Natural Product Sciences
7(1) : 9~12 (2001)

Broad Spectrum Anti-bacterial Activity of the Leaves, Stem and Root Barks of *Myristica subabulata*

M. R. Khan,* M. Kihara and A. D. Omoloso

Department of Applied Sciences, Papua New Guinea University of Technology
P.M.B. Lae, Papua New Guinea

Abstract – A number of *Myristica* species are used in herbal medicine for a variety of ailments. The methanol extracts of the leaves and the stem and root barks of *Myristica subabulata* were fractionated into petrol, dichloromethane and finally ethyl acetate. These were then screened against a total of 31 micro-organisms comprising of 13 G +ve, 12 G ve, one protozoa and 5 moulds. A broad-spectrum anti-bacterial activity was observed. As compared to fractionated the un-fractionated extracts exhibited much better level of activity.

Key words – *Myristica subabulata*, broad-spectrum antibacterial activity.

Introduction

In most of the third world countries medicinal plants are extensively used in local herbal medicine (Watt and Breyer-Brandwijk, 1962; Kokwaro, 1976; Mshigeni *et al.*, 1991). Due to some times non-availability and mostly high cost of health care in developing countries the positive role medicinal plants can play in providing primary health care in such countries can not be over emphasized (Akerele, 1988).

A number of *Myristica* species are used in local herbal medicines to treat diarrhoea, heart disease, rheumatism, malaria, sciatica, skin infections, internal pains, nose bleed, as diuretic, carminative, stimulant, antispasmodics and as an aphrodisiac (Perry, 1980).

Myristica fragrans is the commercial nutmeg produced mainly in Indonesia and the West Indies. *Myristica argentea* is called the New Guinea nutmeg and is the product of Papua New Guinea. Although *Myristica* nuts are of major commercial importance mainly used as flavoring, fragrance, spices and in pharmaceutical preparation (Smith and Anand, 1984), but there does not seems to be any major systematic biological study reported on the genus. *Myristica fragrans* is reported to have antibacterial activity against *Streptococcus mutans* (Hattori *et al.*, 1986), *Salmonella typhimurim* (Mahmoud *et al.*, 1992), *Staphylococcus aureus* and as an anti-diarrhoea agent used for pig calves (Perez, 1994). *M. argentae* was reported to be active against *Streptococcus mutans* (Stamford *et al.*, 1980) and *M. castaneifolia* demonstrated anti-diarrhoeal properties (Nakatani *et al.*, 1988). The major phytochemicals reported from various *Myristica* species are lignans and alkanones (Hattori *et al.*, 1986; Mahmoud *et al.*, 1992; Perez, 1994; Stamford *et al.*, 1980; Nakatani *et al.*, 1988; Ali *et al.*, 1993). In Papua New Guinea, *Myristica* is represented by 39 species and 9 varieties (Womersley, 1978).

In continuation to our studies on Papua New Guinea medicinal plants (Khan, 1998a; Khan, 1998b; Khan *et al.*, 1998), now we report the anti-microbial activity of the leaves and the stem and root barks of Myristica subabulata. As far as we are aware there is no report of biological activity or phytochemistry on this species.

Materials and Methods

Tested materials – The leaves and the stem and root barks of *Myristica subabulata* Miq. (Myristicaeae) were collected in June 1998 from Bulolo, Morobe Province, Papua New Guinea (PNG). The plant was identified at the Forestry Department, PNG University of Technology, Lae, where a voucher specimen number NGF 7492 is deposited.

Extraction – The dried plant material (20 gm) after pulverisation was extracted with methanol (200 ml) for 3 hours using Soxhlet apparatus. The solvent was removed under reduced pressure and the residue (**C**)

*Author for correspondence.

was suspended in water (50 ml) and successively extracted first with petroleum ether (**P**) (60-80°C) followed by dichloromethane (**D**) and finally ethyl acetate (**E**). The solvents were evaporated and the yields were recorded (Table 1). Phytochemical screening (Harborne, 1984) - *Leaves:* **P** (flavonoids / lignans, triterpenoids, sterols), **D** (flavonoids / lignans, saponins, tannins), **E** (flavonoids / lignans, triterpenoids, sterols). *Stem bark:* **P, D, E** (flavonoids / lignans, triterpenoids, sterols). *Root bark:* **P** (flavonoids / lignans, triterpenoids), **D** (flavonoids / lignans, triterpenoids, sterols), **E** (saponins, sterols).

Used microorganisms – These are given in Table 1 and were obtained from the stock culture of the Microbiology Laboratory of the Department of Applied Sciences in Lae. Cultures were maintained as nutrient agar slants in screw capped bottles and stored at 4°C. Cultures were prepared by transferring a loop full of bacteria from the stock culture into nutrient broth and incubated at 37°C for 24 h except for *Micrococcus roseus* and *Micrococcus lutea* that were incubated at 30°C for 24 h before use. All the bacteria were seeded into nutrient agar plate and incubated for 24 hours. Moulds were transferred into freshly prepared potatoes dextrose agar plates and incubated at 25°C for 3 days.

Anti-microbial activity – Anti-microbial activity was determined by slightly modified disc diffusion

Table 1. Antimicrobial activity of extractives from *Myristica subabulata*[a]

Microorganisms		Leaves				Stem bark				Root bark				Standard[b]			
		C	P	D	E	C	P	D	E	C	P	D	E	Chl	GM	SXT	PG
Bacillus cereus	G+	18	8	0	0	18	8	10	8	20	8	8	8	16	16	00	00
B. coagulans	G+	–	0	0	0	–	10	10	10	–	8	8	10	18	–	–	–
B. megatarium	G+	–	0	0	0	–	10	10	10	–	8	8	10	16	–	–	–
B. subtilis	G+	12	8	0	0	12	8	8	8	20	8	8	8	16	12	00	20
Lactobacillus casei	G+	–	0	8	8	–	8	8	8	–	8	8	8	18	–	–	–
Micrococcus luteus	G+	12	0	0	0	12	10	10	10	12	8	8	8	16	18	16	10
M. roseus	G+	12	8	8	0	12	10	8	10	12	12	8	12	06	16	00	00
Staphylococcus albus	G+	12	8	8	8	12	10	8	8	12	10	8	10	16	06	00	00
S. aureus	G+	12	0	0	0	12	8	8	10	12	8	10	10	18	18	16	04
S. epidermidis	G+	12	8	0	8	12	10	10	10	18	10	8	10	00	18	16	10
Streptococcus faecalis	G+	12	0	0	0	12	8	8	8	12	8	8	12	00	16	00	00
S. pneumoniae	G+	–	0	0	0	–	8	8	8	–	8	12	10	18	–	–	–
S. mutans	G+	–	8	10	14	–	8	12	12	–	12	8	12	18	–	–	–
Agrobacterium tumefaciens	G–	–	8	8	8	–	8	8	8	–	8	10	12	12	–	–	–
Citrobacter freundii	G–	–	0	0	0	–	10	10	10	–	10	10	12	16	–	–	–
Enterobacter aerogenes	G–	–	0	0	0	–	8	8	8	–	8	8	10	18	–	–	–
Escherichia coli	G–	10	8	8	8	12	8	10	10	10	8	8	12	18	18	08	08
Klebsiella pneumonia	G–	–	12	12	12	–	10	10	10	–	10	10	12	0	–	–	–
Neisseria gonorrhoeae	G–	–	10	10	16	–	14	10	14	–	14	10	16	18	–	–	–
Proteus mirabilis	G–	12	0	0	0	12	10	10	10	12	10	8	10	16	18	12	00
P. vulgaris	G–	18	8	0	0	12	8	10	10	12	8	10	12	18	–	–	–
Pseudomonas aeruginosa	G–	–	8	0	8	–	16	12	12	–	10	10	10	24	18	18	00
Salmonella typhi	G–	12	0	8	8	12	12	10	10	12	10	8	14	16	00	00	00
S. typhymurium	G–	12	0	0	0	12	12	10	12	18	10	10	12	16	08	08	00
Serratia marcescens	G–	–	0	0	0	–	8	8	8	–	8	12	12	18	20	18	00
Trichomonas vaginalis	Pz	18	0	0	0	18	10	10	10	18	10	12	12	16	16	00	00
																Gri	
Aspergillus niger	M	–	0	0	0	–	0	0	0	–	0	0	0			10	
Candida albican	M	–	0	0	0	–	0	0	0	–	0	0	0			10	
C. tropicalis	M	–	0	0	0	–	0	0	0	–	0	0	0			12	
Trichophyton mentagrophytes	M	–	0	0	0	–	0	0	0	–	0	0	0			10	
Yields %		16.6	2.6	1.4	1.0	12.4	4.4	1.6	0.8	10.5	0.4	1.0	0.4				

[a]Values are inhibition zone (mm). C, unfractionated ethanol extract; P, petrol (60-80°C) fraction; D, CH_2Cl_2 fraction; E, EtOAc fraction; (conc. 4 mg/disc), G, gram reaction of bacterium; Pz, protozoa; M, mould; –, not tested.

[b]Chl, chloramphenicol (10 μg disc Oxoid B42960), GM, gentamicine (10 μg disc 792947 DIFCO), PG penicillin G (2 units B44234 Oxoid), SXT, sulphamethoxale/trimethoprin (25 μg disc B44241 Oxoid) and Gri, griseofulvin (25 mg/disc).

methods techniques (Barry, 1976; Bauer *et al.*, 1966). The filter paper discs (6 mm) were impregnated with the concentrated extracts (4 mg per disc) and after drying were then placed on agar plates (90 mm) which has been previously seeded with cultures of the organisms. The plates with the organisms were incubated for 24 hours. Four standard antibiotics (chloramphenicol 10 µg disc Oxoid B42960, gentamicin 10 µg disc 792947 DIFCO, penicillin 2 unit B44234 oxoid, and sulphamethoxazole/trimethoprine 25 µg disc B44241) were used for comparison. Zone of inhibitions was measured to the nearest mm.

Results and Discussion

The anti-bacterial activities of the tested materials are shown in Table 1. A total of 31 microorganisms comprising of 13 G +ve, 12 G –ve, one protozoa and 5 moulds were screened against 3 un-fractionated crude extracts (**C**) and 9 fractionated extracts (**P, D, E**). The unfractionated extracts exhibited much better activity as compared to fractionated extracts, an uncommon observation but possible. The activities of the fractions **C** of the three parts were similar. There were no significant differences in the activities of various fractions of the root and stem barks and these were much superior to the leaf extracts which practically lost the activities on fractionation. Good level of activity was observed against *B. cereus* and *T. vaginalis* by all the **C** extracts. Strong activity was exhibited by the root bark **C** extract against *B. subtilis, S. epidermidis, S. typhymurium* and the leaves **C** extract against *P. vulgaris*. For any further anti-microbial study it is important to use the unfractionated crude extract for better activity. The activity exhibited by the three parts of the plant is broader and in some cases better than the standards used.

References

Akerele, O., Medicinal plants and primary health care: an agenda for action. *Fitoterapia* **LIX**, 355-363 (1988).

Ali, S., Read, R.W. and Sotheeswaran, S., Arylpropanoids from *Myristica castaneifolia* (Myristicaceae). *Australian J. Chemistry* **46**, 1421-1424 (1993).

Barry, A.C., in "Standard diffusion disc methods for antibiotic susceptibility of common rapid-growing bacterial pathogens." Park Press, Baltimore, USA, 1976, pp. 2-9.

Bauer, A.W., Kirby, W.M.M., Sherries, J.C., Truck, M., Antibiotic susceptibility testing by a standardized disc method. *Am. J Clin. Pathol.* **45**, 493 (1966).

Harborne, J.B., Phytochemical Methods. 2 ed., Chapman and Hall, London - New York, 1984, pp. 55, 84, 120.

Hattori, M., Hada, S., Watahiki, A., Ihara, H., Shu, Y., Kakiuchi, N., Mizuno, T. and Namba, T., Studies on dental caries prevention by traditional medicines. X. Anti-bacterial action of phenolic components from mace against *Streptococcus mutans. Chem. Pharm. Bull.* **34**, 3885-3893 (1986).

Khan, M.R., Anti-microbial activity of some *Diospyros* species. *Fitoterapia* **LXIX**, 471-474 (1998a).

Khan, M.R., Cytotoxicity assay of some *Bignoniaceae. Fitoterapia* **LXIX**, 538-541 (1998b).

Khan, M.R., Komine, K. and Omoloso, A.D., Antibacterial activity of some Annonaceae Part I. *Fitoterapia* **LXIX**, 367-369 (1998).

Khan, M.R., Komine, K. and Omoloso, A.D., Anti-microbial activity of *Uvaria cordata. Fitoterapia* **LXIX**, 177-179 (1998).

Kokwaro, J.O., Medicinal Plants of East Africa, East African Literature Bureau, Kampala, Nairobi, Dar es Salaam, 1976.

Mahmoud, I., Alkofahi, A. and Abdelaziz, A., Mutagenic and toxic activities of several spices and some Jordanian medicinal plants. *Int. J. Pharm.* **30**, 81-85 (1992).

Mshigeni, K.E., Nkunya, M.H.H., Fupi, V., Mahunnah, R.L.A. and Mshiu, E.N. (eds.) Traditional Medicinal Plants. Proceedings of International Conference, Arusha, Tanzania, February 18-23 (1990), 1991.

Nakatani, N., Ikeda, K., Kikuzaki, H., Kido, M. and Yamaguchi, Y., Diaryldimethylbutane lignans from *Myristica argentea* and their anti-bacterial action against *Streptococcus mutans. Phytochemistry* **27**, 3127-3129 (1988).

Perez, C. and Anesini, C., Anti-bacterial activity of alimentary plants against *Staphylococcus aureus* growth. *American J. Chinese Medicine* **22**, 169-174 (1994).

Perry, L.M., Medicinal Plants of East and South-East Asia, Attributed Properties and Uses. MIT Press, Cambridge, Massachusetts and London, 1980, pp. 279-280.

Smith, A.E. and Anand, N., The United Kingdom Markets for Cloves, nutmeg, Mace, cinnamon and Cassia. Tropical development Institute (ODA), London, 1984, pp. 15-16.

Stamford, I.F., Bennett, A. and Greenhalf, J., Treat-

ment of diarrhoea in calves with nutmeg. *Veterinary Record* **106**, 289 (1980).

Watt, J.M. and Breyer-Brandwijk, M.G.B., Medicinal and poisonous plants of Eastern and Southern Africa. 2nd Edition, E. and S. Livingston, Ltd. Edinburgh and London, 1962.

Womersley, J.S., Handbooks of the Flora of Papua New Guinea, Volume I. Melbourne University Press, 1978, pp. 181-215.

(Accepted December 15, 2000)