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Review Article

Extraction and Isolation of Phytochemicals from *Kaempferia rotunda* Linn. (White Turmeric) for Pharmacological Application: A Review

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ABSTRACT

Kaempferia rotunda (white turmeric) is an indigenous plant from Southeast Asia that is traditionally used for human health. The plant has been widely used, especially in the main and tuberous parts of the rhizome, which are rich in essential oils. This review examines the effects of various extraction methods and solvents on the phytochemical composition of *K. rotunda* as well as the pharmacological activities of the phytochemicals from the extracts. Several databases such as Scopus, Pubmed, and Google Scholar were used to search for published articles within the last ten years using specific keywords. The outcomes showed that *Kaempferia rotunda* is extracted by several extraction methods, such as maceration, reflux, and soxhletation using suitable organic solvents. Meanwhile, the maceration method is mostly preferred to extract its phytochemical compounds due to its ease in technicalities and the acquisition of isolated compounds. Various phytochemicals in each solvent extraction exhibit a variety of pharmacological activities, including antioxidants, antielastases, antithyrosinases, UV protection, antibacterial, antimutagenic, anticancer, antinociceptive, antihyperglycemic, antiallergic, antiandrogenic, anthelmintic, and wound healing. Benzyl benzoate, crotepoxide, 5-hydroxy-7-methoxyflavanone, 7-hydroxy-5-methoxyflavanone7-dihydroxyflavanone, and methyl-D-galactopyranoside (lectin specific) were isolated by chromatography method to determine the phytochemicals responsible for its pharmacological activities and mechanism. Subsequently, their pharmacological activities were tested *in vitro*. The findings of this study demonstrate the relationships among several elements, such as extraction methods, solvent, duration of extraction, pharmacological activities of extracts, and isolated phytochemicals from *K. rotunda*.

***Keywords*:** *Kaempferia rotunda*, Isolation, Pharmacological activity, Phytochemicals, Solvent extraction, White turmeric.

Introduction

Kaempferia rotunda Linn. is known as white turmeric or kunir putih gombyok in Indonesia, while it is called Bhumicampaka in India, where it has been used in ancient traditional Ayurvedic medicine for thousands of years. Similarly, the plant has also been traditionally used in Indonesia as a treatment for diarrhea, and colds. Its rhizomes are used for treating obesity.[1-3] *Kaempferia rotunda* is a member of the Zingiberaceae family that has not been fully explored compared to *K. galanga* (kencur) and *K. pandurata* (temu kunci). Different solvent extraction methods are expected to provide a general overview of the class of compounds in the solvent that are responsible for specific biological activities.[4] The extracts and their separated components from *K. rotunda* have been shown in previous research to have a variety of pharmacological properties, including anticancer, antioxidant, antibacterial, antiandrogenic, wound healing, and antihyperglycemic.[5-7]

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These bioactive compounds and the various pharmacological activities they have offer insight and prospects for the development of modern drugs of Indonesian origin, or Obat Modern Asli Indonesia (OMAI), which are anticipated to be less harmful than conventional medications.[8] The phytochemical content of the *Kaempferia* has been linked to anticancer, antibacterial, and antioxidant effects in previous research.[4] However, their extraction method and the biological activities of isolated compounds have not been reported. It is well known that the *Kaempferia* species contains a lot of essential oils, camphor, and methoxiflavone compounds. Additionally, pinostrobin (5-Hydroxy-7-methoxyflavanone) isolated from its chloroform fraction of methanolic extract inhibited T47D cancer cells. The methanolic extract was obtained by maceration.[7] Meanwhile, it was reported in a study by Diastuti et al.,[1] that the main poisonous component of *K. rotunda* essential oils is benzyl benzoate, which was tested using the Brine Shrimp lethality assay with an LC_{50} value of 173.49 µg/mL.
The present review was aimed at exploring the effects of the different extraction methods and solvents on the phytochemical constituents of *K. rotunda.* More so, the pharmacological activities of the bioactive compounds from the extracts were examined.

Materials and Methods

In this review, several databases such as Scopus, Pubmed, and Google Scholar were consulted. During the search, the keywords, "pharmacological activity" and "*Kaempferia rotunda*" were used. The primary criterion for choosing references is the original research

articles published within the last ten years that provide details on the extraction procedure and solvent, in addition to a pharmacological activity test. In addition, information on isolated phytochemicals obtained from bioassay-guided and isolation methods as well as their pharmacological activities was also included. The exclusion criteria were articles with unclear methods. The details of the literature selection are presented in Figure 1.

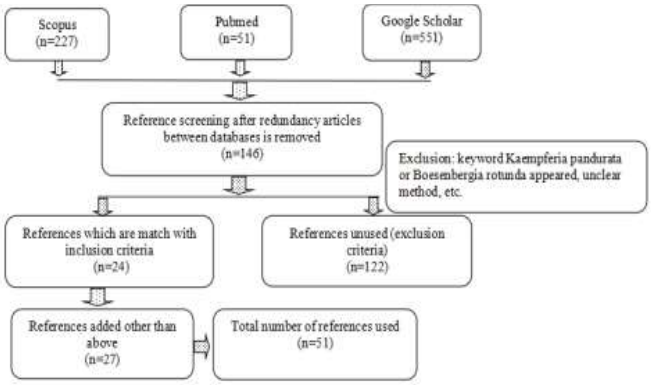

Figure 1: Literature selection process.

Results and Discussion

Extraction methods for Kaempferia rotunda

The initial step in developing a medicinal plant into a drug is to evaluate its ability to manifest a pharmacological activity.[9–11] Meanwhile, the pharmacological effects of a medicinal plant are influenced by its phytochemical compounds.[12,13] Extraction is the first step that determines the discovery of bioactive compounds in medicinal plants. The method employed must satisfy criteria such as cost efficiency, environmental friendliness, and ease of use.[14] Extraction is the process of separating the dissolved metabolites from the produced phytochemical substances and leaving the undissolved residues.[15] With the aid of an appropriate solvent extraction technique, it also offers the highest level of the phytochemical compounds. Table 1 details the extraction methods used for *K. rotunda* with specific solvents and the pharmacological properties of the extracts.

Maceration is a method that has been widely adopted and used in medicinal plant research. The stages involve the soaking of the powder of the simplicia in a closed container with a solvent at room temperature for a certain period. This is followed by a periodic agitation process that is expected to disrupt the simplicia cell wall and release phytochemical compounds that can dissolve in the extraction solvent.[15,17] The maceration extraction technique addressed in this review article is the standard method that uses a variety of solvents to identify many isolated chemicals responsible for their pharmacological activities. It has been demonstrated that the TLC profiles of the ethanol and ethyl acetate extracts of *K. rotunda* obtained with the maceration method differ. In a polar solvent such as methanol: chloroform (5:1), the extract was separated into seven compounds, but more compounds were obtained in a semi-polar solvent.[18] The difference was influenced by the polarity of the extraction solvents. The TLC chemical profile of the ethanol extract did not reveal the non-polar molecule at Rf 0.167 that was present in the ethyl acetate extract. In a different study, qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, and terpenoids in n-hexane extract from *K. rotunda* macerated for 24 hours. It was also reported that these compounds were also present in the ethanol and ethyl acetate extracts.[19] Conversely, Suphrom et al.,[2] showed that 3 days of hexane maceration produced different compounds. As analyzed by GC-MS, the groups of volatile compounds such as monoterpenes, benzyl derivatives, sesquiterpenes, hydrocarbons, diterpenes, crotepoxide fatty acid esters, and sterols were detected. The analysis revealed that the highest content in the extract was benzyl benzoate (18.92%), followed by pentadecan (10.90%), and crotepoksid (2.68%). Anthraquinone glycosides were also present when ethyl acetate and water at 30-40^{o}C were employed for maceration for 72 hours. [20] Reflux is a method of extraction that uses heat at the boiling point of the solvent. This method is more efficient than maceration because it requires a short time and less solvent. However, the disadvantage is that it cannot be used to isolate thermolabile compounds. A study that employed the reflux extraction method for *K. rotunda* with 96% ethanol for 1 hour revealed the presence of flavonoids, steroids/ terpenoids, and essential oils with a yield of 8.36%. [21] This was significantly higher compared to the results of the maceration using ethanol as a solvent, with a yield of 1.62%.[22] The low yield with maceration may be due to the shorter time of maceration, which was 30 minutes.[22,23] The types of compounds present in the reflux method were also relatively more diverse. This is because high temperatures promote solubility and diffusion while simultaneously having the potential to decompose thermolabile substances.[24,25]

Soxhletation is an extraction technique using continuous heat. A high temperature is used in this extraction process. The prolonged extraction time may cause the degradation of thermolabile compounds.[15,26,27] This method is an integration of reflux and percolation methods, which use a solvent that is always new.[24] A study conducted using soxhletation showed that methanol and water extracts of *K. rotunda* contain steroids, including *β*-sitosterol, stigmasterol, and less polar phenolic compounds such as chalcone.[28] The extraction yield by this method was observed to be the highest compared to other extraction methods, where the methanol extract of *K. rotunda* had a yield of 8.5%.[29] Meanwhile, the occurrence of a repetitive cycle allows for higher levels of the extracted compounds and generally non-polar compounds found in the soxhletated extract. The process is expected to continue until the flowing solvent leaves no residue. The advantage of the soxhletation method is that large quantities of samples can be extracted with little solvent and do not require a filtration step.[30]

The outcomes of the extraction of natural materials are influenced by the extraction methods, solvent selection, temperature, extraction time, and size of the particle of the extracted plant part.[24] In solvent extraction, the choice of solvent is crucial and is influenced by the kind and part of the plant, the properties of the target compounds, and the availability of solvent.[4] Selectivity, cost, recoverability, solubility, viscosity, and safety must all be taken into account. A good performance of an organic solvent occurs when the polarity value is close to that of the solute or vice versa. The polarity of the solvent and the dissolved components vary, which has an impact on the chemical components in plants that control variation in yield.[19] This is demonstrated by the extraction of curcumin using different solvents.

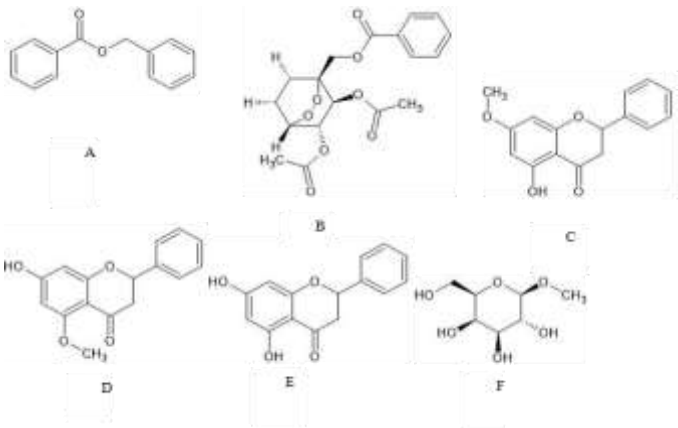

Figure 2: Phytochemical compounds isolated from *Kaempferia rotunda* rhizome and tuber. A: Benzyl benzoate;[51] B: Crotepoxide;[51] C: 5-hydroxy-7-methoxyflavanone (pinostrobin); D: 7-hydroxy-5-methoxyflavanone;[43] E: 5,7-dihydroxyflavanone;[43] F: Methyl-β-D-galactopyranoside (specific lectin).

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Table 1: Extraction methods and the pharmacological activities of the extracts from *Kaempferia rotunda*

Extraction	Solvent	Duration	Phytoconstituents	Activity	Ref.
Maceration	Water	72h	alkaloids, flavonoids, saponins, anthraquinone glycosides	Antimicrobe	20
	Methanol	72h	alkaloids, flavonoids, saponins, terpenoids, steroids	Antimicrobe	20
	Methanol	48h	-	Antihyperglycemic, antinociceptive	7
	Ethanol	24h	Alkaloids, flavonoids, terpenoids	Antioxidant, antityrosinase	19
	Ethanol	24h	-	Antibacterial, antioxidant	31
	Ethanol	30 min	Flavonoids	Antioxidant, UV protecting	22
	Ethanol	-	Alkaloids, flavonoids, terpenoids	Anticancer	18
	Ethyl acetate	24h	Alkaloids, flavonoids, terpenoids	Antioxidant, antityrosinase	19
	Ethyl acetate	24h	-	Antibacterial, antioxidant	31
	Ethyl acetate	-	flavonoids, terpenoids, alkaloids, tannins	Anticancer	18
	Ethyl acetate	72h	alkaloids, flavonoids, terpenoids, steroids, anthraquinone glycosides	Antimicrobe	20
	Hexane	3d	Monoterpene, benzyl derivative, sesquiterpene, hydrocarbon, diterpene, ester of fatty acid, cyclohexane diepoxide, sterol	Antiandrogenic	2
	Hexane	24h	Alkaloids, flavonoids, terpenoids	Antioxidant, antityrosinase	19
	Hexane	24h	-	Antioxidant, antimicrobe	31
	Hexane	72h	Alkaloids, terpenoids, flavonoids, steroids	Antimicrobe	20
	Purified with Petroleum ether, then extracted with ethanol	-	-	Antiplanctonic, antibiofilm	39
Reflux	water	3h	-	Antiallergic	36
	ethanol	3h	-	Antiallergic	36
	ethanol	1h	Saponins, flavonoids, triterpenoids, volatile oil	Antioxidant, antielastase	21
Soxhlet	water	-	Syringic acid, quercetin, stigmasterol, β-sitosterol, Flavonoids, crotepoxide, chalcones, protocatechuic acid,	Wound healing	28
	methanol	-	Some hydrocarbons, syringic acid, β-sitosterol, stigmasterol, chalcones, quercetin, protocatechuic acid, flavonoids, crotepoxide	Wound healing	28
	methanol	-	stigmasterol, syringic acid, some hydrocarbons, flavonoids, procatechuic acid, β-sitosterol, flavonols, crotepoxide, chalcones, quercetin	anthelmintic	29

Maceration of *K. rotunda* rhizomes using hexane, ethyl acetate, and ethanol (with a ratio of 1:7) for 24 hours revealed that ethanol was the most selective solvent compared to ethyl acetate and hexane, with the maximum level of curcumin dissolving in ethanol at 1.92 µL/mL.[31] This study showed that polar solvents such as water, methanol, and ethanol are generally used in the extraction of polar compounds, while nonpolar solvents such as hexane and petroleum ether are used in the extraction of nonpolar compounds.[16,30] From most references cited in this review, the ethanol group is considered the solvent that exhibits the most pharmacological activities. The option for subsequent phases such as fractionation to isolation is made possible by the capacity of an organic solvent to extract certain phytochemical components.

Extraction time is another important aspect that affects the outcome of the extraction, and it is determined by the high solids to solvent ratio. A high ratio will likely result in a large number of dissolved compounds in the sample, which will increase the concentration but consequently increase the extraction time.[24] A study found that a longer extraction period produced the maximum yield of patchouli alcohol. The study revealed that by increasing extraction time, some compounds were reduced, while others increased, and some components remained

unaffected. The extraction time also affected the profile of the compounds produced.[32] Extraction time is important in minimizing the energy and cost of the extraction process.[33] A study conducted by Kavak,[34] showed that the total phenolic content (TPC) of the compound, which was visible after 30 minutes of the extraction procedure, did not change significantly after 90 minutes. Therefore, the optimal extraction time is crucial to determining the effectiveness of the extraction process and its effects.[35]

Pharmacological activities of Kaempferia rotunda extracts

Qualitative analysis and phytochemical screening of the extracts showed the presence of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, etc. (Table 1). These compounds were reported to have pharmacological activities such as allergy, antihyperglycemic, anti-cancer, anti-tumor, antifungal, and anti-proliferation.[36–38] *Kaempferia rotunda*, especially the tuber, can be consumed directly. The plant has traditionally been used as an antitumor agent, for stomach aches, and diarrhea. Also, it facilitates breastfeeding and herbal concoctions after delivery.[39] *Kaempferia rotunda* is rich in flavones, epoxides, essential oils, and other phytochemicals that have promising biological activity and pharmacological effects.[40,41] A total of 9 polyoxygenated cyclohexane derivatives, which include the crotepoxide compound, were isolated from *K. rotunda*. This compound had an antifeedant effect against the larvae of *Spodoptera littoralis*.[42] Meanwhile, isolated pinostrobin and two flavone compounds, such as 7-hydroxy-5-methoxyflavanone and 5,7-dihidroxyflavanone were active as antimutagenic (pinostrobin) and inhibited the growth of breast cancer cells.[38,43] Tables 1 and 2 depict the pharmacological activities of the extracts and phytochemicals from *K. rotunda*.

Antimicrobial activity of Kaempferia rotunda extracts

Alkaloids, flavonoids, saponins, anthraquinone glycosides, terpenoids, and steroids were reportedly present in the water, methanol, ethyl acetate, and n-hexane extracts produced by the maceration procedure. These extracts inhibited seven types of pathogenic bacteria that attack the respiratory tract.[20] Ethyl acetate extract had the highest inhibitory activity on *L. acidophilus* with an inhibition zone of 17.3±0.57 mm, followed by *S. pneumonia* (16.6±0.28 mm), *S. pyogenes* (16.6±0.28 mm), and *P. aeruginosa* (15.3±0.28 mm), respectively. In the ethyl acetate extract screening, terpenoids and steroids predominate, but anthraquinone flavonoids, alkaloids, and glycosides showed positive results while being less dominant. The low content of flavonoids or phenolic compounds in the ethyl acetate extracts further suggests that these substances may not be responsible for the effects.[44] Other studies also reported antimicrobial activity of ethyl acetate extract,[31] which had the highest activity in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli,* with inhibition zone values of 5.32±0.12 and 5.21±0.01 mm, respectively.

De-lipidation of ethanol extract with petroleum ether is a common practice to remove fatty compounds. This purified extract exhibited antimicrobial activity against *Staphylococcus aureus* with an MIC_{50} of 0.5 µg/mL, antibiofilm formation of *S. aureus* with an IC_{50} value of 0.125 mg/mL, and biofilm degradation activity against *P. aeruginosa* and *S. aureus* with the same IC_{50} value of 0.5 mg/mL.[39] The alkaloids, flavonoids, and terpenoids, which are dissolved in ethanol, are thought to be responsible for the effect.[19] The antibacterial activities of the isolated compounds from acetone extract are detected using the Kirby Bauer diffusion method. The results showed that crotepoxide and benzyl benzoate compounds have weaker activities in inhibiting four pathogenic bacteria, namely *Escherichia coli*, *Enterococcus aerogenes*, *Bacillus cereus*, and *Staphylococcus aureus* compared to the crude acetone extract and n-hexane fraction. Benzyl benzoate showed moderate activity in inhibiting *B. cereus* with an inhibition zone of 5.9–9.9 mm at a concentration of 50–500 µg/mL. Furthermore, inhibitory zones of 5.2–7.0 and 6.1–9.1 mm were recorded against *E. coli* and *S. aureus*, respectively at a benzyl benzoate concentration of 100–500 µg/mL, and an inhibitory zone of 8.9 mm against *E. aerogenosa* at a concentration of 500 µg/mL. Meanwhile, crotepoxide did not have activity against other bacteria, it exhibited a moderate antibacterial activity against *E. aerogenes* at a concentration of 100–500 µg/mL with an inhibitory zone of 6.1–8. 6 mm and *B. cereus* at 500 µg/mL with an inhibition zone of 7.0 mm. The study demonstrated that the isolated compounds were not necessarily more active than the crude extracts. This phenomenon might be due to the presence of metabolites having a synergistic effect in the extracts, to inhibit the growth of these bacteria.[1]

Anti-hyperglycemic potential of Kaempferia rotunda extracts

Kaempferia rotunda rhizome methanolic extract has been tested in mice for the effect of lowering blood glucose by administering the extract at doses of 50, 100, 200, and 400 mg/Kg BW. In comparison to the conventional drug (glibenclamide) at a dose of 10 mg/Kg BW, the administration of the extract at a concentration of 400 mg/Kg BW resulted in the greatest reduction in blood glucose (39.5%). In the study, the decrease in blood glucose levels was achieved individually by the extract or in combination. The extract may contain compounds that increase the potential of insulin secretion in the pancreas to control blood glucose levels.[7]

Antinociceptive action of Kaempferia rotunda extracts

The antinociceptive action was demonstrated in a study using test animals and a methanol extract dose of 400 mg/Kg BW (an effect that was dose-dependent). When compared to control aspirin, which reduced stomach stretching by 73.4% at the same dose, this extract reduced it by 69.4%. The effect was observed through acetic acid-induced writhing tests. Chemical compounds that can reduce stomach constriction indicate that they have an analgesic effect through inhibition of prostaglandin secretion. The antinociceptive effect of *K. rotunda* extract provided evidence of its rhizome's traditional use as pain relief due to bumps, bruises, and headaches.[7]

Antioxidant capacity of Kaempferia rotunda extracts

To evaluate the antioxidant capacity of *K. rotunda* extract, a total of three extracts were compared. In this investigation, the ethanolic extract outperformed the ethyl acetate and hexane extracts in terms of their ability to inhibit DPPH (2,2-Diphenyl-1-picrylhydrazyl) radicals, with an IC_{50} value of 67.95 µg/mL. The ethanol extract had the highest TPC, at 5.11 µg/mL, while the ethyl acetate and hexane extracts came in at 3.33 and 1.98 µg/mL, respectively.[31] The antioxidant activity is due to phenolic compounds, which bind oxygen to avoid the oxidation process. Phenolic compounds also bind metals that catalyze oxidation reactions.[31,45] In another study, the ethanolic extract of *K. rotunda* rhizome obtained by reflux method also showed antioxidant activity. The extract, which contained saponins, flavonoids, triterpenoids, and volatile oils, demonstrated weak activity against DPPH radicals (IC_{50} 193.71 µ/mL) as determined by the DPPH method.[1] With the reflux method, the quantity of the flavonoid or saponin compounds that serve as electron donors may be low or even damage due to heating. This necessitates a quantitative assessment of the total flavonoid content (TFC) before embarking on the DPPH testing.[46] On the other hand, the highest inhibition level was obtained from the ethyl acetate extract from *K. rotunda* rhizome, with a value of 84.02% compared to the 16.95% inhibition of hexane and 19.92% of ethanol solvents. These three extracts, namely hexane, ethyl acetate, and ethanol extracts, were also tested on ABTS radical scavenging activity, yielding values of 67.24, 307.18, and 280.18 mg TEAC/g extract, respectively, by quantitatively analyzing the Trolox Equivalent Antioxidant Capacity (TEAC) in each gram of extract.[19] In other studies, the antioxidant capacity of *K. rotunda* ethanolic extract was analyzed by DPPH, ABTS, and FRAP methods. The results showed that the highest capacity was from the FRAP method, at 119.6±3.86 µM Trolox/gram of dry simplicia. [22]

Antityrosinase/ UV protecting/ and antielastase activities of Kaempferia rotunda extracts

Tyrosinase is a key enzyme that is responsible for brownish spots and aging on human skin. The hexane, ethyl acetate, and ethanolic extracts of *K. rotunda* were reported to inhibit the activity of the tyrosinase enzyme with an IC_{50} >12.5 µg/mL. In the study, Kojic acid was used as a positive control, yielding an IC_{50} value of 0.01 µg/mL. The TFC detected in the three extracts may inhibit the synthesis of melanin and tyrosinase as key enzymes. However, the strength of the inhibition of the tyrosinase enzyme depends on the concentration of the compounds

in each type of solvent and the extraction method used.[19] In other investigations, the ethanol extract of *K. rotunda* rhizome was tested as a sun-protecting agent with a spectrophotometric method. The study also evaluated the TPC and antioxidant capacity through ABTS, DPPH, and FRAP assays.[22] The results showed that the ability of *K. rotunda* ethanol extract as sun protection started at SPF 40-100 and did not show any effect at SPF 20. This showed that the TFC and antioxidant capacity are directly proportional to the ability to function as a sun protector. Elastase is one of the enzymes that destroys the extracellular matrix components in the skin.[47] When the elastase level increases, it accelerates the skin aging process. These levels are influenced by an increase in reactive oxygen species (ROS), which causes oxidative stress and triggers the enzyme elastase. The ability of *K. rotunda* extract to inhibit the elastase enzyme level has been reported.[21] The results showed 40.82% inhibition, which is lower than that achieved by *Curcuma zedoaria*, with a value of 49.24%. In Java, these two plants are called white turmeric. In the study, the elastase enzyme was 85.72% inhibited by the control substance, epigallocathecingallat (EGCG). The lower inhibition of *K. rotunda* ethanolic extract was due to the lower antioxidant activity. Antioxidants and antielastases are frequently employed as measures to test the early signs of skin aging.

Anthelmintic properties of Kaempferia rotunda extracts

One of the traditional uses of *K. rotunda* rhizome is as an anthelmintic agent. According to Agrawal et al.,[29] a 100 mg/mL methanol extract of *K. rotunda* was more effective than a conventional drug, piperazine citrate, in killing *Pheretima posthuma* and *Ascardia galli* worms. It was hypothesized that the phenolic substance in the methanolic extract is responsible for the action.

Antiallergic characteristics of Kaempferia rotunda extracts

The test of antiallergic activity was evaluated by determining the effects of the extracts on the inhibition of β-hexosaminidase expression in RBL-2H3 cells. It was discovered that both ethanol and water extracts inhibited the enzyme release. The β-hexosaminidase enzyme is contained within granules that are secreted by mast cells, where histamine is stored. It is expressed together with histamine when it is immunologically activated and causes an allergic reaction. The inhibition of the enzyme is used as a marker of degranulation in an allergic reaction. In the study, the ethanol extract showed stronger inhibitory activity with an IC_{50} value of 70.12 µg/mL compared to the water extract, which had an IC_{50} value of >100 µg/mL.[36] However, further research was recommended to determine the mechanism of the extract and the bioactive compounds that are responsible for these effects.

Wound healing activity of Kaempferia rotunda extracts

A study demonstrated that both aqueous and methanolic extracts of *K. rotunda* showed significant wound healing activity in albino rats on days 4, 8, and 12. In the study, the test animals were categorized into two dose groups, anesthetized with ether, and two vertebral incisions were applied on the shaved back skin. The wound was closed with sutures and opened on the 7th day, while the breaking strength was measured in anesthetized mice on the 11th day after the wound. A significant improvement in the incision wound healing was shown by the two treatment groups that were administered aqueous and methanolic extracts at concentrations of 250 and 500 mg/Kg BW, respectively. Both extracts affected the rate of contraction time of the excision wound.[28] These findings support the traditional usage of *K. rotunda* for bruise relief and wound healing.

Antiandrogenic effect of Kaempferia rotunda extracts

Studies on the chemical compounds in hexane extract fractionated with ethanol and dichloromethane showed that the compounds had an inhibitory effect on the hormone testosterone. The study showed that hexane extract exhibited the strongest inhibition (IC_{50} 0.43 µg/mL) compared to the dichloromethane fraction (IC_{50} 1.17 µg/mL) and ethanol (IC_{50}> 10 µg/mL) against the positive control of ethinylestradiol (IC_{50} 0.26 µg/mL).[2] Furthermore, the chemical compounds contained in hexane extract as analyzed by GC-MS showed that they are more complex than those in dichloromethane and ethanol fractions. The hexane extract contains higher monoterpene and sesquiterpenes than the other two extracts. The study showed a correlation between antiandrogenic activity and terpenoid content. Similarly, the antiandrogenic effects of sesquiterpenes isolated from *Curcuma aeruginosa* (germacrene) have also been reported.[48]

Anticancer/ antitumor/ antimutagenic/ antiproliferation activities of Kaempferia rotunda extracts

According to Ahmed et al.,[37] the tuberous rhizome portion of *K. rotunda* contained a particular lectin (methyl-D-galactopyranoside) that was extracted. This compound exhibited an *in vitro* anti-tumor activity. The lectin inhibited the activity of Ehrlich Ascites Carcinoma (EAC) cells at pH between 6-9, at 30-80°C in EAC mice. Meanwhile, other types of lectins also showed an anti-proliferative effect by inhibiting EAC cells *in vivo* with 51 and 67% inhibitory effects on animals that were administered at doses of 1.25 and 2.5 mg/kg BW/day, respectively, for 5 consecutive days.[49] A study on lectin activity was also reported through their ability to inhibit SW480 and SW48 human colon cancer cells by 67 and 59%, respectively, at a concentration of 1 mg/mL. The evaluation of lectin activity on the proliferation and growth of EAC cancer cells has also been reported. Islam et al.,[50] demonstrated the anti-proliferation, and anticancer mechanisms of this compound based on cancer cell morphology, analysis of the cell cycle, and apoptotic protein expression in SW480 and SW48 cells. Lectin inhibited the proliferation of colorectal cancer cells (SW48 and SW480) by induction of apoptosis as confirmed by fluorometric assays, flow cytometric studies, caspase inhibitors, and various protein expressions. Mitochondrial intrinsic pathway apoptosis was activated followed by the administration of the lectin.

Ethanol and ethyl acetate extracts of *K. rotunda* were reported to be cytotoxic on HELA cells. According to the investigation, the ethanol extract exhibited a more potent cytotoxic activity (IC_{50} 16.39 µg/mL) than the ethyl acetate extract (IC_{50} 127.9 µg/mL). Cisplatin (IC_{50} 1.28 µg/mL) was employed in the investigation as a positive control.[18] Meanwhile, in the another study, it was reported that three flavone compounds, namely 5-hydroxy-7-methoxyflavanone (pinostrobin), 7-hydroxy-5-methoxyflavanone, and 5,7-dihydroxyflavanone isolated, from the methanol extract of *K. rotunda* have antimutagenic effects on male Balb-C mice (8–12 weeks) induced by cyclophosphamide.[43] DNA gene mutations, which occur during carcinogenesis and also play a role in the pathogenesis of chronic degenerative disorders, have been highlighted as one of the causes of cancer. In the study, the *in vivo* antimutagenic activity of the isolated compounds and methanol extract was investigated using the bone marrow micronucleus assay method. The results showed that the three flavone compounds had inhibitory effects of 56.5, 93, and 96.5% at a dose of 30 mg/ Kg BW, whereas they exhibited stronger inhibition at a dose of 60 mg/Kg BW (96.5-100%).[43] At dosages of 300 and 600 mg/kg BW, the crude methanol extract also demonstrated antimutagenic properties, inhibiting 55 and 80% of gene mutases, respectively. Because crude extracts contain a variety of flavone compounds that work synergistically, they have significant antimutagenic activity.

Isolated compounds of Kaempferia rotunda

According to Woerdenbag et al., the mono and sesquiterpene contents of essential oils from *K. rotunda* is significantly limited, while aromatic (benzoates and salicylates) and aliphatic compounds are predominant.[3] In the study, the essential oil samples were separated into two fractions, namely hydrocarbons and oxygen-containing compounds, which were analyzed by GCMS. The results showed that benzyl benzoate was the most abundant component, with a total of 69.7% in the main part of the rhizome, and 20.1% in the tubers. Another study reported different isolation methods of benzyl benzoate from *K. rotunda*. The study reported the hexane layer of acetone extract of *K. rotunda* rhizome, which was fractionated by vacuum liquid chromatography (VLC) using an n-hexane gradient solvent, the mixture of n-hexane: chloroform (with a ratio of 7:3, 6:4, 5:5, 2:8, and 1:9), chloroform, and ethyl acetate as displayed in Table 2. Subsequently, the fractions obtained were monitored by TLC and similar compound profiles were combined. A total of seven fractions were further separated by column chromatography with hexane: chloroform (9:1) as the mobile phase.

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Table 2: Extraction, isolation, and bioactivity of compounds isolated from *Kaempferia rotunda*.

	Extraction			Isolation		Activity	Ref.
Isolated compound	**Method**	**Solvent**	**Duration**	**Method**	**Solvent**		
Benzyl benzoate	maceration	Acetone	-	Vacuum column chromatography, gradient eluted, then purified by column chromatography, recrystallization	n-hexane, the mixture of n-hexane:chloroform (7:3, 6:4, 5:5, 2:8 and 1:9) and n-hexane:chloroform (9:1) (CG)	antibacterial	1
Crotepoxide	maceration	Acetone		By column chromatography	n-hexane, chloroform, ethyl acetate (5:5:1)	antibacterial	1
5-hiydroxy-7-methoxyflavanone (pinostrobin)	Maceration	Methanol		Vacuum column chromatography, recrystallization (ethyl acetate fraction) Recrystallization (hexane fraction) VLC Recrystallization (chloroform fraction)	n-hexane, n-hexane:ethyl acetate (gradient system), ethyl acetate, acetone, then last one is methanol n-hexane, n-hexane-ethyl acetate (gradient system), ethyl acetate, acetone, then last one is methanol	antimutagenic	43
7-hydroxy-5-methoxyflavanone	Maceration	Methanol		column chromatography gravitation (from chloroform fraction)	Hexane: ethyl acetate (6:4)	antimutagenic	43
5,7-dihydroxyflavanone	Maceration	Methanol		Recrystallization From ethyl acetate and hexane fraction		antimutagenic	43
Lectin	Maceration	Tris-HCl buffer 150 mM NaCl (500 ml buffer was used for 100 gram tuber) at pH 8.2	-	glucose-Sepharose (glucose linked to epichlorohydrin-activated sepharose-4B), by chromatography column of QA cellulose, verified by gel electrophoresis, affinity chromatography glucose-Sepharose	Buffer Tris HCl pH 8,2 then add sodium buffer saline acetate pH 4,6 ; sodium chloride salt gradient, TBS 10 mM, pH 8,2	Antiproliferation, antifungal, antibacterial, bacterial agglutinating, Anticancer (Ehrlich ascites carcinoma), anticolon cancer	37,49,50

The results demonstrated that fraction 1 had the highest yield, with a value of 9.5 g, to produce pure benzyl benzoate in the form of a colourless oils (543 mg yield). [51]

Moreover, several investigations reported the extraction of crotepoxide and benzyl benzoate using the semi-polar fraction (ethyl acetate) of acetone extract. The isolation was conducted by the VLC method using a solvent gradient n-hexane system: ethyl acetate (8:2, 7.5:2.5, 7:3, and 0:10), which resulted to five sub-fractions, namely F1 (0.05 g), F2 (0.1 g), F3 (0.3 g), F4 (0.9 g), and F5 (1.3 g). The crotepoxide was further isolated from F5 by the column chromatography method (Figure 2B).[51] Atun et al.,[43] also isolated antimutagenic compounds from the methanolic extract (Figure 2C, D, and E) of *K. rotunda*. The first compound, namely 5-hydroxy-7-methoxyflavanone (pinostrobin), a colourless crystal that has a flavanone framework with substituted methoxyl and hydroxyl groups based on elucidation data using the HMBC instrument, was obtained. This compound was from sub-fraction A (3.5 g), which was isolated from the ethyl acetate fraction with VLC gradient eluent. Meanwhile, the second isolated flavone compound was 7-hydroxy-5-methoxyflavanone, which appeared as a pale-yellow crystal. Compound 2 was from sub-fraction B (10 g) isolated by column chromatography using hexane: ethyl acetate (6:4) as eluent to obtain 48 fractions. Afterward, the compounds from sub-fractions 13-21 were combined, concentrated, and recrystallized to give a pale-yellow crystal of 7-hydroxy-5-methoxyflavanone, namely compound 2, which was 1.2 g. The third compound, 5,7-dihydroxyflavanone, was from the chloroform fraction, which was further isolated by VLC using a gradient solvent system to give 20 sub-fractions. The compound was found to be produced by sub-fractions 15–18.[43] According to the study, cytotoxic activity was elevated in compounds having hydroxyl groups at the C-7 position. The presence of this group in compound 3 suggests the ability of the compound to inhibit MCF-7 cancer cells, HCT 116, and Ca ski, with the strongest

activity against A549. Methyl-β-D-galactopyranoside (specific lectin; Figure 2F) was also isolated from the tuber of *K. rotunda* and reported to exhibit antibacterial and anticancer activity on colon cancer cells and EAC.[37,49,50] The isolation of purified specific lectins from the tubers of *K. rotunda* was started by homogenization using Tris HCl buffer pH 8.2. Subsequently, the supernatants were separated by affinity chromatography on the glucose-sepharose column,[49,50] ion exchange (QA-cellulose), and hydrophobic (phenyl-sepharose) chromatography.[37] A total of 500 grams of *K. rotunda* tubers produced 2,500 mg of crude protein. Furthermore, 15 mg of pure protein was obtained through the affinity chromatography purification technique.[49]

Conclusion

The findings of this review reveal that extraction is the first step that determines phytochemical composition in medicinal plants. The class of phytochemicals produced varies according to the type of extraction solvent, methods, and time. Extraction methods and solvents play an important role in obtaining different classes of compounds and separating bioactive constituents from the rhizome of *K. rotunda*. Maceration is the most widely used method for extracting the compounds that are responsible for pharmacological effects. The solvent of choice to extract different classes of chemical compounds with pharmacological activities is ethanol.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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