. The results of MIC values indicated that the oils inhibited all microorganisms tested. The gram-negative bacteria (*Escherichia coli*) was more sensitive to the leaf part oil than gram-positive bacteria (*Staphylococcus aureus*). The lowest MIC value is 0.0094 mg $mL^{-1}$.

TABLE-4
ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS FROM LEAF AND FLOWER PARTS OF *Agastache rugosa*

| Organisms | MIC[a] (minimum inhibitory concentration) | |
|---|---|---|
| | Leaf part | Flower part |
| *Staphylococcus aureus* | 0.0378 | 0.021 |
| *Escherichia coli* | 0.0094 | 0.021 |
| *Blastomyces albicans* | 0.0378 | 0.042 |

[a]Values given as mg $mL^{-1}$

The oils showed fungicidal activity against *Blastomyces albicans*, the oil of leaf part was sensitive than the flower part. *Blastomyces albicans* exist in mucous membrane of oral cavity, upper respiratory, intestinal tract, vagina *etc*. Meanwhile, *Staphylococcus aureus* and *Escherichia coli*. exists in upper respiratory and food, large intestine, respectively. So the essential oils could be developed as an antimicrobial agent in food industry. Identification of the active composition of the essential oils and its mode of action are required for future drug development.

When the oils was added to the culture medium, the growth rates of tested organisms were found to significantly decrease as compared to the control cultures. In some cases, the oils showed the same type of antimicrobial activity compared to penicillin, while, the oils showed high activity in some other cases than the standard reference antibiotics (Table-5).

TABLE-5
ANTIMICROBIAL ACTIVITY OF STANDARD ANTIBIOTICS

| Organisms | MIC[a] (minimum inhibitory concentration) | | |
|---|---|---|---|
| | Penicillin | Gentamycin | Fluconazole |
| *Staphylococcus aureus* | 0.03 | | |
| *Escherichia coli* | | 0.1 | |
| *Blastomyces albicans* | | | 25.00 |

[a]Values given as mg $mL^{-1}$

With the GC-MS analysis, we can investigation the active antimicrobial compositions of the flower and leaf parts from *A. rugosa*, Xinjiang, in the future research. Meanwhile, offer the clinical treatments and other uses of *A. rugosa*.

## ACKNOWLEDGEMENTS

Xinjiang Uygur Autonomous Region Education Department Project (Grant No. XJEDU 2008I17); Xinjiang Autonomous Region Nature Funding (Grant No. 200821129).

## REFERENCES

1. M. Hudaberdi and X.L. Pan, Introduction of *Agastache rugosa* (Fisch. et Mey) of Xinjiang (4th of Flora Xinjiangensis), Xinjiang Science and Technology Publishing House Publ, Xinjiang, pp. 237-239 (2004).
2. Uighur Medicine Standard (upper volume), Xinjiang Uygur Autonomous Region Health Department Edits, Xinjiang Health Science and Technology Publishing House Publ (K). Xinjiang, pp. 387-388 (1993).
3. H.M. Oh, Y.J. Kang, Y.S. Lee, M.K. Park, S.H. Kim, H.J. Kim, H.G. Seo, J.H. Lee and K.C. Chang, *J. Ethnopharmacol.*, **103**, 229 (2006).
4. H.K. Kim, H.K. Lee, C.G. Shin and H. Huh, *Arch. Pharm. Res.*, **22**, 520 (1999).
5. S.Y. Yoon, S.K. Eo, D.K. Lee and S.S. Han, *Arch. Pharm. Res.*, **6**, 438 (1994).
6. W.R. Bidlack, S.T. Omaye, M.S. Meskin and D. Topham, Phytochemicals as Bioactive Agents. Lancaster: Technomic Publishing Company, pp. 106-110 (2000).
7. M.L. Faleiro, M.G. Miguel, F. Ladeiro, F. Venâncio, R. Tavares, J.C. Brito, A.C. Figueiredo, J.G. Barroso and L.G. Pedro, *Lett. Appl. Microb*., **36**, 35 (2003).
8. S. Shin, *Pharm, Res*., **26**, 389 (2003).
9. S. Shin and C.A. Kang, *Lett. Appl. Microbiol.*, **36**, 111 (2003).
10. S. Shin, *Arch. Pharm. Res*., **27**, 295 (2004).
11. C.H. Xiao, Chemical of TCM (2nd), Shanghai Science and Technology Publishing House, Shanghai, pp. 595-597 (1996).
12. X.Y. Zhou, H.Y. Gong, T.H. Xu and S.G. Tian, *Phcog. Mag*., **6**, 278 (2010).
13. National Committee for Clinical Laboratory Standards, Performance Standards for Antibacterial Susceptibility Testing (9th International Supplement), M 100-S9 (1999).
14. Z.Y. Guan, A.L. Wang and J. Li, Experiment Technique of Medical Microbiology, Chemical Industry House, China, pp. 115-117 (2006).