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Research Paper

Antiulcer and Antibacterial Evaluations of Illicium Verum Ethanolic Fruits Extract (Ivefe)

Medical science

KEYWORDS : Illicium verum, antiulcer, antibacterial, MIC, MBC.

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ABSTRACT

Illicium verum, or commonly known as "bunga lawang" is widely used in Malaysia for cooking. Current study was carried out to evaluate the potential antiulcer and antibacterial activity within Illicium verum ethanolic fruit extract (IVEFE). Four groups of rats were pre-treated with carboxymethyl cellulose (negative control group), omeprazole 20mg/kg (positive control group), 250 and 500 mg/kg of IVEFE an hour before absolute ethanol were administered to induce gastric ulcer. An hour later, the rats were sacrificed, and the evaluation of ulcer areas, pH of gastric content, mucus production and histology of gastric mucosa were done. Antibacterial activity, Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract was performed. Negative control group exhibited severe mucosal injury, while the groups pre-treated with the IVEFE shows milder injury. This is consistent with the increment of mucus production and pH of gastric content, as well as histology findings. Antibacterial activity studies showed zone of inhibition ranging between (7.0-11.0mm), MIC (4.69-9.38 mg/ml) and MBC (4.69-9.38 mg/ml) were evaluated. As conclusion, Illicium verum possess antiulcer and antibacterial activities. However, further studies need to be taken to evaluate the potential of this plant as a replacement of therapy.

Introduction

According to World Health Organization (2012), antimicrobial resistance is defined as the ability of the microorganisms to survive exposure to antimicrobial drugs such as antibacterial, antifungal and antiviral drugs. High frequencies of antimicrobial resistance would reduce the effectiveness of the drugs and leads to a risk of complications and fatal outcome (Ahmed et al 2012; Jamal et al 2012).

Gastric ulcers have affect huge number of populations and these lesions has been considered as a new "plague" of 21st century by some authors (Moraes De Carvalho et al, 2010). According to Dorland's medical dictionary, ulcer is medically defined as a local defect or excavation of the surface of an organ and tissue produced by sloughing of necrotic inflammatory tissue.

Illicium verum Hook.f, from the family of Magnoliaceae was originated from China and Vietnam, but can easily be found throughout Asia. It is an evergreen tree living in cooler tropics and subtropics (Glob in Med). It produces unique star-shaped fruits with five to ten boat-shaped sections radiating from the center, tough skinned and are rusty in colour (Encyclopedia of spices, 2003).

It is commonly known as star anise in English, bunga lawang in Malay and Indonesia, stemanis in German, anice stellato in Italian and ba chio in Chinese. This plant is used traditionally in Chinese, Carribean and Latino populations as an infusion for the treatment of infant colic (Ize-Ludlow D et al, 2004). The fruit is used widely in cooking and also as flavoring agent in candies, chewing gums, pickles and sometimes were chewed to help in the digestion as well as breath sweetener (De M et al, 2001).

Previous researches has showed that the plant possesses antifungal (Yazdani D et al, 2009), antimicrobial (Zhaoming L et al, 2009; De M et al, 2001), antiseptic (Sung-won L et al, 2003) and anti-HIV (Wen-Tong S et al, 2007) activities.

A study on the chemical constituents has been done on the plant previously. It reveals the composition of 76.93% of the component is anethole and the rest of 10.22% is P-allylanisole (Zhaoming L et al, 2009). It is suggested that most of the medicinal properties that lies in the star anise may probably comes from the anethole present (De M et al, 2009).

Illium verum Hook. F. is considered safe as it does not contains potent neurotoxins such as unisatin, neoanisatin and pseudoanisatin. However, it still contains toxic compounds named veranisatins A, B and C but in low concentration. It is safe to be taken by adults, but caution needs to be taken as significant quantities may be enough to cause neurologic reactions to infants (Ize-Ludlow D et al, 2004).

Materials and Methods

Sample collection and identification:

Fresh plant materials were collected from Ethno Resources Sdn Bhd, Sungai Buloh, Selangor Malaysia. The plants then were identified by comparison with the voucher specimens deposited at the Herbarium of Rimba Ilmu, Institute of Science Biology, University of Malaya, Kuala Lumpur.

Experimental animals:

Sprague Dawley adult rats weighed between 150g-200g were obtained from the Experimental Animal House, Faculty of Medicine, University of Malaya. The selected animals were maintained at a controlled temperature of 22-24°C with a 12 hours light/dark cycle and were fed with a standard diet and water ad libitum. The rats were randomly divided into 4 groups of 6 rats each and were placed in separate cage (one rat per cage) with wide-mesh wire bottoms to prevent coprophagia during the experiment. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Medicine, University of Malaya with Ethic no. PM/27/07/2010/MAA (R). Throughout the experiment, all the animals were received human care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institute of Health.

Microbial cultures

Laboratory isolates of pure culture of Gram positive (Staphylococcus epidermidis, Bacillus sibtilis and MRSA) and Gram negative (Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa) organisms were obtained from Microbiology Laboratory, Department of Molecular Medicine, Faculty of Medicine, University of Malaya. The isolates were separately cultured on nutrient agar plate for 24 hour. A colony of each test microorganism was sub cultured in 10mL nutrient broth and was incubated at 37°C overnight. Sub cultured organisms from the broth were then used for the experiment.

Omeprazole :

Omeprazole was used as the reference anti-ulcer drug, and was obtained from University Malaya Medical Centre (UMMC) Pharmacy. The drug was then dissolved in carboxymethyl cellulose (0.5% w/v CMC) and was administered orally to the rats with the concentration of 20mg/kg body weight (5 ml/kg) according to the recommendation of (Abdulla et al., 2010).

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Plant extraction
Fresh plants were tap washed followed by washing with distilled water and shade-dried for 7-10 days. The dried plants then were finely powdered using electrical blender. 100g of the fine powder were soaked in 500ml of 95% ethanol in conical flask for 3 days. After 3 days, the mixture was filtered using a fine muslin cloth followed by filter paper (Whatman No. 1) and was distilled under reduced in an Eyela rotary evaporator (Sigma-Aldrich, USA).

Antimicrobial activity
Extract preparation
The extract was dissolved into absolute ethanol inside the sterile micro centrifuge tubes. Three concentrations of the extract solution used were 50mg/ml, 100 mg/ml and 150 mg/ml.

Disc diffusion method
The medicinal plant extracts were tested in vitro against six bacterial species (reference strain) in which three of them are Gram positive: Staphylococcus epidermidis, Bacillus subtilis, MRSA, and the other three are Gram negative: Klebsiella pneumonia, Escherichia coli and Pseudomonas aeruginosa by the disc-diffusion assay (Bauer et al., 1966).

Anti-ulcer activity
Gastric ulcer-induction by absolute ethanol:
The rats were fasted for 24 hours before the experiment (Mahmood et al., 2010), but were allowed free access to drinking water up until 2 hours before the experiment. Negative control group was orally administered with solvent (5% Tween 80, 5ml/kg). Positive control group received oral doses of 20 mg/kg Omeprazole in distilled water (5ml/kg). Experimental groups were orally administered with crude extract in solvent (5% Tween 80, 5ml/kg) at doses of 250 mg/kg and 500 mg/kg. One hour after this pre-treatment, all groups of rats were administered with absolute ethanol (5ml/kg) in order to induce gastric ulcers (Mahmood et al., 2010). The rats were sacrificed 1 hour later (Ketuly et al., 2011) under an overdose of xylazin and ketamine anesthesia and their stomachs were immediately excised.

Measurement of mucus production:
Gastric mucus production was measured in the rats that were subjected to absolute ethanol-induced gastric lesions. The gastric mucosa of each rats was obtained by gentle scraping of the mucosa with a glass slide and the collected mucus were weighed by using a precision electronic balance (Wasman et al., 2010; Ketuly et al., 2011)

Measurement of acid content of gastric juice (pH):
Samples of gastric contents were analyzed for hydrogen ion concentration by pH metric titration with 0.1 N NaOH solutions using digital pH meter (Abdulla et al., 2010).

Gross gastric lesions evaluation:
Ulcers of the gastric mucosa appear as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Gastric mucosa of each rats were examined for any damage. The ulcers were measured by a planimeter under a dissecting microscope (1.8x). The ulcerated area was measured by counting the sum of small squares (2mm x 2mm) which covers fully the length and width of each ulcer band. The ulcer area (UA) then was calculated by using the formula:

UA (mm2) = sum of all small squares x 4 x 1.8

The inhibition percentage (I%) was calculated by using the formula under the recommendation of (Wasman et al., 2010).

(I%) = [(UAcontrol – UAtreated) ÷ UAcontrol] x 100%

Histological evaluation of gastric lesions:
Specimens of the gastric walls of each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sectioning of the stomachs was done at a thickness of 5 µm and was stained with hematoxylin and eosin for histological evaluation (Ketuly et al., 2011).

Statistical analysis
All data except for antibacterial activity were expressed as mean ± standard error mean (SEM). The statistical significance of differences between groups was assessed using one-way ANOVA. A value of $p<0.05$ was considered significant.

Results
The antimicrobial activity of Illicium verum is summarized in Table 1. Illicium verum shows antibacterial activity towards all bacteria tested except for a few gram negative bacteria. The plant extract showed the highest zone of 11mm when used in concentration of 150mg/ml against Escherichia coli and no zone was seen in any concentrations against Kliebsella pneumonia and Pseudomonas aeruginosa.

Table-1 Disc diffusion method (zone of inhibition)

PLANT	ORGANISM	ZONE OF INHIBITION (mm)				
		Positive control	Negative control	50 mg/ml	100 mg/ml	150 mg/ml
Illicium verum Hk.f	*Klebsella pneumonia*	23.0	-	-	-	-
	Pseudomonas aeruginosa	23.0	-	-	-	-
	Bacilus subtilis	17.0	-	7.5	8.5	9.5
	Escherichia coli	24.0	-	8.0	9.0	11.0
	Staphylococcus epidermidis	23.0	-	-	7.0	7.0
	MRSA	17.0	-	8.0	8.0	8.0

1Positive controls : (vancomycin : MRSA, Staphylococcus epidermidis, Bacilus subtilis) ; (imipenem : Pseudomonas aeruginosa, Klebsiella pneumonia) ; (ampicilin : Escherichia coli)

2 Negative control : Absolute ethanol.
Gross evaluation of ethanol-induced gastric lesion model is shown below. Results shows that the rats pre-treated with the plant extracts has significant reduction of ulcer areas as compared to the rats pre-treated with only CMC. Flattening of the mucosa folds were also observed grossly in most of the rats pre-treated with the plant extracts. The reduction of the ulcer areas were comparable with the standard drug used for this experiment, Omeprazole. (Figure 1).

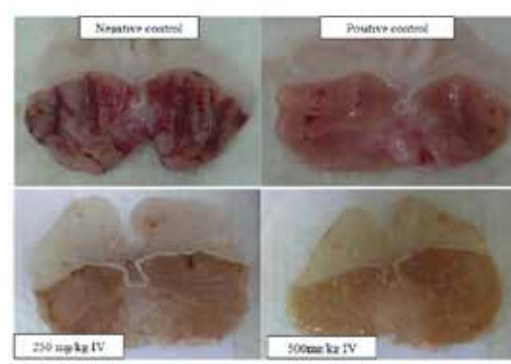


Figure-1: Gross evaluation of ethanol-induced gastric lesion

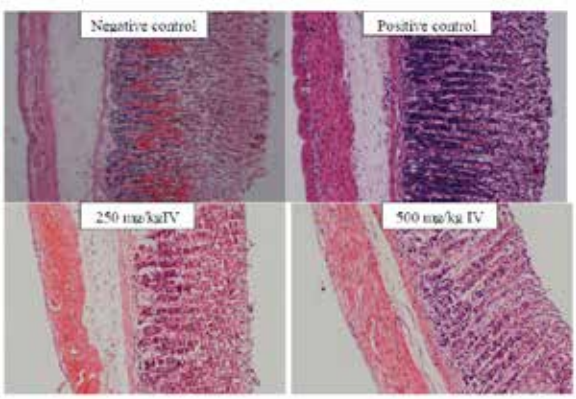
Ulcer area is characterized by hemorrhagic bands on the gastric mucosa. Negative control group shows extensive visible hemorrhagic bands on the mucosa indicating severe mucosal injury. With this, we ensure that the ethanol causes the formation of gastric ulcer.

On the other hand, rats pre-treated with Omeprazole (positive control group) shows mild injuries to the gastric mucosa as compared with the negative control group. Generally, all groups that were pre-treated by plant extracts show the reduction of the ulcer areas. Higher concentration of extract (500 mg/kg) groups show more reduction of ulcer areas compared to the lower concentration of extract (250 mg/kg) groups of rats. The effects of the plant extracts were significant and dose-dependent.

Histology evaluation
Histological evaluation has been done on all gastric tissues of

all groups of treatment. The negative control group, which has been pre-treated with CMC only, showed extensive damage to the gastric mucosa, edema together with infiltration of leucocytes at the submucosal layer. Positive control group on the other hand, showed less damage, absence of edema and reduction in leucocyte infiltration to the submucosal layer. Rats which has been pre-treated by the extracts has also shown better protection as compared to the ones in negative control group. (Figure 2).

Figure 2: Histological evaluation on all gastric tissues of all groups of treatment.



CONCLUSIONS

In conclusion, Illicium verum exhibits some degree of anti bacterial activities against Staphylococcus epidermidis, E.coli, Bacillus subtilis and MRSA. However, it did not exhibit antibacterial activities against Klebsiella pneumonia and Pseudomonas aeruginosa.

This plant protects the gastric mucosa by increasing gastric mucous production, decreasing the acidity of gastric content, reducing the ulcer areas and inhibiting the infiltration of leucocytes to the submucosal layers. However, the exact mechanism of action for antibacterials and antiulcer properties of these plants are still unknown. Deeper studies should be done to determine the exact active principles that contribute to these properties should be done.

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