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ANTIMICROBIAL ACTIVITIES OF THE PETROLEUM ETHER, METHANOL AND ACETONE EXTRACTS OF *KAEMPFERIA GALANGA* L. RHIZOME

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Abstract

Crude extracts of the rhizome *Kaempferia galanga* L. with petroleum ether, acetone and methanol were screened for antimicrobial activities against two Gram-positive and three Gram-negative pathogenic bacteria *i.e. Bacillus* sp., *Pseudomonas* sp., *Escherichia coli, Salmonella* sp. and *Shigella sonnei*. The zones of inhibition produced by the crude extracts of *K. galanga* petroleum ether, acetone and methanol extracts against pathogenic bacteria were found between 0-20, 0, 0-15 and 6-11 mm respectively. Acetone extract showed no activity whereas methanol and petroleum ether extracts of the plant exhibited significant activity. Therefore, methanol and petroleum extract was further repeated to confirm the bioactive principles. The minimum inhibitory concentration (MIC) of methanol and petroleum extracts was measured against Gram-negative *i.e. E. coli, Salmonella* sp., *Shigella* sp., and Gram-positive *i.e. Bacillus* sp., *and Pseudomonas* sp., the values were found to be for between 2-16 µg/ml.

Key word: Plant extract, Antimicrobial, Bacteria, *Kaemperia galanga*

mvi ms‡¶c: *Kaempferia galanga* Gi ivBR‡gi †cUwjqvg B_vi, GwmUb Ges wg_vb‡j Acwi‡kwaZ wbhvm Øviv `BvU Mvg c‡RwUf Ges wZbwU Mvg wb‡MwUf e¨vK‡Uwiqv h_v, *Bacillus* sp., *Pseudomonas* sp., *Escherichia coli, Salmonella* sp. Ges *Shigella sonnei*- †`i Dci AYRxe cwZ‡ivaK Kvh¶gZv †`Lv nq| *K. galanga*-Gi †cUwjqvg B_vi, GwmUb Ges wg_vb‡j Acwi‡kwaZ wbhvm c¨v_‡RwbK e¨vK‡Uwiqvi Dci 0-20, 0-15 Ges 6-11 wgwgt Bmnwemb †Rvb Drcb K‡i| GwmU‡b Drcb wbhv‡mi †Kvb KvhKvixZv †`Lv hvq bv, Ab¨w`‡K wg_vbj Ges †cUwjqvg B_v‡i wbhv‡mi KvhKvixZv ˆewkócY †`Lv hvq| mZivs wg_vbj Ges †cUwjqvg B_v‡i cwß wbhv‡mi Kvh¶gZv wbY‡qi Rb¨ cbivq cix¶v Pvjv‡bv nq| wgwbgvg BbwnweUvix Kb‡mb‡Ukb (MIC)-Gi cwigvc Mvg wb‡MwUf e¨vK‡Uwiqv, *E. coli, Salmonella* sp., *Shigella* sp., Ges Mvg c‡RwUf e¨vK‡Uwiqv, *Bacillus* sp., *and Pseudomonas* sp.-‡Z 2-16 µg/wgwjUvi cvIqv hvq|

Introduction

Plants have provided a good source of anti-infective agents; emetine, quinine, and berberine remain highly effective instruments in the fight against microbial infections. Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases including opportunistic AIDS infections. With more than 25% of the pharmaceuticals in use today derived from natural products (Williams and Lemke, 2002; Rahman et al., 2004). Plants containing protoberberines and related alkaloids, picralima-type indole alkaloids and garcinia biflavonones have been found to be active against a wide variety of micro-organisms (Iwu et al., 1999). The overall quantities of plants used medically, in one way or another, are large. Plant based antimicrobials represent a vast untapped source for medicines. Continued and further exploration of plant antimicrobials needs to occur (Farnsworth et al., 1991). The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phytomedicines (Iwu et al., 1999).

Knowledge of the biological activities and or chemical constituents of medicinal plants are desirable not only for the discovery of new therapeutic agents, but also for information in discovering new sources of other economic materials (Khaleqzzaman et al., 2002). Phyto-chemical investigation of medicinal plants including biochemical tests of their crude extracts and isolate pure chemical compounds could provide such knowledge (Lewington, 1990). The scientists are giving top most priority in search of appropriate to combat the diseases. Natural products have been a major source of new drugs (Vuorelaa et al., 2004).

Kaemperia galanga L. (Zingiberaceae) is an acaulescent perennial that grows in southern China, Indochina, Malaysia, India and Bangladesh (Kanjanpothi et al., 2004). The rhizomes of the plant, which contains essential oils, have been used in a powdered form for indigestion, cold, pectoral and abdominal pains, headache. Its alcoholic maceration has also been applied as liniment for rheumatism (Keys, 1976; Lieu, 1990). In Chinese medicine, *K. galanga* rhizomes have been used as an aromatic stomachic and also as incense.

K. galanga is widely distributed throughout Bangladesh. Its rhizomes are considered stimulating, expectorant, carminative and diuretic. Mixed with oil, the rhizomes of this plant used as a cicatrizant (Ghani, 1998). For its medicinal importance, in the present investigation attempts were made to investigate the anti-bacterial activities of *K. galanga* rhizome extracts

Materials and Methods

Extraction: K. galanga plant was collected from the Sheikhpara bazaar, Jhenidah, Bangladesh. After collection, the rhizome was cleaned. After cutting in to small pieces, they were dried under shade. When the rhizome was properly dried and pulverized into a coarse powder, five grams of rhizome powder was weighted and 20 ml each of the solvent (petroleum ether, acetone and methanol) was added. The powder samples with solvent were placed in water bath shaker for 5-6 hours. The crude extracts were then filtered. The extracts were air dried after filtration to concentrate.

Antibacterial screening: Five organisms, (three Gram-negative *i.e. E. coli, Salmonella* sp., *Shigella sonnei* and two Gram-positive *i.e. Bacillus* sp. and *Pseudomonas* sp.) were used in the present study to determine the antibacterial activity of the crude extracts. Ten ml of distilled water was taken into the screw cap tube and pure colony of freshly cultured bacteria was added in to the tube and vortexed. The optical density (OD) was measured with the colorimeter and microbial population was confirmed to be within in 10^7 ml^{-1} to 10^8 ml^{-1}. This suspension was used as inoculum. Then *in vitro* antibacterial activities of the test samples were carried out by disc diffusion method (Bauer et al., 1966; Barry, 1980). In the disc diffusion method, nutrient agar (High media, India) was used as culture media and the discs were placed aseptically over the bacterial culture on nutrient agar plates and incubated at 37^{o}C for 24 hours. After incubation for 24 hours, the zone of inhibition around the discs was measured by millimeter scale. Discs were impregnated with each treatment and control was assayed on duplicate agar medium plate for *E. coli, Salmonella* sp., *Shigella sonnei, Bacillus* sp. and Pseudomonas sp. The antibacterial activities were determined by measuring the diameter of the zone in mm. The experiment was replicated two times to confirm the reproducible results. Sterile, blank paper discs was impregnated with only sterile solvent (methanol, acetone and petroleum ether) and used as negative control each time. Standard Ampicillin (10 μg/disc), Ciprofloxacin (5 μg/disc) and Cefalaxin (30 μg/disc) were used as positive control for comparison of the antibacterial activity. Minimum Inhibitory Concentration (MIC) value was determined in present study following the serial dilution technique according to Reiner, (1982).

Results

All of the extracts were insensitive to *Shigella sonnei, Bacillus* sp. and *Pseudomonas* sp. Compared to other extracts, petroleum ether crude extract showed the highest activity (inhibition zone 20 mm) against *E. coli,* (Fig. 1). However, no activity was found against rest of the tested organisms (Table-1). Methanol extracts produced 15mm zone of inhibition and 10mm against *E. coli* and *Salmonella* sp.

respectively. On the other hand no activity was found for acetone extracts against all tested organism. Therefore, The MIC value was determined only with petroleum ether and methanol extracts. Different concentration of methanol extracts produced zone of inhibition against *E. coli* and *Salmonella* sp. (Table-2). The MIC values of methanol extract against *E coli* and *Salmonella* sp. were found to be 2µgml^{-1} and 16µgml^{-1} respectively. For petroleum ether, the MIC value against *E. coli* was found to be 4µgml^{-1} (Table-2). Negative control (disc containing only solvent *i.e.* petroleum ether, acetone, and methanol) exhibited no zone against five different organisms (*E. coli*, *Salmonella* sp., *Shigella sonnei, Bacillus* sp. and *Pseudomonas* sp.) All the positive control showed antibacterial activity against tested bacteria

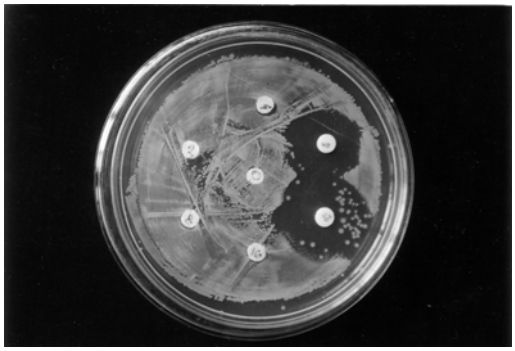

Fig. 1. Antimicrobial activity of the petroleum ether extracts of accani against *E coli.*

Table 1. Activity of methanol, petroleum ether and acetone extract of rhizome of *Kaemperia galanga* L. on *E. coli*, *Salmonella* sp., *Shigella sonnei., Bacillus* sp. and *Pseudomonas* sp.

Bacteria	Methanol for accane crude; mm	Petroleum ether for accane crude; mm	Acetone for accane crude; mm	Negative control	Positive control; mm		
					AMP(10)	CIP(5)	CFX(30)
E. coli	15(-)	20(-)	+	+	20	25	30
Salmonella spp.	10(-)	+	+	+	15	20	25
Shigella sonnei	+	+	+	+	10	15	20
Bacillus spp.	+	+	+	+	12	15	24
Pseudomonas spp.	+	+	+	+	14	18	27

Zone of inhibition (-); Growth (+); AMP (10) = Ampicillin (10 µg/disc); CIP (5) = Ciprofloxacin (5 µg/disc); CFX (30) = Cefalaxin (30 µg/disc)

Table 2. Comparison of minimum inhibitory concentration (MIC) values of petroleum ether and methanol and acetone extract of rhizome of *Kaemperia galanga* against *E. coli, Salmonella* sp., *Shigella sonnei., Bacillus* sp. and *Pseudomonas* sp.

Bacteria	Petroleum ether extract of accane (µgml^{-1})										Methanol extract of accane (µgml^{-1})									
E. coli	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+
Salmonella sp.	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	+	+	+
Shigella sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bacillus sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pseudomonas sp.	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	512	256	128	64	32	16	8	4	2	0	512	256	128	64	32	16	8	4	2	0

Discussion

Members of the family Zingiberaceae have attracted continuous phyto-chemical interest due to

their considerable importance as a natural species or as medicinal plants. Well known examples for the use as spices are the rhizome of the Z*ingiber offcanal* (ginger) or *Curcuma longa* (Syn. *C. domestica*) and *K. pandurota* are on the other hand important medicinal plants used in the folk medicine of Southeast Asia for the treatment of stomach discomfort as expectorant or as antiseptic for wound (Pandji et al., 1993).

The constituents of the rhizomes of *K. galanga*, hitherto reported, have included cineol, borneol, 3-carene, camphene, kaempferide, cinnamaldehyde, P-methoxycinnaamic acid, ethyl cinnamate, and ethyl P-methoxycinnamate (Nakao and Shibu, 1924). Ethyl P-methoxycinnamate was reported to inhibit monoamine oxidase (Noro *et al.,* 1983). The methanolic extract of *K. galanga*, which identifies as ethyl cinnamate, ethyl P-methoxycinnamate and P-methoxycinnamic acid, showed larvicidal activity against the second stage larva of dog roundworm, *Toxocara canis* (Kiuchi et al., 1988). Kiuchi et al., (1988) found that the rhizome extract exhibited Epstein-Barr virus (EBV) activation inhibitory activity when screened for anti-tumour promoter activity using the short term assay of inhibition of 12-O-tetradecanoyl phorbol-13-acetate (TPA) induce EBV early antigen in Raji cells. Chu et al., (1998) found that *K. galanga* extract exhibited amebicidal activity *in vitro* against three species of *Acanthomoeba: A. culbertsoni*, *A. castellanii*, and *A. polyphaga* that were not lytic for normal macrophage culture.

Acetone extract was found insensitive against all tested bacteria, while the methanol extracts showed the significant antibacterial activity against *E. coli* and *Salmonella* sp. but not *Shigella* sp., *Bacillus* sp., and *Pseudomonas* sp. The diameter of the highest zone of inhibition of methanol extract were found 15mm in cases against *E. coli*. Similarly methanolic extract of accane crude produced 10mm zone of inhibition against *Salmonella* sp. The petroleum ether extract demonstrated highest antibacterial activity against *E. coli.* The zone of inhibition was found to be 20 mm in this case, whereas no activity of this extract was found against rest of the tested bacteria. Generally, low polar substances, such as oil, fats, waxes, hydrocarbons, glyceroides are washed by the acetone extracts. It is well known that low polar substances contain less or no medicinal active compound (Willims and Lemke, 2002). This may be the reason for the low antibacterial activity of the acetone extracts.

For the comparison of the plant extracts activity positive control (different type of antibiotic disc) and negative control (only solvent absorbing disc) was used. The negative control showed no activity against all tested bacteria. The positive control showed significant antibacterial activity against the all bacteria.

Conclusion

As methanol and petroleum ether crude extracts exhibited an antibiotic potential against *E. coli*, it proved that traditional use of *K. galanga* has scientific basis. Further investigation is necessary to find out the active compounds and to conform their bioactive principles.

References

Barry AL. 1980. Procedure for testing antimicrobial agent in agar media. In Antibiotica in laboratory medicines, ed. V Lorian, pp1-23. Willims and Wilkins Co. Baltimore.

Bauer AW, Kibry WMM, Sherris JC, Turck M. 1966. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 45, 493-496.

Chu D M, Miles H, Toney D, Ngyuen C, Marciano-Cabral F. 1998. Amebicidal activity of plant extracts from Southeast Asia on *Acanthamoeba spp*. Parasit Res, 84, 746-752.

Farnsworth NR, Akerele O, Heywood V, Soejarto DD. 1991. Global Importance of Medicinal Plants. In Conservation of medicinal plants, ed. O Akerele, V Heywood, H Synge, pp 25-51.Cambridge Univ. Press, Cambridge, UK.

Ghani A. 1998. Medicinal plant of Bangladesh. 2nd edn. Asiatic Society, Dhaka, Bangladesh.

Iwu MW, Duncan AR, Okunji CO. 1999. New antimicrobials of plant origin. In Perspectives on new crops and new uses, ed. J Janick, pp. 457-462. ASHS Press, Alexandria, VA.

Kanjanpothi D, Panthong A, Lertprasertsuke N, Taesotikul T, Rujjanawate C, Kaewpinit D, Sudthayakorn R, Choochote W, Chaithong U, Jittpakdi A, Pitasawat B.

2004. Toxicity of crude rhizome extract *kaempferia galanga* L. (Prph Hom). J Ethnopharm 90, 359-365.

Keys JD. 1976. Chinese herbs: Their Botany, Chemistry and Pharmacodynamics. ed. E Charles. Tuttle Company, Inc. Tokyo.

Khalequzzaman M, Gazi MMR, Das BC. 2002. Antimicrobial activities of the Rhizome and Leaf Extracts of *Zingiber cassumunar* Roxb. Bangladesh J Genet Biotechnol 3, 35-40.

Kiuchi F, Nakamura N, Tsuda Y, Kondo K, Yoshimura H. 1988. Larvicidal principles in *Kaempferia* rhizome. Chem Pharm Bull 36, 412-415.

Lewington A. 1990. Plants for people. Natural History Museum, London. pp. 135-136.

Lieu VD. 1990. Medicinal plant in Vietnam. Ha Noi 35, 239.

Nakao M, Shibu C. 1924. Yakugaku Zasshi 44, 913.

Noro M T, Kuroyanagi M, Ueno A, Fukushima. 1983. Monoamine oxidase inhibitor from the rhizomes of *Kaempferia galanga* L. Chem Pharm Bull 31, 2708-2711.

Pandji C, Grimm C, Wray V, Witte L, Proksch P. 1993. Insecticidal constituents from four species of the zingiberaceae. Phytochem 34(2), 415-419.

Rahman A, Parvez MAK, Parvin T, Paul DK, Sattar MA. 2004. Antimicrobial activity of three bioactive compound from the stream bark of *Piper chaba* Hunter. Bioscien Res 1(1), 16-20.

Reiner R. 1982. Antibiotica. -An Introduction. ed. F Hoffman, pp23-30 pp. La Roche and Co. Ltd. Switzerland.

Vuorelaa P, Leinonenb M, Saikkuc P, Tammelaa P, Rauhad JP, Wennberge T, Vuorela H. 2004. Natural products in the process of finding new drug candidates. Curr Med Chem 11(11), 1375-89.

Williams D A, Lemke T H. 2002. Antimicrobial activity of three bioactive compounds from the stem bark of *Piper chaba* Hunter. Biosc Res 1(1), 16-20.