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Review Article

Review of Isolation Methods, Chemical Composition and Biological Activities of *Curcuma aeruginosa* Roxb Rizhome

Ririn Suharsanti[1,2], Puji Astuti[3], Nunung Yuniarti[4], Subagus Wahyuono[3]*

[1]*Doctoral Program of Pharmaceutical Science, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia*
[2]*STIFAR "Yayasan Pharmasi", Letjend Sarwo Edie Wibowo KM 1, Semarang, Indonesia*
[3]*Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia*
[4]*Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Gadjah Mada, Indonesia*

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ABSTRACT

Curcuma aeruginosa Roxb rhizome is known as *temu ireng* or *temu hitam* in Indonesia and has been widely used as a traditional medicine in Asia. There have been many studies to reveal the chemical content of *Curcuma aeruginosa* Roxb rhizome, some of which are influenced by the isolation methods. Several separation and purification methods were identified including essential oil distillation, extraction and chromatography. This study aims to describe, analyze and discuss the isolation methods of chemicals compounds *Curcuma aeruginosa* Roxb rhizome, how the techniques influence the types and composition of the components and to examine which compounds have potential biological activities. The method used for review articles is explored several databases such as PubMed and Scopus to identify and download abstracts, original articles, and research papers related to extraction followed by fractionation, isolation, and chemical components of *Curcuma aeruginosa* Roxb. The inclusion criteria used were the article year between 2010-2020, the part used for the study was the rhizome, there was a discussion of isolation process, components that have been isolated and their biological activity. The exclusion criteria used were articles whose samples were only up to the extraction stage and articles whose methods were unclear. Chemical compounds of *Curcuma aeruginosa* Roxb rhizome can be carried out in various ways, including steam distillation, water distillation, extraction, fractionation to isolation by chromatography, especially column chromatography with elution gradient. Germacron is the most isolated and has several biological activities.

***Keywords*:** *Curcuma aeruginosa* Roxb, Rhizome, Isolation, Chemical composition, biological activity.

Introduction

Curcuma aeruginosa Roxb has a broad geographical scope in Indonesia. Traditionally, the part of *Curcuma aeruginosa* Roxb used as medicine is the rhizome. *Curcuma aeruginosa* Roxb rhizome is used to treat abdominal pain, obesity and rheumatism, asthma and cough, scurvy and mental disorders.[1] In the ancient Indonesian herb library, *Curcuma aeruginosa* Roxb rhizome is used for the treatment of roundworms or pinworms, scabies, sores, weight loss, postpartum or menstruation problems and gout.[2] There are many benefits of *Curcuma aeruginosa* Roxb rhizome based on several studies reported. These includes antiandrogenic[3], hair growth promoter[4,5], antinociceptive,[6] anticancer,[7] antimicrobial,[8,9] antifungal,[10] repellant,[11] antiasthmatic,[12] anthelmintic,[13] uterine relaxant,[14] anti-inflammatory,[15] and anticancer.[16] There have been many studies to reveal the chemical content of *Curcuma aeruginosa* Roxb rhizome. Earlier research form Indonesia conducted before 2000 revealed that compounds that were isolated from *Curcuma aeruginosa* Roxb included aerugidiols,[17] then curcumenol, P-eudesmol, Isocurcumenol, curcione, humuladiene, and curcumenon in other research.[18]

*Corresponding author. E mail: subagusw_fa@ugm.ac.id
Tel: +6281804374550

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In China, curcumenol and isocurcumenol is also found in the rhizome of *Curcuma aeruginosa* Roxb plus other compounds such as germacrone and curzerenone.[19] *Curcuma aeruginosa* Roxb rhizome is also distilled to obtain essential oils such as 1,8-cineole, camphor, curcumenol, isocurcumenol, curzerenone, zedoarol, and filranogermenone.[20] Each researcher performs the isolation of the chemical composition of *Curcuma aeruginosa* Roxb in various ways. Methods used to obtain essential oils from *Curcuma aeruginosa* Roxb can be hydro and steam distillation, but also other methods such as microwave-assisted hydrodistillation or fractional vacuum liquid chromatography. The choice of method for separating chemical components from plants must pay attention to the properties of the compounds to be separated. Separation can be done by a simple method such as liquid-liquid extraction and can be continued with more sophisticated methods. If the components in the sample are too many, chromatographic techniques can be used. In addition to essential oils isolated by distillation, several chemical components of *Curcuma aeruginosa* Roxb rhizome have been isolated by chromatographic method. Although *Curcuma aeruginosa* Roxb is a rhizome that has been used for a long time and has been widely researched, no one has yet reviewed. This is important because the active compound in *Curcuma aeruginosa* Roxb can be selected as a marker that has been investigated for various biological activities. This study aims to discuss the isolation method of *Curcuma aeruginosa* Roxb rhizome's chemical components and bioactive compounds from each of the techniques used and see which compounds in *Curcuma aeruginosa* Roxb rhizome are most potent against certain biological activities. Rhizome of *Curcuma aeruginosa* Roxb has various activities in various forms, among others, extracts, essential oils, and isolates. Each form has a different biological

activity. Germacrone is an isolate from Curcuma aeruginosa Roxb which has a lot of biological activity and is the most isolated.

Materials and Methods

In this review, the authors explored several databases such as PubMed and Scopus to identify and download abstracts, original articles, and research papers related to extraction followed by fractionation, isolation, and chemical components of *temu ireng* (*Curcuma aeruginosa* Roxb). The keywords used during the search for information was *Curcuma aeruginosa* Roxb in the article title only. The inclusion criteria used were the article year between 2010-2020, the part used for the study was the rhizome, there was a discussion of isolation process, components that have been isolated and their biological activity. The exclusion criteria used were articles whose samples were only up to the extraction stage and articles whose methods were unclear.

Results and Discussions

Curcuma aeruginosa Roxb

Curcuma aeruginosa Roxb in the local language is known by several names, including temu ireng (java), koneng hideung (Sunda), temu ereng (Madura), dan temu irang (Sumatra), and temu hitam (Minang). This plant comes from Burma, then spread to other tropical areas, especially in Indonesia. *Curcuma aeruginosa* Roxb grows wild in teak forests, in fields and in other places to a height of about 1750 m above sea level. Wet-trunked plant, up to 2 m high. The leaves are oval. The flowers are white or slightly reddish white. The rhizome, when cut into pieces, looks like a blue/grey circle. According to the cabe puyang inherited from the ancestors' book, the rhizome of *Curcuma aeruginosa* Roxb can be used for intestinal worms, scabies, ulcers, weight loss, postpartum or menstruation problems and gout.[2] Meanwhile, *Curcuma aeruginosa* Roxb rhizome is known as blue or grey like a ring when the rhizome is split has been used as an anti-diarrheal and antifungal in Indian medicine.[10,21] In Vietnam, the rhizome of *Curcuma aeruginosa* Roxb is also used in traditional medicine for antibacterial, anti-gastric, anti-inflammatory treatment.[22]

Based on research conducted on *Curcuma aeruginosa* Roxb in Indonesia, the shape of the rhizome of *Curcuma aeruginosa* Roxb can be divided into four different types. Rhizome type I is round in clusters, type II is round spread horizontally and type III is oval horizontally and type IV is oval spread vertically.[23] *Curcuma aeruginosa* Roxb obtained from 20 different locations in Indonesia showed differences in phenolic content, flavonoid content and DPPH scavenging activity. This difference indicate that the samples obtained from different locations belong to different genetic groups. This diversity can be used in breeding programs to produce quality and standardized *Curcuma aeruginosa* Roxb.[24] In another study, to dentify the best source of curcuminoid content from ten different locations from where *Curcuma aeruginosa* Roxb was grown under the same conditions, a metabolite study was carried out at different locations. Beringharjo, Gunung Kidul, Muara Bungi and Pakem locations produce high curcuminoids. Therefore, for a commercial scale, this *Curcuma aeruginosa* Roxb source site should be chosen for future breeding programs that will produce high curcuminoid compounds.[25] The *Curcuma aeruginosa* Roxb rhizome has three different parts. They are: primary rhizome (PR), secondary rhizome (SR) and tertiary rhizome (TR). PR and SR *Curcuma aeruginosa* Roxb are the most widely used parts for treatment. Extraction of primary rhizome with 70% ethanol has antioxidant and cytotoxic activities and also contains secondary metabolites such as tannins, triterpenoids, and saponins.[26] In another study, the variability of total phenolic *Curcuma aeruginosa* Roxb rhizome ranged from 29.08 - 46.92 mg GAE/g, and total flavonoids ranged from 21.31-33.81 mg QE/g. Madura, Ciampea Bogor, Beringharjo, Gunung Kidul, Klewer, and Pakem were the six accession sampling locations that resulted in high yields of extracts, total flavonoids, and total phenolics. Rhizome shape, rhizome fresh weight, rhizome dry weight, and number of shoots can be used as important selection factors in breeding *Curcuma aeruginosa* Roxb to obtain extracts and high phenolic content.[27]

Methods of extraction and purification

Based on the reviewed journals, essential oils are obtained by the distillation method. Steam and hydro distillation are typical methods used to extract essential oils from plant materials.[28] The principle of the distillation method is based on the difference in boiling points for the separation of liquids from a mixture so that the substance which has a lower boiling point evaporates first. In one distillation cycle, the mixture evaporates followed by a cooling process due to the presence of a condenser and then liquid condensation. Liquids with a lower boiling point will evaporate first, substances that have a higher boiling point will evaporate later. The steam distillation method used for the distillation of *Curcuma aeruginosa* Roxb rhizomes produced the largest yield reaching 1.99%.[29] Meanwhile, the steam distillation method in another article reviewed resulted in a low yield of only 0.19%.[8] Although both were carried out at the same duration, 6 hours, the greatest yield was possible because the materials used were more and fresh (Table 1). Fresh ingredients will produce a greater essential oil yield than the powder form. Based on Table 1, the highest essential oil yield was 1.99% and used for antibacterial and teeth biofilm degradation tests. In this study, Curcuma aeruginosa rhizome essential oil showed a higher potential as an antibacterial agent than stem and leaves oil.[30] However, the yield of essential oil was more in *Curcuma aeruginosa* Roxb rhizome and it was identified that 1,8-cineol (13%) was the largest component. The major components of essential oil with the steam distillation method were cycloisolongifolene, 8, 9-dehydro-9-formyl (35.29%) and dihydrocostunolide (22.51%).[8] Steam distillation works by lowering the system pressure so that the distillation results are far below the initial boiling point. Since the vapour is under pressure, the temperature can be carefully adjusted to provide maximum extraction rate with minimum thermal decomposition.[31] In the steam distillation process, initially hot steam flows through the fresh material and opens a cavity where the essential oil is then evaporated. The steam is cooled because there is a condenser attached so that a distillate in the form of essential oil is produced in the upper layer.[28] In Table 1, it can be seen that not only steam distillation is used to obtain essential oils but also hydrodistillation (Table 1). Clevenger is a device used for distillation of water in the form of a long tube, at the end, the steam will condense and recover and the distillate, in the form of essential oil, is removed from the top of the hydrosol.[32] The disadvantages of this method are that a large amount of material is needed and the extraction time is very long.[33] Based on Table 1, the yield of essential oil with hydro distillation is about 0.03 to 0.35%. This essential oil yield result is smaller than essential oil yield by steam distillation.[30] This may occur because the ingredients used are less, although the distillation duration is longer up to 5 hour.[5,34] A study on *T. pallescens* leaves showed that the duration of hydrodistillation time affected the essential oil composition and bioactivity. However, it is still more efficient if the essential oil is produced in a short time.[35] The largest yield of 0.35% was produced by the hydrodistillation method which used the highest quantity of ingredients (3 kg) even though the duration of the distillation was only 5 hours. This is in stark contrast to the lowest yield because the lowest yield also used as much fresh material (3 kg) with the hydrodistillation process which produces the highest yield.[36] The ingredients used in the hydrodistillation are fresh, but the dry powder is also used in the hydrodistillation process of *Curcuma aeruginosa* Roxb rhizome and produces 0.18% essential oil yield[22] better than the yield of essential oils with fresh ingredients which produce 0.03% yield.[36] In hydrodistillation of *Curcuma aeruginosa* Roxb rhizome, the results of the identification of the major components were different for each article. The main components identified were camphor (29.39%) and germacrone (21.21%),[9] monoterpenes (21.47%) in the form of *β*-pinen and 1,8 cineol,[34] 1,8-cineol (22.65%) and germacrone (17.70%),[5] tropolone (18.1%) and eucalyptol (17.9%),[36] β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%).[22]

Fresh or dried plant parts that are not damaged by heating are recommended using the method most frequently used, hydrodistillation.[37] Although wet and dry materials can be distilled by water, the quality of material, and essential oil content after harvesting are higher than those contained in the storage of materials at a certain time and temperature.[38]

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Table 1: Chemical Composition of Curcuma aeruginosa Distillation

No.	Material (Rhizome)	Method	Time	Yield (%)	Identification			Identification Result	Biological Activity	Ref.
					Instrument	Temperature setting (°C)	Helium gas flow rate (mL/min)			
1	5 kg (fresh)	Steam distillation	6 hours	1.99	GC-MS	80 and 250°C	20	1,8-cineol (13%), *β*-pinene, 3,6-dimethyl-5-isopropenyl benzofuran, camphene, isoborneol, alcanfor, β-elementse, 2,2,5-trimethyl-2'(h)-5',6'-dihydropyrano[3',4',9] indan-1-one	Antibacterial biofilm degradation	30
2	1.8 kg (powder)	Steam distillation	6 hours	0.19	GC-MS	50 and 250°C	5	cycloisolongifolene, 8, 9-dehydro-9-formyl (35.29%) and dihydrocostunolide (22.51%), germacrone (6.50%), oxygenated monoterpenes (5.92%), β-elements (4.76%), alloaromadendrene oxide-(2) (4.07%), aromadendrene oxide-(2) (2.40%), α-bulnesene (2.14%), and eudesma-4,11-diene (1.13%), eucalyptol (3.98%), l camphor (1.32%) and isoborneol (0.62%), caryophyllene, *β*-cubebene, and xanthinin	Antimicrobial	8
3	3 kg (fresh)	Hydro distillation	5 hours	0.35	GC-MS	70 and 250 °C	0.99	camphor (29.39%), germacron (21.21%), 1,8-cineole (2.68%), α-pinene, β-pinene, 2-heptyl alcohol, camphene, limonene, 2-nonanol, borneol, endo-borneol, germacren A, germacren B, terpinen-4-ol, β-elemente, trans-caruophyllene, α-humulene, curzerene	Antimicrobial	9
4	2 kg (fresh)	Hydro distillation	15 hours	0.2	GC-MS	nd	nd	monoterpenes (21.47%) are mainly β-pinene and 1,8-cineol, sesquiterpenes (73.88%) are mainly germacorn (total 26 compounds)	hair-growth and lightens skin	34
5	1 kg (fresh)	Hydro distillation	15 hours	0.2	*GC-MS and HPLC For germacrone	40 and 260 °C	1	1,8-cineol (22.65%), germacrone (17.70%), and furanodiene (11.40%), α-pinene, β-pinene, germacrene A, germacrene B, germacrene D, camphene, terpene-4-ol, α-terpene-4-ol, 2-undecanone, β-elemente, β-caryophyllene, γ-cadinene, β-selinene, α-selinene, zingiberene, β-bisabolene, α-cadinene, δ-cadinene, caryophyllene oxide, guaiol furanodiene, furanoelemene, curcumenol, isocurcumenol, elementoic acid, dehydrocurdione (total 28 compounds)	hair growth promoter	5
6	3 kg (fresh)	Hydro distillation	4 hours	0.03	GC-MS	60 and 250 °C	0.6	tropolone (18.07%), eucalyptol (17.90%), camphor (5.31%), curcumol (5.69%) and (Z, Z) -3.6-nonadienal (3.86%) (total 30 compounds)	cytotoxicity	36
7	500 g (powder)	Hydro distillation	3 hours	0.18	GC-FID, *GC-MS	40 and 260 °C	1	β-pinene (21.9%), neocurdione (16.1%) and curcumol (15.2%), β-elemente (6.6%), myrtenyl acetate (6.1%), β-caryophyllene (4.9%), α-pinene (3.4%) and α-selinene (3.3%)	no activity tested	22

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Table 2: Chemical Composition of Curcuma aeruginosa Isolation

No	Identification					Identification		Instrument	Identification Result	Biological activity	The Most Potent Isolate	Ref.
	Method	Temperature setting (°C)	Duration	Solvent	Volume	Method	Solvent					
1	Maceration	room temperature	3 days	n-Hexan	2,5Lx3	Quick column chromatography (10× 8 cm) with gradient elution sequentially resulted from 17 fractions and finally recrystallized	hexane, CH_2Cl_2, and methanol as the mobile phase	NMR and MS	germacrone(1), zederone(2), dehydrocurdione(3), curcumenol(4), zedoarondiol(5) and isocurcumenol(6)	Antioxidant	germacrone	3
2	Maceration	room temperature	nd	Methanol	nd	VLC column with gradient elution and continued to be separated and purified by RC, CC, and preparative TLC	hexane:EtOAc, EtOAc: MeOH, and finally, MeOH	LC–MS	turmeronolA(1),turmeronol B (2), germacron-13-al (3), and furanogermenone (4), isoflavone (chromene) group compound, i.e.,7 butoxy-4-methyl-3pentyl-2H-chromen-2 one(5), 3-butyl-7hydroxy 4-methyl-6- pentyl-2H chromen-2-one (6),isobutyl-4-methyl-7 pentyloxy-2H-chromate-2-one(7), 4,8-dimethyl-7-octyloxy-2H chromen-2-one(8), 2-decyl-5 hydroxy-4H chromen-4-one(9), and 1-(4-isopropyl-2,2-dimethyl-7-propoxy-2H-(chromate-6-il)- etanon(10), isoflavone (chromena) group compounds (11-16)	anti-androgenic	the extract is more potent than the isolate	46
3	Maceration	room temperature	3 days	n-Hexan	6Lx3	Column chromatography (Column: 10x13 cm) with gradient elution. Finally recrystallization with methanol	Hexane/CH_2Cl_2 (100:0 to 0:100) followed by a CH_2Cl_2 to MeOH gradient (100:0 to 0:100)	melting point, UV (MeOH), IR (KBr) , 1H NMR (CDCl3, 400 MHz), EI-MS	Germacrone	hair growth promoter	germacrone	40

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4	Maceration	room temperature	3 days	n-Hexan	10Lx3	Column chromatography (Column: 10x13 cm) with gradient elution. Finally recrystallization with methanol	Hexane/CH2Cl2 (100:0 to 0:100) followed by a CH2Cl2 to MeOH gradient (100:0 to 0:100)	TLC, HPLC, NMR, and MS	Dehydrocurdione (1) curcumenol (2) and germacrone (3)	Activity Against Human Cancer Cell Lines	germacrone	5
5	Maceration	room temperature	nd	Methanol	nd	by vacuum liquid chromatography (VLC) and purified by repeated column chromatography on silica gel eluted with various solvent systems	partitioned in hexane, chloroform, and ethyl acetate. the chloroform fraction was continued for isolation with various solvent (nd)	UV, IR, and NMR	Aeruginon (1) and curcumenon (2)	Anti nociceptive	nd	7
6	Maceration	room temperature	3 days	Methanol	4Lx3	VLC, open column chromatography, crystallization	VLC = hexane (5 L),chloroform (5 L) , EtOAc (5 L), acetone (5 L) and MeOH (5 L), CC EtOAc gradients	TLC, IR(KBr), H-NMR	Germacrone	no activity tested	germacrone	50
7	ultrasonic extraction	room temperature	3 hours	Methanol	nd	Liquid liquid extraction (1), CC column chromatography) (2)	(1) =water, hexane, and CH2Cl2 , (2)= hexane/EtOAc (gradient) and also hexane/CH2Cl2 (50/1),acetone/water(3/1),hexane/EtOAc (50/1), MeOH/water(4/1) to get next sub and sub sub fraction	1H NMR, 13C NMR, CDCl3), and ESI-MS	Pyrocurzerenone (1), Dehydrochromolaenin (2), Curzeone (3), Linderazulene (4), Curzerenone (5),8,12-Epoxy-1(10),4(15),7,11-germacratetraen- 6-one (6)	no activity tested	nd	43
8	Maceration	room temperature	nd	Methanol	nd	Liquid liquid extraction (1),	(1)= hexane (Hex.) and ethyl acetate	melting point, UV	Flavone	no activity tested	nd	47

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chromatography) (2), recrystallized(3)	(EtOAc). (2)=gradient of hexane in EtOAc up to 100%, followed by an increasing gradient of MeOH up to 100%, (3)= hexane and dichloromethane	(MeOH), IR (KBR),1H NMR,13C-NMR

Nd = no detected

When compared to hydrodistillation, the steam distillation method is considered to be superior because the distillate will be free from organic solvents, there is no partition after the process, the equipment is cheap, and can be used on an industrial scale. However, there are drawbacks to steam distillation such as the presence of components that are degraded due to heat and require a long extraction time.[39] Both steam and hydro distillation, produce oil-containing monoterpene and sesquiterpenes compounds. There are monoterpene hydrocarbons (32.2%), oxygenated monoterpenes (6.8%), sesquiterpene hydrocarbons (20.4%), oxygenated sesquiterpenes (33.1%), and monoterpenes (2.1%).[22] Based on Table 1, it can be concluded that the GC-MS identification of the essential oil *Curcuma aeruginosa* Roxb contained monoterpenes with the highest percentages are 1,8-cineol[5,9,30,35] and β-pinene.[23,35] Meanwhile, eucalyptol which is a synonym for 1,8-cineol was also detected as a major compound.[36] Whereas for sesquiterpene, the most frequently occurring and was germacrone[5,9,34] whose structure can be seen in Figure 1.

On this decade, a lot has been explained about the extraction and purification of compounds present in *Curcuma aeruginosa* Roxb. The extraction method of *Curcuma aeruginosa* Roxb powder and purification to obtain certain compounds are summarized in Table 2. Generally, the extraction used at the beginning of the isolation process is maceration. The solvent mostly use in the maceration process is the polar solvent, methanol. Apart from methanol, the maceration process also uses a non-polar solvent, n-hexane.[3,40] In Table 2, using methanol produces more identifiable compounds than maceration using n-hexane. Maceration is the extraction stage. Extraction is the first step to isolating compounds from natural materials and a simple, easy, and often used technique is maceration.[41] Maceration is carried out simply by immersing the material (either powder or unpulverised) in the appropriate solvent for 3 days. The solvent will replace the existing air cavities in the sample due to the process of drying the material. Filtering is carried out after 3 days to separate the residue from the extract (filtrate) to concentrate extract.[41] Based on to Table 2, does not mention how much extraction yield is produced so it cannot be discussed further the comparison between the amount of solvent and the extracted material with the yield produced, while the temperature and duration of maceration are almost the same, carried out at room temperature and for 3 days. A study concluded revealed that the large yield was influenced by the length of maceration time and temperature.[42] Ultrasound-assisted extraction (UEA) can also be used as an alternative to maceration.[43] The mechanical effect of the UEA method increases the surface contact between the permeability of the sample cell wall and the solvent thereby affecting the release of the compound and increasing the mass transport of the solvent into the plant cell so that the extraction time and amount can be reduced.[41]

After macerated, *Curcuma aeruginosa* Roxb rhizome extract was further separated by chromatography before the compounds were identified (Table 2). Column chromatography is a widely used method when separating compounds in *Curcuma aeruginosa Roxb*. The experimental procedure is carried out with loose silica, about 6-7 cm and put into the column and flattened, the stationary phase in the column is withdrawn by vacuum and the mobile phase is compressed to a height of about 4.5–5.5 cm, examining the column from the air cavity, the solvent will melt down. If the column is packed properly, the solvent will drop to a horizontal line. The sample to be separated is dissolved in a solvent with a suitable low boiling point (eg ethyl acetate, methanol) and previously adsorbed on silica.[44] Table 2 shows that the separation and purification stages using column chromatography use an elution gradient. In gradient elution, the mobile phase composition will vary during sample separation, for example changing from 0 to 100%. Gradient elution requires special chromatography equipment and also has several advantages for many separations. The elution gradient can be thought of as a small series of isocratic steps.[45] Column chromatography using the mobile phase gradient method is very useful for samples whose chemical components cannot be separated easily by the isocratic method. In gradient elution, the mobile phase composition changes so that the analysis time is reduced and the quality of the separation is improved. The eluent in the separation of compounds by column chromatography on the *Curcuma aeruginosa* Roxb rhizome chemical components was different. From the data on the isolation of compounds from *Curcuma aeruginosa* Roxb in Table 2, there are 3 n-hexane extracts followed by isolation of the compounds. The results of maceration of *Curcuma aeruginosa* Roxb powder with n-hexane solvent were further processed by fast chromatography column with elution gradient using n-hexane, dichloromethane, and methanol solvents to produce 17 fractions coded CA1-17.[3] The *Curcuma aeruginosa* Roxb n-hexane extract in another study was fractionated by fast column chromatography with an elution gradient using n-hexane/dichloromethane mobile phase followed by another elution gradient with a mobile phase dichloromethane to methanol. The fraction obtained from separation by column chromatography was dissolved, evaporated and crystallized with n-hexane at 4°C. White germacron crystals are produced after two recrystallizations with methanol.[40] In another study, *Curcuma aeruginosa* Roxb n-hexane extract was separated by a fast chromatography column with an elution gradient using the same mobile phase as in previous research.[40] The identification results produced 3 main sesquiterpene compounds, namely dehydrocurdione (1) curcumenol (2), and germacrone (3).[5] Apart from n-hexane extract, *Curcuma aeruginosa* Roxb was mainly extracted with methanol as a solvent (Table 2).

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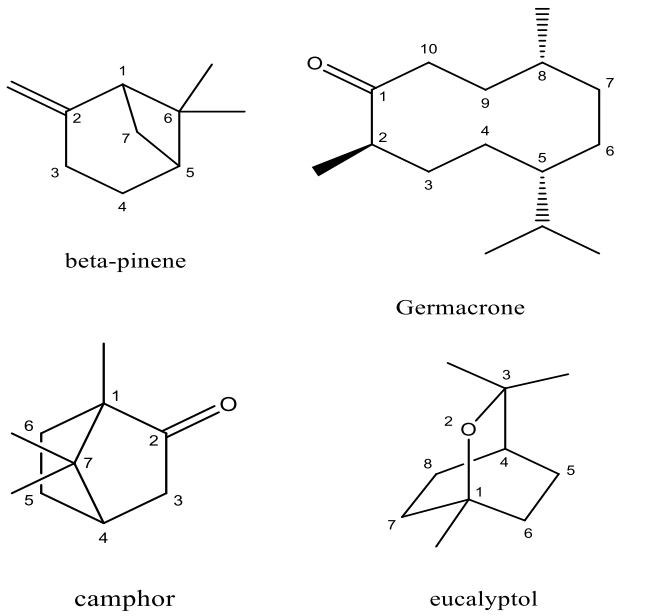

Figure 1: Chemical Structure of Major Compounds in *Curcuma aeruginosa* Roxb Rhizomes

A methanol extract of *Curcuma aeruginosa* Roxb in one study was separated by VLC with n-hexane mobile phase, followed by a gradient mixture of n-hexane: ethyl acetate, and finally with MeOH and it produced 17 fractions. The fractions that produced the same chromatogram pattern after being identified by TLC were combined and became 3 fractions G, H, I. The G fraction was continued to be separated and purified by (Radial Chromatography) RC, (Column Chromatography) CC, and preparative TLC and produced 1-10 compounds, whereas compounds 6-11 have never been reported in black mice (*Curcuma aeruginosa* Roxb) or other species in the genus Curcuma.[46] Methanol extract, in another study, was fractionated with n-hexane, chloroform, and ethyl acetate as solvents. After being tested, the isolation activity was continued by isolating the compound from the chloroform fraction. The chloroform fraction of *Curcuma aeruginosa* Roxb was separated and repeated by extensive chromatographic purification, and the resulting compounds (1) and (2) were brown oils.[7] Germacrone was again identified in ethanol extract in a study on the isolation of compounds from methanol extract *Curcuma aeruginosa* Roxb. Germacrone isolation is carried out through a very long stage. The methanol extract was eluted using the isocratic method with n-hexane, chloroform, ethyl acetate, acetone, and methanol solvents to obtain fractions 1-5 from each solvent. Based on the activity screening, fraction 1 was continued to the isolation stage with VLC gradient elution using n-hexane-ethyl acetate as mobile phase. The obtained fractions 1–48 (n-hexane), fraction 49–54 (n-hexane:ethyl acetate, 9:1), fraction 55–58 (n-hexane:ethyl acetate, 8: 2), fraction 59–62 (n-hexane:ethyl acetate, 5:5) and 63–68 (ethyl acetate). From the 68 fractions produced, the chromatogram pattern was tested by TLC, the same fractions were collected and subfractions 1 to 5 (SF1-5) were produced. After being tested again for activity, SF 3 was the most potent so that it proceeded to the crystallization stage from n-hexane into a colorless rod-shaped crystal, germacrone which is a sesquiterpene.[6] The methanol extract produced from ultrasonic extraction was also separated using a chromatography column but previously separated by LLE (liquid liquid extraction). The viscous extract was partitioned with LLE to give the water fraction, n-hexane fraction, and dichloromethane. The dichloromethane fraction was separated by elution gradient column chromatography with n-hexane: ethyl acetate as mobile phase, then with dichloromethane: methanol to produce D1-8 fractions. The D1 fraction was separated into D1A-D1D by isocratic elution using the mobile phase n-hexane: dichloromethane (50:1) and then n-hexane: ethyl acetate (20:1). The subfraction of D1B was separated into D1B1- D1B3 by acetone: water (3:1) mobile phase chromatography column. Purification of the D1B1 fraction produced isolates (1), (2), and (4). By column chromatography using n-hexane: ethyl acetate (50:1) as mobile phase, the D1D subfraction was separated into D1D1-D1D4. Purification of the D1D2 fraction chromatography with the hot phase of Methanol: water (4:1), followed by n-hexane: ethyl acetate (50:1).[43] In all the articles that discussed the isolation of compounds from the rhizome of *Curcuma aeruginosa* Roxb, sesquiterpenes are the compounds that can most often be isolated and identified. However, other studies have succeeded in isolating compounds other than sesquiterpenes. Initially, the methanolic extract was partitioned with n-hexane and ethyl acetate with LLE. The ethyl acetate fraction was continued to separate the compounds in it by column chromatography with an elution gradient using n-hexane: ethyl acetate as the mobile phase followed by 100% methanol so that 14 A-N subfraction was obtained and finally flavone compounds were obtained.[47] The bioassay-guided isolation is used in the process of isolating active compounds in natural materials by combining solvent-solvent partitioning and column chromatography and then testing its biological activity from all fractions until the isolates are obtained. Bioassay- guided isolation is also defined as a protocol for isolating pure chemicals of natural origin by fractionation with bioassay guidance, full stop which means separation step-by-step based on differences in physico-chemical properties and carrying out activity tests on the results of the separation, followed by the next separation and testing stages. Only the active fraction was fractionated and subjected to further activity tests.[48] Sesquiterpene compounds dominated the isolation results. In all the isolation processes of Curcuma aeruginosa Roxb rhizomes, the mobile phase which is widely used in gradient separation is n-hexane: ethyl acetate and n-hexane and dichloromethane are used in a gradient up to 100% methanol. Almost all of the separation processes listed in Table 2 produce sesquiterpene germacrene compounds. Germacrone is included in the sesquiterpenes compound which has the most potent activity when tested in several biological activities such as antiandrogenic activity,[3,40] hair growth promoter[5] and antinociceptive.[6] On the contrary, the isolate has a lower activity than the extract.[46] Apart from germacorn, in the reviewed article there was a new lactone sesquiterpenes activity from *Curcuma aeruginosa* Roxb, namely aeruginon and curcumeron which were isolated from the chloroform fraction of *Curcuma aeruginosa* Roxb which had not toxic to T-47D, Vero cells and Hela S3 (IC50> 500µg/mL) and low cytotoxic activity against Ca-ski and MCF-7 (IC50 <100µg / mL).[7] Some of the major compounds that were isolated and potent in several activities, are shown in Figure 1.

Biological activities of Curcuma aeruginosa Roxb

Rhizome of *Curcuma aeruginosa* Roxb has various activities in various forms, among others, in the conditions of extracts, essential oils, and isolates. For the condition of the extract, has been studied:

a. Anthelmintic activity by calculating the time of death of *F. gigantica* and histopathological studies. *Curcuma aeruginosa* Roxb extract had an effect on worm death within 75, 57, and 48 minutes. Based on histopathology, *Curcuma aeruginosa* Roxb extract was the cause of tegument damage. Tegument is an important organ in the process of respiration and absorption of nutrients.[13]
b. *Curcuma aeruginosa* Roxb rhizome with *Morinda citrifolia* fruit show potency as anti-inflammatory agents based on inhibition in LPS-induced RAW 264.7 cells.[15]
c. *Curcuma aeruginosa* Roxb extract was reported as a new natural source of anti-asthmatic agents. In the research that has been carried out, the decrease in spasmolytic activity in guinea pig tracheal tone due to the extract had better results and had a significant difference with the negative control (p=0.022).[12]
d. The cytotoxic effect of *Curcuma aeruginosa* Roxb extracts assessed by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay at 200 g/mL was 12.1 ± 2.9. *Curcuma aeruginosa* Roxb showed apoptotic cells 57.7 ± 3.1% using CytoTox-ONE™ assay.[16]
e. *Curcuma aeruginosa* Roxb has been studied and reported to have a relaxing effect on uterine muscles. The extract was reported to inhibit contraction in the presence of induced oxytocin, so the extract could be used as a tocolytic agent to prevent premature

birth. The extract is also able to inhibit contraction due to induction by PGF2, so the extract can also be used for the treatment of dysmenorrhea. Based on other supporting studies. The rhizome of *Curcuma aeruginosa* Roxb contains alpha-pinene and sesquiterpenes.[14]

f. Antiobesity via lipase inhibitor using porcine pancreatic lipase (PPL) enzyme. *Curcuma aeruginosa* Roxb has lipase inhibitor activity of 29.6 ± 0.2%.[49]

In the form of essential oil, *Curcuma aeruginosa* Roxb has several activities, including:

a. The antimicrobial activity of *Curcuma aeruginosa* Roxb essential oil using disc diffusion and micro dilution methods was tested against several microbes including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Cyptococcus neoformans*. *Curcuma aeruginosa* Roxb oil exhibits mild antimicrobial activity. Based on the identification data of compounds in the oil, cycloislongifoline, 8.9-dehydro-9-formyl (35.29%), and dihydrocostunolide (22.51%) were the main compounds in *Curcuma aeruginosa* Roxb oil..[8]

b. *Curcuma aeruginosa* Roxb rhizome essential oil has antibacterial activity against the growth of *S. mutans.* The essential oil of the rhizome of *Curcuma aeruginosa* Roxb showed higher antibacterial activity than the essential oil from stems and leaves with a minimum inhibitory power of 15.63. Oil from *Curcuma aeruginosa* Roxb rhizome can be used as an antibacterial agent in pharmaceutical preparations of mouthwash or toothpaste to prevent dental caries..[30]

c. *Curcuma aeruginosa* Roxb essential oil has cytotoxic activity against brine shrimp and MCF-7. The cytotoxic activity of *Curcuma aeruginosa* Roxb essential oil was lower than Doxorubicin. According to the identification of the essential oil of *Curcuma aeruginosa* Roxb rhizome, the main compounds were curcumol (5.7%), eucalyptol (17.9%), and tropolone (18.1%). [36]

Apart from extracts and essential oils, *Curcuma aeruginosa* Roxb isolates have also been tested for various activities. Germacrone is an isolate that has been tested for various biological activities. Extraction with n-hexane *Curcuma aeruginosa* Roxb. fractionated by rapid column chromatography with elution gradient using n-hexane: dichloromethane as mobile phase followed by another elution gradient with dichloromethane as mobile phase to methanol. The fraction obtained from the separation by column chromatography was dissolved in an evaporator and crystallized with n-hexane at 4° C. White germacron crystals were produced after two recrystallizations with methanol.[5] Germacrone has superior activity compared to other compounds in the rhizome of *Curcuma aeruginosa* Roxb on several biological activities such as antiandrogenic,[3] hair growth promoter,[5] antinociceptive,[6] and anticancer.[7]

Conclusion

Isolation of the chemical components of *Curcuma aeruginosa* Roxb rhizome can be carried out in various ways, including steam distillation, water distillation, extraction, fractionation to isolation by chromatography, especially column chromatography with elution gradient. The essential oil yield range (from distillation) is 0.03 to 1.99%. The chemical composition of the essential oil distillation is a monoterpene with the highest percentages being 1,8-cineol and β-pinene. Meanwhile, eucalyptol which is a synonym for 1,8-cineol was also detected as a major compound. The sesquiterpene compound identified with the highest percentage was germacrone. The essential oil from the hydro and steam distillation of *Curcuma aeruginosa* Roxb has the potential for several biological activities such as antimicrobial, antibacterial-biofilm degradation, lightening skin, a hair growth promoter, and cytotoxicity effect. Germacrone isolated from the rhizome of *Curcuma aeruginosa* Roxb is also a potent isolate with several biological activities such as anti androgenic activity, hair growth promoter, and antinociceptive. From the review of the articles, it is not possible to obtain data on compounds that are truly unique in *Curcuma aeruginosa* Roxb as an analytical marker compound.

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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