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Isolation of curcumin compounds in Temulawak Rhizome (xanthorrhiza Roxb)

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ABSTRACT
The curcumin compounds in Temulawak Rhizome (xanthorrhiza Roxb) have been isolated and identified. This research aims to identify curcumin compounds in ginger rhizomes (xanthorrhiza Roxb) by combining several methods including (1) Extraction, (2) Thin layer chromatography, (3) column chromatography and (4) FTIR (Fourier Transform InfraRed). Based on comparing the sample's Retention Factor (RF) value with the standard curcumin compound, the results were identical, and the positive sample contained a curcumin compound. The results of the Infrared spectrum can be assumed that the sample is a flavanols group, The IR findings that there is OH group at wavelength 3686.08, a CH_2 alkyl group at wavelength 3020.2, a carbon double bond group C=C at wavelength 2400.95, a carbon and oxygen double bond group C=O at wavelength 1625.21, a bond group Single carbon with C-C carbon at wavelength 1525.03. At wavelength 1475.64, the bond group between carbon and oxygen C-O is identified at wavelength 1363.24.

Keywords:
Curcumin; Temulawak Rhizome; Thin Layer; Chromatography; Infrared; Retention Factor

Introduction
Temulawak, which belongs to the Zingiberaceae family, contains essential oils and curcuminoids. Temulawak (Curcuma xanthorriza Roxb.) is commonly found in tropical forests. (Dicky et al., 2016). Temulawak also breeds on dry land around settlements, especially on loose soil, so its rhizome fruit can quickly grow to be significant. (Farida et al., 2018). The part used from the temulawak plant is the rhizome. This rhizome smells good and tastes bitter slightly spicy (Hanwar et al., 2020).

Traditionally, temulawak rhizome is used to improve digestion, increase appetite in children, decaying gallstones, facilitate breast milk, promote digestion, reduce fever, reduce kidney stones, and reduce cholesterol. In Indonesia, temulawak is known by various regional names, for example, koneng gede (Sunda), temulawak (Sumatra and Java), and temu radish (Madura) (Panjaitan et al., 2022). Temulawak rhizome contains curcumin, essential oils, starch, protein, fat, cellulose, and minerals (Syamsudin et al. 2019). Metabolites in temulawak rhizomes that support health benefits include curcuminoids and essential oils.

Curcuminoids consist of yellow curcumin compounds, desmethoxycurcumin (a yellow dye, a derivative of heptanoids), and bisdemethoxycurcumin. Only in turmeric rhizome was bisdemethoxy in curcuminoids. Curcumin is one of the active compounds isolated from the rhizome of Curcuma xanthorrhiza (curcuma) (Vikri et al., 2022). However, based on recent research, curcumin can also be isolated from Curcuma zedoaria and Curcuma aromatica. Curcumin is produced naturally from Temulawak rhizomes and two other curcumin analogues, namely demethoxycurcumin and bisdemethoxycurcumin.

Curcumin is produced from temulawak rhizomes in the most significant amount compared to demethoxycurcumin and bisdemethoxycurcumin. The components contained in the curcuminoids analyzed from the chromatography results are curcumin, demethoxycurcumin, and bisdemethoxycurcumin. It is called demethoxy curcumin because of the loss of one methoxy group in the curcumin structure. In contrast it is called bisdemethoxycurcumin because of the loss of two methoxy groups in curcumin (Kesumayadi et al., 2021).

The degree of polarity between curcumin, demethoxycurcumin, and bisdemethoxycurcumin is due to the loss of the methoxy group in the curcumin structure. Of the three components above, namely curcumin, demethoxycurcumin, and bisdemethoxycurcumin, the most polar is bisdemethoxycurcumin. This is because of the three, the bisdemethoxy curcumin molecule is the smallest, thereby increasing the compound's polarity (Nanda et al., 2020)

From the Thin Layer Chromatography (TLC) experiment prior to column chromatography, curcumin was at the top position on the thin layer chromatography plate; below it was demethoxycurcumin, and bisdemethoxycurcumin at the bottom. (Kautsari et al., 2021). This shows that curcumin is more non-polar than the other two compounds, while bisdemethoxycurcumin is more polar than the other two compounds (Ramandha et al., 2023).

Identification of secondary metabolites in the chloroform fraction of temulawak (Curcuma xanthorrhiza) rhizome using UV-Vis spectrophotometer, IR spectrometer, ^{1}H-NMR spectrometer, ^{13}C-NMR spectrometer, HMQC, HMBC and COSY. The results of the analysis by Thin Layer Chromatography (TLC) showed the presence of single stains with the eluent ratio n-hexane : ethyl acetate = 6:4 (Rf = 0.4), petroleum ether:ethyl acetate = 6 : 4 (Rf = 0.286) and petroleum ether:ethyl acetate = 8 : 2 (Rf = 0.086) (Indriani and Ramandha, 2023).

The UV-Vis spectra provide absorption with maximum wavelengths at 271 nm and 418 nm. The IR spectra showed the presence of –OH groups, C=C, C=O groups, aliphatic C-H, and C-O groups. The ^{1}H-NMR spectra showed the presence of aliphatic protons, aromatic protons, and methoxy protons. The ^{13}C-NMR spectra showed the presence of carbonyl C=O, C=C, and C-O. Based on data analysis of UV-Vis spectrophotometer, IR spectrometer, ^{1}H-NMR spectrometer, ^{13}C-NMR spectrometer, HMQC, HMBC, and COSY it can be concluded that the compound isolated from curcuma rhizome (Curcuma xanthorrhiza) in the chloroform fraction indicates that the compound belongs to the curcumin group, namely demethoxycurcumin.

This research uses several methods including: (1) Extraction, (2) Thin layer chromatography, (3) column chromatography and (4) FTIR (Fourier Transform InfraRed). This is done to streamline costs and to get better results.

Methods

This research uses maceration methods, solvent evaporation, Thin Layer Chromatography (TLC) separation, Column Chromatography and FTIR (Fourier Transform InfraRed)

The tools and materials used in this research are: (1)Beaker; (2)Test tube; (3)Volumetric flask; (4)Pipette; (5)Glass jar funnel; (6)The eluent, namely CH2Cl2 : MeOH (97 : 3); (7)Fraction of Curcumae domesticate rhizome; (8)Pencil; (9)Capillary pipe; (10)Filter paper; (11)Preparative TLC absorbent 0.5 - 2 mm thick and usually 20 x 20 cm in size; (12)Stirring rod; (13)Clamp; (14)Stative; (15)Glass bottles; (16)Vacuum pump; (17)Curcuma; (18)Silica Gel; (19)Cerium Sulfate ($CeSO_4$); (20)n-Hexane; (21)Ethyl Acetate; (22)Methanol 96%; (23)Alcohol 70%; (24)Aluminum foil; (25)Filter paper; (26)chloroform.

Procedures

1. Sample maceration

   One hundred grams of ginger powder samples were weighed, soaked in a glass jar using 96% methanol for three days, filtered using a funnel and filter paper, and left for one week to dry the precipitate from the filtering results. The results of the maceration are then evaporated using a rotary evaporator
2. Identification by Thin Layer Chromatography (TLC) by:
   a) Tools and materials are prepared.
   b) Mark the absorbent (usually silica gel) using a pencil.
   c) Put the eluent into a beaker.
   d) Spotting of the extract of the Curcumae domesticate rhizome fraction was carried out by dissolving the extract in a small amount of solvent. The extract is spotted in bands with the narrowest possible spacing because the separation depends on the width of the bands. A good solvent for dissolving the extract is a volatile/essential solvent
   e) Development of the preparative TLC plate was carried out in a glass vessel.

f) The vessel is kept saturated with the expanding solvent with the help of filter paper placed around the vessel's inner surface.
g) After the sample stain rises, remove the plate from the glass vessel and let it dry; then, the results are viewed under UV light with a wavelength of 254 nm.
h) The formed stain bands are marked with a pencil to make it clearer to separate the stains.

3. After TLC has been carried out, it contains a UV lamp, which helps detect the location of the separated bands in compounds that absorb ultraviolet light. To detect compounds that do not absorb ultraviolet light, cover the plate with a piece of glass and spray both sides with a sprayer. Separation by Column Chromatography:
   a) Prepare a column for chromatography, insert cotton and filter paper into the column and close the column valve.
   b) Pour the eluent (chloroform) up to half of the column, and add the silica gel that will be used to the desired amount.
   c) Remove the eluent (chloroform) up to 1 cm above the surface of the silica gel. Observe carefully. Pay attention that there are no air bubbles in the silica gel. The column is ready to use.
   d) Slowly open the column faucet so the eluent (chloroform) will flow out.
   e) Record the first time after dropping the eluent (chloroform).
   f) The fraction that comes out is collected every 2 mL; note the minute a few drops or fractions containing the first analyte start to come out (the first and last minutes for the same analyte).
   g) And so on for the next analyte.
   h) Attention: Do not add eluent (chloroform) too late; do not let the silica gel above the eluent (chloroform) run out.

After the band has been exposed non-destructive, the colorless compound with the adsorbent is scraped off the glass plate. This method is useful for separating mixtures of several compounds so that pure compounds are obtained.

4. The purification results via column chromatography are then identified using an FTIR tool. The FTIR tool will display graphical results which in this research are discussed in the discussion

Results and Discussions

In this experiment, the first step taken was the maceration process (soaking ginger powder in order to facilitate the separation of curcumin from ginger, where the ginger sample used was in the form of 100 grams of fine powder with 1 liter of methanol, the maceration process was carried out for three days, then filtered so the result obtained is ginger filtrate. The ginger filtrate obtained from the maceration results is carried out by evaporation of the filtrate for four days so that the ginger extract does not contain solvent from the previous maceration results.

After the maceration process, the eluent was identified by Thin Layer Chromatography (TLC) with three different eluents, namely methanol which is polar; chloroform which is non-polar; and n-hexan which is non polar; this was done to find out the best eluent to do, separation on column chromatography.

After TLC was carried out using three different eluents with three times each spot on the TLC plate, the TLC plate that had been sprayed was put into a chemical glass containing different eluents; it was seen that the best results for the separation were in the chloroform eluent which is semi-polar, because in temulawak the major compound is curcumin, where the polarity of curcumin is more soluble in chloroform. (Risthanti et al., 2019)

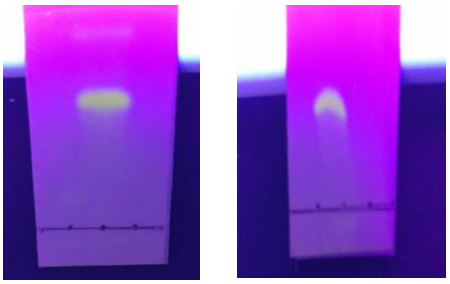
A separation method using thin-layer chromatography was carried out to test the presence of curcumin. The eluent used to elute the sample in this TLC is the same as the eluent used in column chromatography, namely chloroform. In order to separate the sample properly, the eluent will elute the sample on the TLC plate upwards due to capillary forces. TLC was carried out using three plates, where the first plate was carried out for three different fractional spottings: the first with fractions 5, 10 and 15, the second plate with fractions 20, 25 and 30 and the third plate, namely fraction 35. Figure 1 show the identification TLC.

(a) (b) (c)

Figure 1. (a) Identification results with UV spots on the plate with samples in columns 5, 10 and 15, (b) Identification results with UV spots on the plate with column samples 20, 25 and 30, (c) Identification results with UV spots on the plate with column 35 samples.

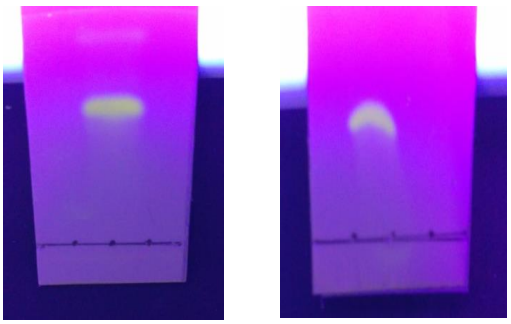
From the results of spot readings or stains on the TLC plate, which had been moistened with chloroform solution eluent and put in a 254 UV lamp to see the separation, after viewing it in a 254 UV lamp, it was known that on the first plate with three different fraction spots (5,10,15) there one spot or one stain each formed on spots 10 and 15. In contrast, on spot five, there is no spot (see observational data), the results on the second TLC plate with fractions (20,25,30) have one spot each parallel of these three fractions, and the results on the third TLC plate with fraction 35 where the results obtained when viewed in a 254 UV lamp there is one spot.

Of the three TLC plates, the results of this column chromatography were compared so that the best spots were seen on the second and third TLC plates, namely spots on fractions 20, 25, 30 and 35. Therefore, a combination or mixture was carried out into two fractions, where the fractions first were a mixture of fractions 20-30 and the second fraction of fractions 31-36. Then TLC was carried out using one TLC plate with two spots (fractions 20-30 and fractions 31-36); from the TLC results and visible in the UV lamp 254 there were each one spot. Figure 2 show spor result of TLC.



(a) (b)

Figure 2. (a) Spot results on the plate with a mixed column sample of 20-30, (b) Spot results on the plate with a mixed column sample of 31-36



(a) (b)

Figure 3. (a) Spot results on the plate with a mixed column sample of 20-30, (b) Spot results on the plate with a mixed column sample of 31-36

By comparing the distance of the spot or spot and the distance of the solvent, the Rf value of each spot will be obtained, where the Rf for the first spot (20-30) is 0.56 while the Rf for the second spot (31-36) is 0.52. From these two spots, the Rf of the two is not much different; this is because, most likely, these two spots have the same pure compound, curcumin. Figure 3 show spot result TLC.

After the standard test is carried out, the purity test is carried out. For this purity test, at least three different types of eluents are used; the first is with chloroform eluent, the second is with chloroform + n-hexane eluent and the third is using chloroform + ethyl acetate eluent.

Because by using chloroform eluent, it has been done and got the results, then it is continued by using two eluents. The first TLC plate used the eluent (chloroform + n-hexane), and it was sufficient to use the fraction (20-30) as a sample because the two spots showed the same Rf as the curcumin standard. The results of the TLC seen on the first TLC plate with UV 254 lamp using eluent (chloroform + n-hexane) there is one spot or one stain on each spot (see observational data) with Rf on the spot (20-30) is 0.24 while the standard Rf is 0.16, the two Rf values are very different. Figure 4 show comparioson of the TLC result with standard compounds.

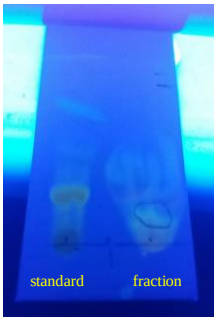


Figure 4. TLC results of comparison of Standard Compounds and column fractions 20-30 with chloroform + n-hexane illuene

The curcumin compound obtained is not pure, possibly influenced by the eluent used, so the polarity for the sample absorption is different. The results on the second TLC plate using chloroform + ethyl acetate eluent on the spot (fraction 20-30) show that two spots appear. The first spot contained in the spot (fraction 20-30), which is 0.7, indicates that the compound obtained is curcumin because it has the same Rf as the standard, but the results obtained are not maximally pure. There are still visible other spots show in figure 5.

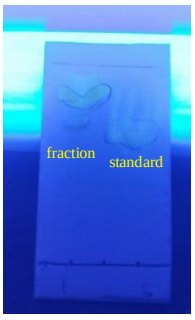


Figure. 5 TLC results of comparison of Standard Compounds and column fractions 20-30 with chloroform + ethyl acetate illuene

Curcumin is an active compound found in turmeric in the form of a polyphenol with the chemical formula $C_{21}H_{20}O_6$. Curcumin has two tautomeric forms: ketone and enol. The ketone structure is dominant in solid form, while the enol structure is found in liquid form. Curcumin is a compound that interacts with boric acid to produce a red compound called rosocyania.

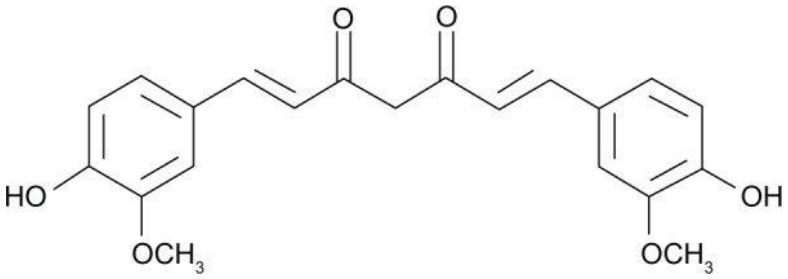


Figure 6. structure of curcumin

The IR findings from Figure 7 show that there is OH group at wavelength 3686.08, a CH_2 alkyl group at wavelength 3020.2, a carbon double bond group C=C at wavelength 2400.95, a carbon and oxygen double bond group C=O at wavelength 1625.21, a bond group Single carbon with C-C carbon at wavelength 1525.03. At wavelength 1475.64, the bond group between carbon and oxygen C-O is identified at wavelength 1363.24. In Figure 7 it can be seen that there are still many other peaks that are indicated as impurities. The presence of impurities is caused by purification that has yet to be carried out optimally.

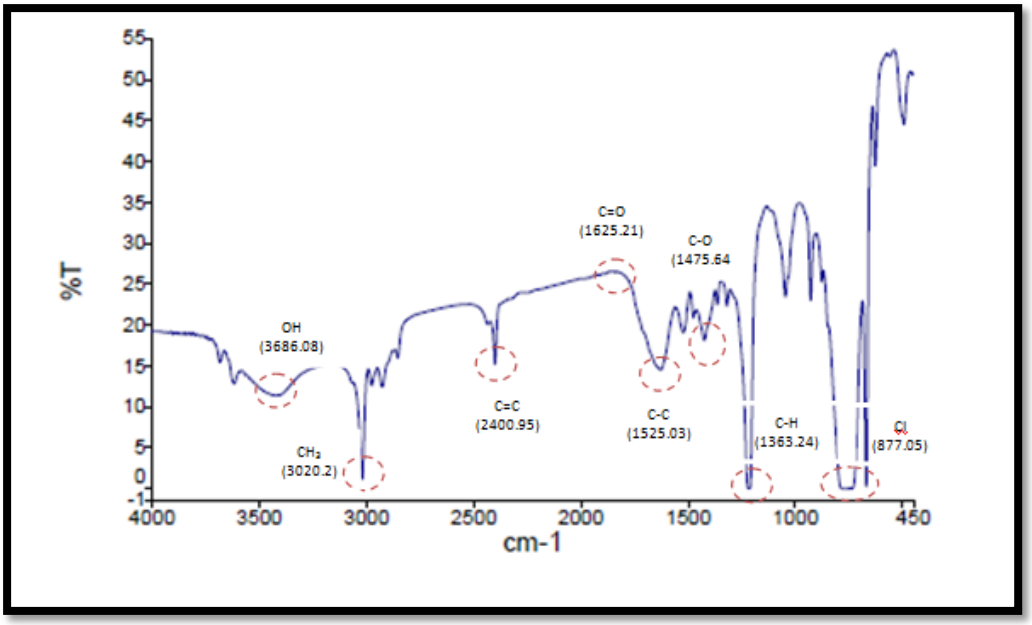


Figure. 7. FT-IR spectrum from xanthorrhiza Roxb

Conclusion

The isolated compound obtained is a curcumin compound; this can be seen from the Rf value of the compound obtained which is the same as the Rf value of the standard curcumin compound. An indication of the presence of curcumin in temulawak can be seen in the peaks that appear on the IR identification. But there are still spurious peaks which are impurities. Suggestions for future researchers to modify the purification process in order to obtain pure curcumin compounds in temulawak

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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