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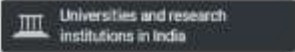
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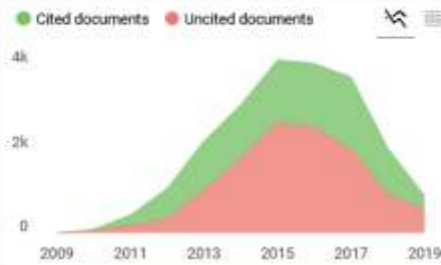
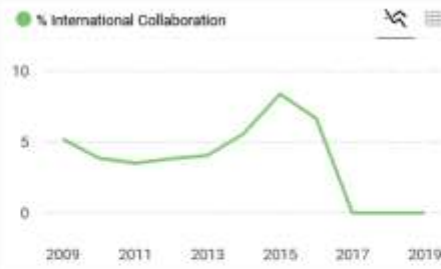
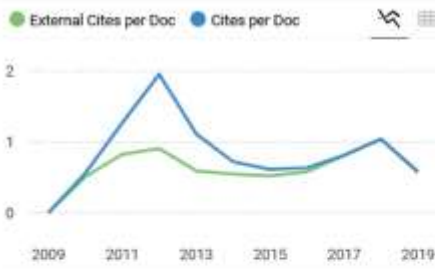
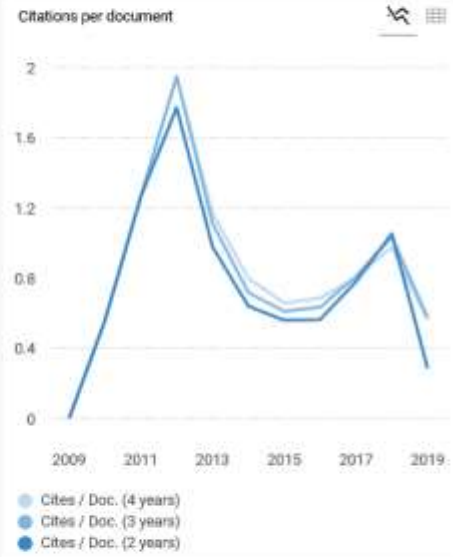
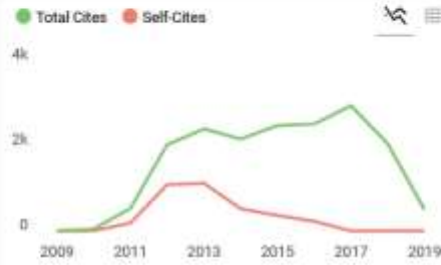
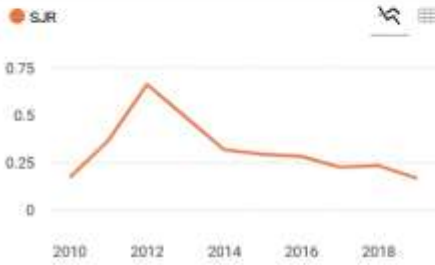
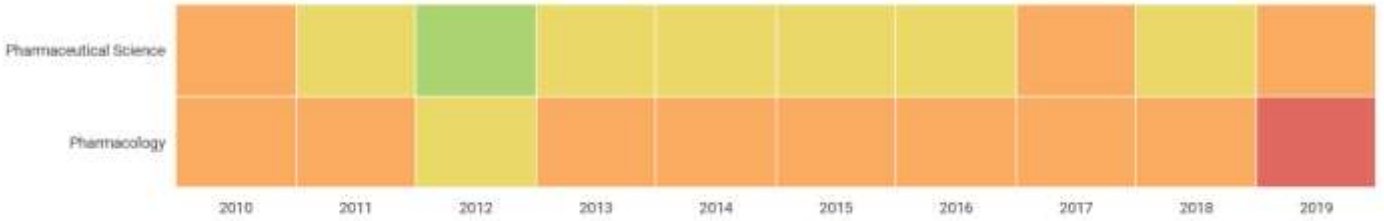
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PHOSPHOLIPID COMPLEX AS A CARRIER OF *KAEMPFERIA GALANGA* RHIZOME EXTRACT TO IMPROVE ITS ANALGESIC ACTIVITY

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ABSTRACT

Preparation of phospholipid complex of *Kaempferia galanga* rhizome extract using phosphatidylcholine was intended to improve the bioavailability of its constituents. Characteristics and analgesic activity of the extract and its marker compound, ethyl *p*-methoxycinnamate (EPMS), were compared to their phospholipid complex (F.Extract and F.EPMS). Characteristics of the free form and their complexes were analysed by DTA and SEM. Their analgesic activity was determined using writhing test. The complex showed a better analgesic activity compared to the free form of both extract and EPMS at an equivalent dosage.

Keywords: *Kaempferia galanga*, Ethyl-*p*-methoxycinnamate, Phospholipid complexes, Analgesic activity

INTRODUCTION

Kaempferia galanga is known as "kencur" and in Java is used in cooking, especially in Indonesian cuisine. A Javanese beverage called "beras kencur" is made from its rhizome. The pasta form of its juice is also used to relief fatigue¹. In Malaysia and Indonesia, this plant is used to make a gargle, the leaves and rhizomes are chewed to treat coughs, or pounded and used in poultices or lotions applied to relieve many topical ailments. The juice of the rhizome is used as an expectorant and carminative, and is often as a part of children's medicine and tonics. The rhizome is also used to treat abdominal pain, swelling and muscular rheumatism².

The major chemical constituents of its volatile oil extracted from dried rhizome were ethyl-*p*-methoxycinnamate (31.77%), methylcinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%)³. Other constituents of the rhizome are cineol, borneol, 3-carene, camphene, kaempferal, cinnamaldehyde, *p*-methoxycinnamic acid, ethyl cinnamate and ethyl *p*-methoxy cinnamate⁴.

Phospholipids are small lipid molecules where glycerol is bonded to two fatty acids, with the third hydroxyl, normally one of the two primary methylenes, bearing a phosphate group⁵. Phospholipids from soy, mainly phosphatidylcholine, are lipophilic substances and readily complex polyphenolic compounds. In this context, phosphatidylcholine, the major molecular building block of cell membranes and a compound miscible in both water and in oil/lipid environments, is well absorbed orally, and has the potential to act as a chaperon for polyphenolics, accompanying them through biological membranes⁶.

Phosphatidylcholine is a major constituent of cell membranes and it is freely compatible with other nutrients, and when coadministered may enhance their absorption. Several studies have demonstrated that complexation of phospholipid with phytoconstituents increases their bioavailability^{7,8}. Based on the properties of phospholipid mentioned above and also the analgesic activity of the plant extract, effect of phospholipid complexes were evaluated and compared against their free form in acetic acids-induced writhing test in mice.

MATERIAL AND METHODS

Plant material

The *Kaempferia galanga* fresh rhizomes used in this study were authenticated and supplied by Purwodadi Botanical Garden, Indonesia. Voucher specimens from the plant samples were deposited at the Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Airlangga University,

Indonesia. The rhizomes were dried at 40°C in hot air oven for 24 hrs to remove any moisture present.

Preparation of extracts

Dried powders of the rhizomes were extracted using 70% ethanol with reflux in a water bath at 40°C for 6 hours. The extract were filtered and evaporated under vacuo to dryness with a rotary evaporator and then placed in an oven at 40°C until constant weight was obtained. The final weights of all extracts were determined. The yield was defined as the percentage of the final extract per dried rhizomes weight and was found to be 14.77%.

Preparation of phosphatidylcholine complexes of extract of *Kaempferia galanga*/EPMS and its characterizations

The PC complexes were prepared at molar ratio 1:1 of EPMS contained in the extract and phospholipid (MW EPMS=206.24; MW PC=760.09). The weighed amount of extract and phospholipid were taken into a 250 ml round bottom flask and absolute ethanol was added. The mixture was refluxed at a temperature not exceeding 60°C for 2h, and an adsorbent (i.e. cab-o-sil) was added to the resulting clear solution. Afterwards, the mixture was evaporated under vacuo to remove the solvents. The resulting complex was kept in an amber colored glass bottle, purging with nitrogen gas to avoid oxidation of lipids during storage, and sealed tightly before stored at 4°C. These experiments were performed in triplicate.

Differential thermal analysis (DTA)

Thermograms of extract, EPMS, and PC in their single components and PC complexes were characterized using a Differential Thermal Analysis (DTA) (Mettler Toledo, Switzerland). The investigations were carried out over the temperature range between 30–280°C at a heating rate of 10°C min⁻¹.

Scanning electron microscope (SEM)

The PC complex of extract and EPMS were characterized by a Scanning Electron Microscope (SEM) (Zeiss EVO@MA10, England) to observe their particle shape and surface morphology.

Experimental animals

Male ICR mice weighing 20-25 g were obtained from the Animal Laboratory Airlangga University, Surabaya, Indonesia. At the beginning of experiments, animals were housed in plastic cages, maintained under 12 h dark light cycles in a temperature-controlled room. They were allowed to adapt to this environment for a period of 1 week before the experiments. Food and water were available *ad libitum*. The study was conducted in accordance with Ethical

Commission on Animal Research of Airlangga University. Every effort was made to minimize the numbers and any suffering of animals used in the experiments.

Acetic acid induced writhing in mice

The method of Koster, et al.⁹ was used to evaluate the antinociceptive activity. Thirty mice were randomly divided into six groups each containing five animals. Equivalent dose of the extract, F.Extract, EPMS and F.EPMS used in this test (30 mg EPMS/kg) was administered orally to each mouse 30 min. before intraperitoneal injection of 0.6% acetic acid in normal saline (10 ml/kg body weight) to induce the characteristic writhing. Cosolvent (10 ml/kg PO) and aspirin (100 mg/kg PO) were given to mice in the control and reference groups, respectively. The mice were observed and counted for the number of abdominal constrictions and stretching in a period of 0-30 min. The responses in the treated groups were compared with those of animals in the control group. The percentage of inhibition of the number of writhing was calculated.

RESULTS AND DISCUSSION

Characterization of phospholipid complex

Differential thermal analysis (DTA)

Thermal analysis was used in order to monitor physical changes (e.g. crystalline transitions, fusion, vaporization, and adsorption) or chemical changes (e.g. dehydration, decomposition) of a sample which occurred as the temperature of a sample increased. Phospholipids (Fig. 1) showed two major peaks at 184.6°C and

195°C. The first (184.6°C) peak is not very sharp, which might indicate melting phase of the non-polar hydrocarbon chain of phospholipids. This melting phase might have occurred in two phases that gave another peak at 195°C respectively, which was relatively sharper. As single compound, EPMS (Fig. 1) showed a sharp endothermic peak at 58.31°C; while in contrast, F.EPMS showed a shifting peak at 178.34°C, which was different from the peaks of the individual components of the complex. Although a small peak still appear referring to the remaining small amount of individual EPMS, it was evident that the original peak of EPMS almost disappeared from the thermogram of PC complex, showed only a small amount of remaining EPMS that was not complexed. For the pure extract, two endothermic peaks were seen in the thermogram. The first peak at 46.5°C might be related with major constituent which contained in the extract i.e. EPMS, and the second broad peak at 127.8°C could be explained as common extract and the lost of water molecules due to dehydration. Interestingly, the similar thermal profile was seen in F.Extract with those that shown in F.EPMS.

Scanning electron micrograph (SEM)

Scanning electron micrographs of the complexes are shown in Fig. 2. The PC complexes, either extract or EPMS were found to be irregular shaped in powdered form. The morphology of their surfaces was seen to be rough. However, its irregular shaped did not affect their flowability as the complex was found to be free flowing particles. The shape and surface morphology, however, might be affected by the type of phospholipids since they are natural components in which their purity grades were determinant factors.

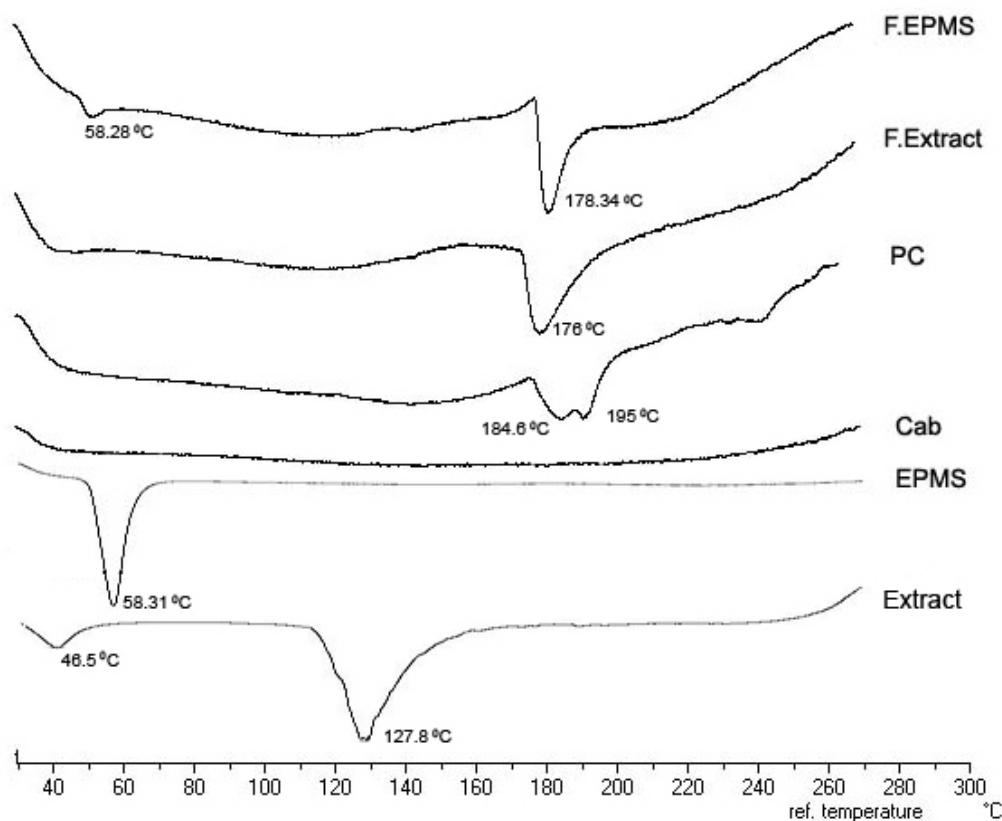


Fig. 1: Thermograms of EPMS, extract Cab-o-sil (Cab), PC, F.EPMS and F.Extract

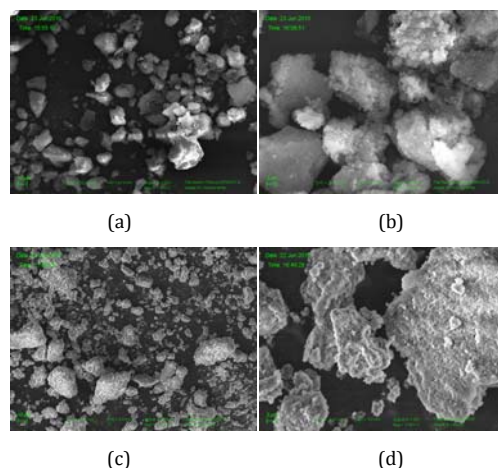


Fig. 2: SEM of PC complex of EPMS: a) 1000X magnification, b) 5000X magnification. PC complex of extract: c) 1000X magnification, d) 5000X magnification.

Acetic acid-induced writhing test

Table 1 shows the effects of the *Kaempferia galanga* extract, EPMS and their phospholipids complex on acetic acid-induced writhing in mice. The oral administration of all samples significantly ($p < 0.001$) inhibited writhing response induced by acetic acid. On comparing the analgesic activity of all drugs in decreasing order it was found to be as: F.EPMS > F.Extract > extract > EPMS \approx aspirin.

In this study, treatment with phospholipid complex, both extract and EPMS, showed better activity than free extract or free EPMS, but the F.EPMS gave higher increase in the analgesic activity than F.Extract if compared with the free form. The enhanced activity of PC complex of both, could be caused by their better absorption due to complexation with phosphotidylcholine.

Table 1: Effect of the extract of *Kaempferia galanga*, EPMS and its phospholipid complexes on acetic acid-induced writhing in mice

Groups	Doses	Number of writhing movements (Mean \pm SEM) 30 min	Percentage of protection
Control	10 ml/kg	75.875 \pm 2.997	-
EPMS	30 mg/kg	45.375 \pm 2.875**	40.20
F.EPMS	equivalent with EPMS 30 mg/kg	22.125 \pm 1.642**	70.84
Extract	equivalent with EPMS 30 mg/kg	37.500 \pm 2.268**	50.58
F.Extract	equivalent with EPMS 30 mg/kg	30.875 \pm 1.642**	59.31
Aspirin	100 mg/kg	46.875 \pm 2.232**	38.22

Values are mean \pm SEM, (n = 5), **: $p < 0.001$, Bunferroni test as compared to control.

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