Journal of Medicinal Plants Research Vol. 4(13), pp. 1269-1272, 4 July, 2010
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR09.388
ISSN 1996-0875 ©2010 Academic Journals

Full Length Research Paper

Phytochemical screening, antioxidant, antibacterial and cytotoxic activities of *Knema angustifolia* extracts

M. Phadungkit*, R. Rattarom and S. Rattana

Faculty of Pharmacy, Mahasarakham University, Kantarawichai, Mahasarakham 44150, Thailand.

Accepted 9 June, 2010

Knema angustifolia **Warb (Myristicaceae) is used in traditional Thai medicine as a whole body tonic agent. The current study sought to evaluate the antioxidant, antibacterial and cytotoxic activities of extracts from this herb and to screen their chemical constituents. Antioxidant, antibacterial and cytotoxic activities were determined by the DPPH scavenging method, the disk diffusion method and the colorimetric method, respectively. Phytochemical screening was performed using standard procedures. The results showed that the ethanol extract had high antioxidant activity with an EC_{50} value of 13.90 ± 1.35 µg/ml. The ethanol and the dichloromethane extracts were active against *Staphylococcus aureus.* The ethanol extract showed a strong cytotoxic activity against the lung cancer cell line with an IC50 value of 4.55 ± 4.60 µg /ml. Phytochemical screening of the extracts showed the presence of condensed tannins, phenolic compounds and triterpenes. In conclusion, the apparent antioxidant and cytotoxic activities of *K. angustifolia* suggest its potential usefulness in the prevention of cancer and other diseases.**

Key words: *Knema angustifolia*, antioxidant, antibacterial, cytotoxicity.

INTRODUCTION

Knema angustifolia Warb. (Myristicaceae) is a species of plant that is distributed widely in Thailand. It is known by the common name as “horse blood” because of the red resin exudated from its bark (Madalena et al., 1990). It is used in traditional Thai medicine as a whole body tonic agent (Chuakul et al., 2004). Other traditional uses include blood tonic and anticancer (Madalena et al., 1990). Despite its common uses in traditional Thai medicine, there has been minimal investigation of its chemical constituents and biological activities.

However, it was reported that the plant from the same genus (*Knema laurina*) is being used to treat inflamed wounds and rheumatism (Ines, 2009). Previous phytochemical studies revealed resorcinols and anacardic acids (6-alkenylsalicylate) in the stem bark of *K. laurina* (Gonzales et al., 1996; Kijjoa et al., 1991). The aims of the current investigation were to screen the chemical constituents and to test the free radical scavenging, antibacterial and cytotoxic activities of the ethanol and dichloromethane extracts of this herb.

*Corresponding author. E-mail: phadang_p@hotmail.com. Tel/Fax: 664-37-54360.

MATERIALS AND METHODS

Plant material

The plant sample (stem) of *K. angustifolia* was collected from the Nongkhai province of Thailand and identified by one of the authors (M. Phadungkit). A voucher specimen has been deposited in the Herbarium at the Faculty of Phamacy, Mahasarakham University, Thailand.

Preparation of the plant extracts

The sample was extracted with petroleum ether (defatting), dichloromethane and 95% ethanol, respectively by the maceration method. The solvents were evaporated by a rotary evaporator yielding the herbal extracts. The dichloromethane and the ethanol extracts were tested for biological activities.

Antioxidant testing by DPPH free radical scavenging assay

The test was carried out using the DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging assay. The free radical scavenging activity of the extracts and standard ascorbic acid solutions in absolute ethanol were determined based on their ability to react with the stable DPPH free radical (Yamasaki et al., 1994). A 750 µL aliquot of the extract (50 to 1000 µg/ml, dissolved in absolute ethanol) was added to 750 µL of DPPH in absolute

Table 1. Antioxidant activity of the herbal extracts (n=3).

	EC_{50}, µg/ml
Dichloromethane extract	42.25 ± 3.66
Ethanol extract	13.90 ± 1.35
Ascorbic acid	4.86 ± 0.89

ethanol (152 µM). After incubation at room temperature in the dark for 20 min, the absorbance of each solution was determined at 520 nm using a ultra-violet (UV) spectrophotometer. The results were expressed as a percentage of inhibition.

Percentage of inhibition (%) = [(Acontrol - Asample)/Acontrol] x 100.

The effective concentration of sample required to scavenge DPPH radical by 50% (EC50 value) was obtained by linear regression analysis of a dose-response curve plotting % inhibition versus concentration.

Antibacterial activity assay

The disk diffusion method was used to determine the antibacterial activity of the herbal extracts against *Staphylococcus aureus* ATTC 25923, *Escherichia coli* ATTC 25922 and *Pseudomonas aeruginosa* ATCC 27853. The test was performed in accordance with NCCLS guidelines with some modification (NCCLS, 2000). Briefly, 100 µl of a suspension containing 10^8 colony-forming units (CFU)/ml of bacteria were spread on Mueller-Hinton Agar (70 ml/plate). The disks (6 mm in diameter) were impregnated with 5 µl of 5 mg/ml crude extract (dissolved in absolute ethanol) and placed on the inoculated agar. Negative controls were prepared using absolute ethanol. Amoxycillin (10 µg /disk) was used as a positive reference standard. The inoculated plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the zone of inhibition (in mm) against the test organisms.

Cytotoxic activity assay

Cytotoxicity of the herbal extracts was measured against the human small cell lung cancer (NCI-H187) cell line using the colorimetric method described previously (Skehan et al., 1990). In brief, cells (5×10^3 cells/ml) were seeded on 96-well plates and cultured in a culture medium containing 10% fetal bovine serum at 37°C for 24 h. The medium was then replaced with serum-free medium containing the herbal extracts at various concentrations (0, 0.5, 5 and 50 /mL). After incubation for 24 h at 37°C under 5% CO_2, the supernatant was removed and the MTT solution (0.5 mg/mL) was added to each well at 4 h prior to the end of the experiment. The formazan crystals that had formed in viable cells were measured at 540 nm using a microplate reader. All experiments were done triplicate. The average data from the triplicates were expressed in terms of killing percentage relative to a negative control. The percentage of inhibition (%) of each of the test samples was calculated according to the following formula:

Percentage of inhibition (%) = [(Acontrol - Asample)/Acontrol] x 100

Cytotoxicity of each sample was expressed as IC50 value. The IC50 value is the concentration of the test compounds that cause 50% inhibition or cell death and was obtained by plotting the percentage inhibition versus concentration of the test compounds.

Phytochemical screening

The dichloromethane and ethanol extracts were freshly prepared and divided into different test tubes. Their various chemical constituents were determined according to the methods described by Trease and Evans (Trease et al., 1989). Classes of compound tested for phytochemical constituents included alkaloids, tannins, phenolics, triterpenes, steroids, flavonoids, saponins, cardiac glycosides and anthraquinones.

RESULTS AND DISCUSSION

Antioxidant activity

The antioxidant activity of the herbal extracts is shown in Table 1. The ethanol extract showed a potent activity while the dichloromethane extract showed a moderate activity when compared to the standard ascorbic acid. Antioxidants act by donating hydrogen to free radical molecules which damage cells and play a role in numberous diseases (Jayaprakasha, 2004). Antioxidants are also thought to protect against coronary heart diseases, skin aging, diabetes and Alzheimer's disease (Cornelli, 2009). Previous studies have shown that potent antioxidants can prevent cancer (Blot et al., 1993; Omenn et al., 1994). Therefore, *K. angustifolia* may warrant further investigation for its ability to promote good health.

Antibacterial activity assay

The antibacterial activity of the herbal extracts, indicated by the size of their zones of inhibition, is summarized in Table 2. Both herbal extracts inhibited only the gram positive bacterium *S. aureus*. The greatest antibacterial activity was detected from the ethanol extract. None of the herbal extracts examined showed antibacterial against *E. coli* or *P. aeruginosa* (gram negative bacteria). These results correlate well with the previous studies which reported that the herbal extracts have a greater activity against gram positive bacteria than gram negative bacteria (Kabuki et al., 2000).This tendency could be explained by the fact that gram negative bacteria possess an outer membrane surrounding the cell wall, which restricts diffusion of bioactive compounds due to the presence of lipopolysaccharide (Burt, 2004).

Cytotoxicity assay

The ethanol extract was found to have a high cytotoxicity with an IC50 value of 4.55 ± 4.60 µg/ml while the dichloromethane extract appeared to be inactive (IC50 >50 µg /ml). The results suggested that polar compounds such as tannins and phenolic compounds play an important role in cytotoxic activity. Previous study showed that phenolic compounds exhibited the cytotoxic activity against human cell lines (Laphookhieo, 2009). In

Table 2. Antibacterial activity of the herbal extracts (n=3).

	Zone of inhibition, mm		
	***S. aureus* ATTC 25923**	***E. coli* ATTC 25922**	***P. aeruginosa* ATCC 27853**
Dichloromethane extract	7.56 ± 0.40	-	-
Ethanol extract	10.67 ± 0.57	-	-
Amoxycillin 10 µg /disk	10.67 ± 0.57	11.83 ± 0.76	6.67 ± 0.57

Table 3. Phytochemical constituents of *K. angustifolia*.

	Dichloromethane extract	Ethanol extract
Alkaloids	-	-
Condensed tannins	+	+ +
Phenolic compounds	+	+ +
Triterpenes	+	+
Steroids	-	-
Flavonoids	-	-
Saponins	-	-
Cardiac glycosides	-	-
Anthraquinones	-	-

- Absent, + small quantities present, + + large quantities present.

addition, the results confirm the potential use of this plant for the treatment of cancer in traditional medicine reported by Madalena et al. (1990).

Phytochemical screening

The results of the phytoscreening assay on the crude extracts are shown in Table 3. Both crude extracts showed the presence of condensed tannins, phenolic compounds and triterpenes but absence of steroids, flavonoids, saponins, cardiac glycoside and anthraquinones. The detected compounds could be responsible for the biological activities. Previous studies indicated that phenolic compounds in herbal medicines can scavenge free radicals (Hsia, 2005). Tannins and phenolic compounds were reported to be potent antibacterial agents (Panizzib, 2002).

Conclusion

The present study indicates that the ethanol extract of *K. angustifolia* posseses the potent free radical scavenging activity, the potent cytotoxic activity against the human small cell lung cancer (NCI-H187) cell line and the moderate antibacterial activity against *S. aureus.* The phytochemical screening demonstrated the presence of different types of compounds including condensed tannins, phenolic compounds and triterpenes which could be responsible for the biological activities. In conclusion, K. *angustifolia* warrants further investigation for its ability to protect against cancer and other diseases.

REFERENCES

Blot W, Li J, Taylor P (1993). Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J. Nat. Cancer Inst. 85: 1483-1491.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods: A review. Int. J. Food Microbiol., 94: 223-253.

Chuakul W, Boonpleng M (2004). Survey on medicinal plants in Ubon Ratchathani province (Thailand). Thai. J. Phytopharm,. 11: 33-54.

Cornelli U (2009). Antioxidant use in nutraceuticals. Clin. Dermatol., 27: 175-194.

Gonzales J, DeOliveira C, Fernandes J, Kijjoa A, Herz W (1996). Further alkyl and alkenylphenols of *Knema laurina* and *Knema austrosiamensis*: location of the double bond in the alkenyl side chains. Phytochem., 43: 1333-1337.

Hsia F, Jer Y, Chao L, Fang R, Yang C, Chin C (2005). Antioxidant activity, cytotoxicity, and DNA information of *Glossogyne tenuifolia*. J. Agric. Food Chem., 53: 6117-6125.

Ines H, Silvia S, Jens N, Katrin F, Katrin P, Klaus R, Ghazally I, Laily D,Ikram MS, Latiff A, Luder W, Frauke Z, Oliver U (2009). Neuroprotection and enhanced neurogenesis by extract from the tropical plant *Knema laurina* after inflammatory damage in living brain tissue. J. Neuroimmunol., 206: 91-99.

Jayaprakasha G, Jaganmohan L, Sakariah K (2004). Antioxidant activities of flavidin in different *in vitro* model systems. Bio. Med. Chem. Lett., 12: 5141-5146.

Kabuki T, Nakajima H, Arai M, Ueda S, Kuwabara Y, Dosako S (2000). Characterization of novel antimicrobial compounds from mango (*Mangifera indica* L) kernel seeds. Food Chem., 71: 61-66.

Kijjoa A, Gonzalez M, Pinto M, Monanondra I, Herz W (1991). Constituents of *Knema laurina* and *Knema tenuinervia*. Planta. Med., 57: 575-577.

Laphookhieo S, Maneerat W, Koysomboon S (2009). Antimalarial and cytotoxic phenolic compounds from *Cratoxylum maingayi* and *Ratoxylum cochinchinense*. Mol., 14: 1389-1395.

Madalena P (1990). Chemical study of *Knema* species from Thailand. Quim. Nova., 13: 243-244.

NCCLS (2000). Performance standards for antimicrobial disk susceptibility tests: approved standard, 7th ed. NCCLS, Wayne, Pa. M2-A7.

Omenn GS, Goodman G, Thomquist M (1994). The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. Cancer Res., 54(7): 2038-2043.

Panizzib L, Caponi C, Catalanoa S, Cionia P, Morellia I (2002). *In vitro* antimicrobial activity of extracts and isolated constituents of *Rubus ulmifolius* J. Ethnopharm., 79: 165-168.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. J. Nat. Cancer Inst., 82: 1107-1112.

Trease GE, Evans WC (1978). A Textbook of Pharmacognosy. 11th ed. Bailliere Tindall Ltd, London p. 784.

Yamasaki K, Hashimoto A, Kokusenya Y, Miyamoto T, Sato T (1994). Electrochemical method for estimating the antioxidative effects of methanol extracts of crude drugs. Chem. Pharm. Bull., 42: 1663-1665.