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PHYSIOLOGY/PHARMACOLOGY

OPTIMISATION AND USE OF K562 CELLS IN LACTATE DEHYDROGENASE (LDH) ASSAY FOR CYTOTOXICITY TESTING

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The optimisation and use of the lactate dehydrogenase (LDH) assay using K562 cells are presented. The LDH assay is an enzymatic release assay that is quantitatively used to measure natural cytotoxicity. It is based on a coupled two-step reaction. In the first step, LDH, a stable cytosolic enzyme that is released upon cell lysis catalyses the oxidation of L-lactate to pyruvate in the presence of NAD^+. In the second step of the reaction, phenazine methosulfate (PMS) acting as a catalyst, uses the newly-formed NADH and H^+, reduced the 2-*p*-iodophenyl-3-*p*-nitrophenyl tetrazolium chloride (INT) to a red formazan product. The amount of formazan formed is proportional to the amount of LDH released into culture medium as a result of cytotoxicity. The optimisation studies involved the optimum number of K562 cells used per well, the concentration of Triton X-100 that permitted maximal LDH activity, the period of incubation of K562 cells and incubation time after the addition of the substrate mixture [5.4×10^{-2}M L(+)lactate, 6.6×10^{-4}M INT, 2.8×10^{-4}M PMS, and 1.3×10^{-3}M NAD in 0.2 M Tris buffer at pH 8.2]. The cell density was 1×10^4 cells/well in 200 µl, and after 4 h incubation, the supernatants were transferred to another plate and absorbance was measured 5 min after addition of the substrate mixture at wavelength 492 nm/630 nm. The maximal LDH activity of K562 was obtained after lysis with 20 µl of 0.9% Triton X-100. There were some background absorbance noise due to phenol red in the media and the spontaneous release of LDH from animal sera. However this can be corrected by including a culture medium background control or by using phenol red-free medium and low percentage of fetal bovine serum. This enzymatic assay is a simple, rapid, inexpensive and convenient technique for use in cytotoxicity assay e.g. natural killer cell activity.

ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF THE AQUEOUS EXTRACT OF *KAEMPFERIA GALANGA* LEAVES IN ANIMAL MODELS

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The present study was performed to determine the antinociceptive and anti-inflammatory activities of aqueous extract of *Kaempferia galanga (K. galanga)* leaves using various animal models. The extract, in doses of 30, 100 and 100 mg/kg, was prepared by soaking (1:10;

w/v) the air-dried powdered leaves (40 g) in distilled water (dH_2O) for 72 h and administered subcutaneously in mice/rats 30 min prior to the tests. The extract exhibited significant ($P < 0.05$) antinociceptive activity when assessed using the abdominal constriction, hot plate and formalin tests with activity observed in all tests occurring in a dose-dependent manner. Furthermore, the antinociceptive activity of *K. galanga* extract was significantly ($P < 0.05$) reversed when pre-challenged with 10 mg/kg naloxone. The extract also produced a significantly ($P < 0.05$) dose-dependent anti-inflammatory activity when assessed using the carrageenan-induced paw oedema test. In conclusion, the present study demonstrated that the *K. galanga* leaves possessed antinociceptive and anti-inflammatory activities and thus supports the Malay's traditional uses of the plant for the treatments of mouth ulcer, headache, sore throat etc.

VASORELAXANT EFFECTS OF CHLOROFORM EXTRACT OF *ANDROGRAPHIS PANICULATA* ON *IN VITRO* RAT THORACIC AORTA

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Andrographis paniculata (AP), of *Acanthaceae*, has been used for centuries in Asia to treat GI (gastrointestinal) tract and upper respiratory tract infections, fever, herpes, sore throat, and a variety of other chronic and infectious diseases. AP has cardio protective property and is familiarly known as "King of Bitters". This plant has no proven side effects and the plants are beneficial in treating cardiovascular diseases. The present study is aimed to investigate the vasorelaxant effect of chloroform extract of AP on rat thoracic aorta. Petroleum ether, chloroform, methanol and water extracts of AP are used in this study. A qualitative study of chloroform extract of AP was carried out by TLC (thin layer chromatography) and fractionated by dry flash chromatography. A HPLC study of chloroform extract of AP is conducted and compared with commercially available standard andrographolide (Sigma Chemicals, USA). Experiments are performed on male Sprague-Dawley (SD) rats for possible vasorelaxing activity of AP by following the methods Gilani *et al*. (1994) and M. Ajay *et. Al*. (2003). Cumulative dose response curves were recorded by using isometric force displacement transducer Model FT 03 and it is connected to Grass Polygraph Model 79D according to the method of Kitchen (1984). Chloroform extract of AP is first suspended in 0.1% PEG then volume made up with Krebs solution. The average of responses to each concentration of the agonist was plotted on the ordinate the logarithm of the concentration of the agonist on the abscissa (Kitchen, 1984). Among all these extracts of AP, chloroform extract is found to be the most potent relaxant effect on noradrenaline induced contraction on rat thoracic aortic ring preparations.

DOES *CENTELLA ASIATICA* POSESS ANTI-VIBRIOS PROPERTY?

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Several scientific reports have documented *Centella asiatica*'s *(C. asiatica)* ability to aid wound healing. Upon treatment with *C. asiatica*, maturation of the scar is stimulated by the production of type I collagen. The treatment also results in a marked decrease in inflammatory reaction and myofibroblast production. Herbalists claim it contains a longevity factor called 'youth Vitamin X' said to be 'a tonic for the brain and endocrine glands' and maintain that extracts of the plant help circulation and skin problems. The isolated steroids from the plant have been used to treat leprosy. *C. asiatica* is used to re-vitalise the brain and nervous system, increase attention span and concentration, and combat ageing. *C. asiatica* also has anti-oxidant properties. However, in the present study, antimicrobial property of aqueous and methanol extracts of *C. asiatica* against *Vibrio* spp. has been revealed. Only aqueous extract of *C. asiatica* was found to inhibit the growth of *Vibrio alginolyticus*, *V. cholerae*, *V. parahaemolyticus* and *V. harveyi*.

THE RENAL ACTIONS OF ANGIOTENSIN II, AND THEIR MODIFICATION BY PARGYLINE

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The renin-angiotensin system (RAS) plays important roles in the control of renal function and mean arterial blood pressure (MABP) and has been implicated in hypertension. It is believed that the sympathetic nervous system (SNS) is involved in the actions of angiotensin II (AII). Pargyline is a drug that can block SNS activity (Fowler *et al*. 1981), so we tested whether it can modify the renal actions of AII. Pargyline (or saline as control) was infused IV into pentobarbitone anaesthetised female rats. Urine was collected via a bladder catheter; glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by the clearance technique; urine Na^+ by flame photometry; and MABP was measured via an arterial catheter. Renal noradrenaline was assayed by the method of Earley and Leonard (1978). After 3.5 h both groups received an IV infusion of AII.

Baseline values (before AII infusion) were:

	Saline infused (control) rats	Pargyline infused rats	P value
MABP (mmHg)	118	82	<0.01
Renal [noradrenaline] (ng/gm)	188	263	<0.02

Thus the dose of pargyline used, was effective. Changes induced by AII (20 ng/kg/min) in the two groups (n = 7 rats/group) were:

	Saline infused (control) rats	Pargyline infused rats	P value
Urine Na+ excretion (µmol/min)	–11.6	–6.4	<0.001
Urine flow (µl/min)	–40.0	–35	<0.01
GFR (ml/min)	–0.5	–0.3	<0.01
ERPF (ml/min)	–2.1	–0.5	<0.01

All changes were evaluated statistically using Student's t-test. These data show that the renal responses to AII are modified by pargyline. This implies that these responses to AII depend on the SNS.

CHANNA STRIATUS (HARUAN) EXTRACTS *IN VITRO* CYTOTOXIC ACTIVITY AGAINST MCF-7 AND HT-29 TUMOUR CELL LINES

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The present study was performed to determine the *in vitro* cytotoxic activity of *Channa striatus (C. striatus)* aqueous, chloroform:methanol (CM; ratio of 1:2 (w/v)), chloroform and methanol extracts against MCF-7 and HT-29 cancer cell lines using the 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay. The fresh fillet of *C. striatus* was soaked in CM (72 h at room temperature) to yield the aqueous and CM extracts while the methanol and chloroform extracts were prepared by soaking the freeze-dried fillet of *C. striatus* separately in the said solvents (72 h at room temperature). The aqueous extract was freeze-dried while the CM, methanol and chloroform extracts were evaporated to dryness and the crude-dried-extract were later prepared in the concentration of 100 µg/ml. Of all the extracts tested, only the CM, chloroform and methanol extracts demonstrated cytotoxic activity against the HT-29 with an IC_{50} of approximately 74, 68 and 73 µg/ml, respectively. Although methanol and chloroform extracts, in particular, caused significant reduction in the percentage of MCF-7 viability, the values obtained did not reach an IC_{50}. Interestingly, all extracts were found to induce proliferation of the normal cell (3T3). In conclusion, this study demonstrated the anticancer potential of *C. striatus* extracts, at least against the HT-29 cell line, as well as its ability to proliferate normal cells, which is in line with traditional claims that *C. striatus* promotes tissue growth.

THE ROLE OF CHEMICAL SYMPATHECTOMY ON RENAL HAEMODYNAMICS OF RATS WITH HYPERTENSION

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This study assessed the impact of sympathectomy and vaso-regulatory mechanisms like renin-angiotensin system (RAS) on renal haemodynamics in normal (WKY) and hypertensive (SHR) rats. Chemical sympathectomy was carried out by the administration of multiple doses of 6-hydroxydopamine (6-OHDA) (50–100 mg/kg i.p.). In acute study, the animals were anesthetised (60 mg/kg i.p., sodium pentobarbitone), blood pressure and renal blood flow (RBF) were measured using a pressure transducer and electromagnetic flowmeter, respectively. Reductions in RBF to electrical stimulation of renal nerve (RNS) and intrarenal administration of noradrenaline (NA), phenylephrine (PE), methoxamine (ME) and angiotensin II (Ang II) were determined. Data was recorded in a computerized data acquisition system and expressed as mean ± s.e.m and compared by two-way ANOVA followed by Bonferroni post-hoc test with a significance level at 5%. In sympathectomised WKY rats, there was significant decrease in the renal vasoconstrictions caused by RNS, NA and PE (all $p < 0.05$) and not to ME and Ang II. In sympathectomised SHR renal vasoconstrictor responses caused by RNS were blunted ($p < 0.05$), while the magnitude of changes induced by ME, PE and Ang II were greater ($p < 0.05$). However, the NA induced changes remained unchanged. In sympathectomised SHR all adrenergic stimuli induced renal vasoconstrictions was greater as compared to that of sympathectomised WKY rats (all $p < 0.05$). The results suggested that in WKY rats, sympathectomy reduced the adrenergically induced sympathetic drive but not the functionality of the α_1-adrenoceptors. In sympathectomised SHR there is some specificity over the subtypes of adrenoceptors involved in mediating the renal vasoconstriction in terms of a selective influence on renal α_1-adrenergic receptors. Meanwhile, sympathectomy did not compromise RAS. In summary, this study showed sympathectomy had varied effect in impeding sympathetic tone in WKY and SHR rats and did not influence the vasoconstrictor action caused by Ang II.

VASCULAR ADRENERGIC RECEPTORS IN THE REGULATION OF ARTERIAL PRESSURE IN A MURINE MODEL OF PRESSURE OVERLOAD LEFT VENTRICULAR HYPERTROPHY

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Left ventricular hypertrophy (LVH) is a potential risk factor for cardiovascular disease related to high mortality and morbidity. The present study investigated the role of peripheral vasculature adrenoceptors in the control of arterial pressure in rats with pressure overload hypertrophy. Age and weight matched Sprague Dawley rats (n = 20) were randomised into three different groups viz. Normal (n = 8), Sham operated (n = 6) and Aortic Banded (n = 6). Metabolic data of all groups was collected in terms of change in body weight, water intake and urine output. Baseline electrocardiographical changes were also recorded on the day of acute study and analysed for heart rate, Q-T interval, QTc (Framingham study), QRS interval, R wave amplitude and R-R interval, using one factor ANOVA. Rats were anesthetised with pentobarbitone sodium i.p (60 mg/kg) and ventilated endo-tracheally to atmospheric air. Right carotid artery was cannulated for mean arterial pressure (MAP) measurements and left jugular vein was perfused with normal saline at a rate of 6 ml/h to administer vasoactive agonists; Noradrenaline (NA), Phenylephrine (PE), Methoxamine (ME) and Angitoensin II (ANGII) at graded doses. Baseline values and changes in the mean arterial pressure after administration of adrenergic agonists and ANGII were recorded using a data acquisition system (power lab) and analysed for statistical variation by two factor ANOVA. At the termination of acute study, animals were euthanised to collect heart and left kidney of each animal to calculate heart to body weight ratio (Hw/Bw) and kidney to body weight ratio (Kw/Bw). ECG analysis revealed significant changes ($p < 0.05$) in heart rate, Q-T interval, QTc, R wave amplitude and R-R interval amongst all groups except between Normal and Sham operated groups, whilst QRS interval remained statistically insignificant amongst all three groups. The baseline values of MAP were statistically significant ($p < 0.05$). Significant changes ($p < 0.05$) observed for NA, PE, ME and ANGII amongst all groups except between Normal and Sham operated groups, suggested a down regulation of α-adrenoceptors in the peripheral vasculature and impaired sympathetic tone in pressure overload left ventricular hypertrophy.

MISCELLANEOUS

THE POTENTIAL OF METHANOL EXTRACT OF *PANDANUS AMARYLLIFOLIUS* ROXB AS NATURAL REMEDY OF CHOLERA

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Pandanus amaryllifolius (P. amaryllifolius) is a tropical plant and used widely in South East Asian for cooking. It is an erect green plant with fan-shaped sprays of long, narrow, blade-like leaves and woody aerial roots. The plant is rare in the wild but cultivated widely for use as a food flavour. The leaves are used fresh or wilted. They have a nutty, botanical fragrance which enhances the flavour of Thai, Malaysian, and Indonesian foods, especially rice dishes. Since *P. amaryllifolius* can be used as food flavor for human consumption, thus, this study was conducted to investigate the antimicrobial property of *P. amaryllifolius* against Gram negative and Gram positive bacteria. In the present study, *P. amaryllifolius* leaf was extracted using distilled water and methanol. The tested microorganisms were *Aeromonas hydrophila, Edwardsiella tarda, Escherichia coli, Citrobacter freundii, Schewanella putrifacien, Streptococcus* sp., *Vibrio cholera, V. harveyi, V. parahaemolyticus* and *V. vulnificus*. The finding of this study showed only methanol extract of *P. amaryllifolius* could inhibit the growth of *V. cholera* and *C. freundii*. Thus, further study should be carried out to find out anti-cholerae compound of methanol extract of *P. amaryllifolius* in the future.

ANTIMICROBIAL PROPERTY OF METHANOL AND AQUEOUS EXTRACT OF *MORINDA CITRIFOLIA* LINN

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Various parts of noni, *Morinda citrifolia (M. citrifolia)* (leaves, flowers, fruits, bark, roots) serve as tonics and to contain fever, to treat eye, skin, gum and throat problems as well as to treat constipation, stomach pain and respiratory difficulties in China, Samoa, Japan, and Tahiti. In Malaysia, heated noni leaves applied to the chest are believed capable of relieving coughs, nausea, or colic. The noni fruit especially is used to treat asthma, lumbago, and dysentery in Indochina. Furthermore, unripe noni fruits can be pounded, then mixed with salt and applied to cut or broken bones. In Hawaii, ripe fruits are applied to draw out pus from an infected boil. Meanwhile, the green fruit, leaves and the root/rhizome have traditionally been used to treat menstrual cramps and irregularities, among other symptoms, while the root has also been used to treat urinary difficulties. Since noni *M. citrifolia* has been widely used as traditional medicine in human, this study was conducted to screen the antimicrobial property of methanol and aqueous extract of

M. citrifolia against fish pathogenic bacteria. The result of the present study showed methanol extract of *M. citrifolia* could inhibit all the tested pathogenic bacteria such as *Vibrio* spp., *Streptococcus* spp. and *Citrobacter freundii*. While aqueous extract of *M. citrifolia* not only can inhibit all tested *Vibrio* spp. and *Citrobacter freundii* but also pathogenic Gram negative bacteria such as *Edwardsiella tarda*, *Citrobacter freundii* and *Schewanella putrifacien*.

INCIDENCE OF ANTIBIOTIC RESISTANT OF FISH PATHOGENIC BACTERIA

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Overuse of broad-spectrum commercial antibiotics greatly hastens the development of antibiotics resistance particularly in aquaculture industry. This study was carried out to investigate the incidence of antibiotic resistance among pathogenic bacteria isolated from diseased fish. Six types of commercial antibiotic were applied in this study: kanamycin, ampicillin, sulphamethoxazole, nalidixic acid, furazolidone and tetracycline. The important finding of the present study showed the incidence of antibiotic resistance among the tested microorganisms was recorded as high as 40%.

EXPLORATION OF ANTIMICROBIAL POTENTIAL OF INDIGENOUS MALAYSIAN PLANT, *TETRACERA SCANDENS IN VITRO*

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This lucubration research has been conducted in order to delve and recognise antimicrobial potential of Indigenous Malaysian Plant namely *Tetracera scandens* (L.) Merr. (Dilleniaceae) *(T.scandens)*; (Local name – 'Pampan' and 'Mempelas'). Traditionally, juice of this plant is generally taken to treat internal pains in the region of Kadazandusun of Kuala Penyu, Sabah, Malaysia. Droplets of water/sap from freshly cut stems are used for eye irritation and juice gathered by smashing the stem is taken to reduce body heat by the indigenous people of the region Tasek Bera in Sungai Pahang, Peninsular Malaysia. Moreover, MeOH-H_2O extract has also been found to exhibit strong XO inhibitory activity with IC_{50} values less than 20 μg/ml. The traditional medicinal value and other folklore claims of 'mempelas' led us to scout its antimicrobial potential. Thence, the screening of antimicrobial activity of petrol, ethanolic and aqueous extracts of leaves and stems was steered by a disc diffusion test against five species of human pathogenic gram-negative and Gram-positive bacteria viz., *Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and three species of fungal strains viz., *Trichophyton rubrum, Microsporam canis* and *Candida albicans.* The significant lethal effects of the different extracts of *T. scandens* against aforementioned microorganisms and phytochemical screening have been described explicitly in this paper.

These findings candidly evince the fact that the polar compounds present in the leaves and stems of 'mempelas' may provide a chemical lead for the synthesis of new derivatives, which might prove to be potential antimicrobial agents having less or no resistant against microorganisms mentioned above.

DESIGNING OF SPECIFIC PRIMER FOR GA PROTEIN

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The appropriate designing of specific primers/oligomers is of the utmost importance for the successful production of PCR amplification. Ideally, assurance of specific primers subject to PCR amplifications will result in fragments of constant length, which uniquely identify genes desired, thus minimizing the differences in PCR amplification efficiency as well as in hybridisation kinetics. Our study is based on investigating the expression of Gαi3 protein and Gαs52 protein in the heart of *Rattus norvegicus* (Wistar-Kyoto rats) facilitated by RT-PCR, thus demanding for specific primers that recognise these Gα proteins. The primers were designed accordingly using online software accessible at *www.genome.wi.mit.edu/cgi-bin/primer3* from Gαi3 and Gαs52 proteins sequences of *Rattus norvegicus* made available in the GenBank database of the National Center for Biotechnology Information (NCBI). Total cellular RNA was extracted from the heart of Wistar-Kyoto rat and subjected to reverse transcription to synthesise first strain of cDNA. The primers were then used in PCR amplification of the cDNA, subsequently; amplified products were analysed on agarose gel. The specific DNA fragments were extracted and purified from the ethidium bromide-stained agarose gel. Then, the DNA fragments were ligated into pGEM®T vector (Promega) and later the recombinant DNA were introduced into *E. coli* JM 109 competent cells (Promega) during transformation. The bacterial cells were let to grow on Luria-Bertani (LB) medium containing ampicillin, IPTG and X-Gal and later these recombinant colonies were subjected to screening. The recombinant plasmids, which enclose the specific DNA fragments were isolated and purified, and were sent for sequencing at 1st BASE Laboratory. In securing the specificity of the primers designed and the targeted DNA fragments amplified, the sequences were compared and identified with other sequences in the GenBank/EMBL database using BLAST command at *www.ncbi.nlm.nih.gov/blast*. The primers designed were found to be specific to the Gα proteins DNA sequences and therefore can be used in RT-PCR.

PRELIMINARY INVESTIGATION OF A POLYSACCHARIDE-PRODUCING BACTERIUM, *SHEWANELLA PUTREFACIENS* ISOLATED FROM MARINE SPONGE, *THEONELLA* SP.

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This study signified the existence of *Shewanella putrefaciens* isolated from marine sponge, *Theonella* sp. identified based on the cellular and morphological appearance, and biochemical characteristics in combination with RapID™ NF Plus System. Eleven isolates have been observed which have the same characteristics such as Gram negative, asporogenous rod, straight or slightly curved, 0.5 x 1.5–2.0 microns in size, motile with peritrichous flagella and grew well in sea water media. Optimal growth was observed in sucrose media incorporated with 3% to 6% of NaCl solution at a temperature of 27°C. These isolates derived from non-human resources which were able to utilise a number of sugars (glucose, arabinose, myo-inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sucrose, trehalose, xylose, galactose and fructose) within period tests time of 24 h to 14 days. Cultivation for bacterial polysaccharide was done using sucrose sea water broth and crude polysaccharides were obtained with weight of 200 mg per 1 l culture media. Molecular weight of isolated bacterial polysaccharide from *Shewanella putrefaciens* with 250.5 kDa has been measured by Gel Permeation Chromatography (GPC). High performance liquid chromatography (HPLC) was used for sugars component investigation. Mannose, glucose, trehalose and galactose were detected in most of the polysaccharide. Detection of sulfate groups for samples was done using Ionic Chromatography (IC). Results showed that polysaccharide isolated from *Shewanella putrefaciens* contained very low concentration sulfate groups.

DRUG PRICES, AVAILABILITY AND AFFORDABILITY IN MALAYSIA

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The medicine price problem has been always a great concern especially in low and middle income countries. Large proportions of the population have limited access to medicine due to its unaffordable price or low availability. Drug prices in Malaysia for instance have hit a record high and consumers are finding it difficult to gain access. In fact drug prices are over and above the international reference pricing, a guide on how much medicines should cost in each country. This report will be discussing mainly about the price, affordability and availability of essential drug in Malaysia. This report consists of introduction concerning the overview of the drug price scenario in Malaysia, content; essential drug price, availability and affordability and discussion; the implication and conclusion; suggestion to overcome this problem. The objectives of this report are to

compare the price of essential drugs in public and private sector, the affordability of drugs in Malaysia, to measure the availability of drug in Malaysia and to compare the price of public sector and private sector with International Reference Price (IRP).

DO METHANOL AND AQUEOUS EXTRACT OF *ZINGIBER OFFICINALE* (ROSCOE) CAN ALTERNATIVE TO COMMERCIAL ANTIBIOTIC FOR AQUACULTURE USE?

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Ginger is commonly used as a spice in cuisines throughout the world. Though commonly referred to as a root, it is actually the rhizome of the monocotyledonous perennial plant *Zingiber officinale (Z. officinale).* Previous medical research indicating that ginger might be an effective treatment for nausea caused by motion sickness or other illness. Currently, the occurrence of antibiotic resistance among pathogenic bacteria in aquaculture cause many commercial antibiotics available in the market not being effective against fish diseases, especially bacteria borne diseases. Due to public health concerns, antibiotics can not be applied in aquaculture especially for food fish culture. Thus, this study was conducted to reveal the existence of antimicrobial property in *Z. officinale* and evaluate the potential of it as an alternative to commercial antibiotic for aquaculture use. In the present study, methanol and aqueous extracts of *Z. officinale* were tested against 27 isolates of bacteria. The results showed methanol extract of *Z. officinale* was able to inhibit the growth of 2 out of 18 isolates of *Edwardsiella tarda* and all the tested *Streptococcus* spp. On the other hand, all the tested microorganisms were resistant to aqueous extract of *Z. officinale*.

POTENTIAL ANTIMICROBIALS FROM PLANTS AGAINST *AEROMONAS HYDROPHILA*, *CITROBACTER FREUNDII*, *EDWARDSIELLA TARDA* AND *ESCHERICHIA COLI* ISOLATED FROM FRESHWATER FISHES

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A total of six types of commonly found plants in Terengganu's area was screened for their antimicrobial properties against *Aeromonas hydrophila, Citrobacter freundii, Edwardsiella tarda* and *Escherichia coli* isolated from freshwater fishes. The plants that were applied in the present study were *Pandanus amaryllifolius* Roxb., *Centella asiatica* L., *Aloe vera* L., *Colocasia esculenta* L. (Schott), *Ipomoea aquatica* (Forssk.) and *Passiflora foetida* L. The leaves of each plant were extracted using methanol and aqueous method. They were then tested for sensitivity test against fish pathogens. Results showed that all tested microorganisms were resistant to both types of extraction methods except for *C. freundii*. *C. asiatica*

(aqueous and methanol extracts) and methanol extract of *P. amaryllifolius* were found to inhibit the growth of *C. freundii*.