# nampu

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# Identification Of Secondary Metabolites Compounds From Nampu (*Homalomena Rosrata* Griff.)

**Fahrauk Faramayuda, Luthfi Fauziah, Ari Sri Windyaswari, Julia Ratnawati**
Division of Pharmaceutical Biology, Jenderal Achmad Yani University, Faculty of Pharmacy. Indonesia.
corresponding author: ramayuda.f@gmail.com

## ABSTRACT

Nampu (*Homalomena rosrata* Griff.) is traditionally used to increase sexual activity for men. This research aims to isolate and identify secondary metabolite compounds in the nampu (*Homalomena rosrata* Griff.). The results of phytochemical screening of raw material, ethanol extract and water fraction showed positive for flavonoid, tannin, polyphenol, quinone, monoterpenoid-sesquiterpenoid, and steroid-triterpenoid groups. The water fraction is hydrolyzed with HCl 2N then fractionated with chloroform. The fractions were monitored by thin-layer chromatography (TLC) method with the addition of KOH 5 %. The results showed a blue spot under U.V. light 365 nm after the addition of KOH 5%. The fraction was purified by a preparative thin-layer chromatography method (TLC-P). The isolates' purity was tested by two dimensional TLC method and TLC with three different phases of mobile phases. The results of the analysis with spectrophotometer UV-Visible shows the wavelength of 278.8 nm and 213.2 nm. The functional group analysis using I.R. spectrometer showed O.H. stretch at wavenumbers 3419.79 $cm^{-1}$, Aromatic C-H at wave number 2922,16 $cm^{-1}$, C = O at the wave number 1672.28 $cm^{-1}$, strain C = C aromatic at the wave number 1585.49 $cm^{-1}$. Based on TLC and spectrophotometric data, isolates are coumarin compounds.

**Keyword**: Nampu (*Homalomena rosrata* Griff.), Isolation, Thin Layer Chromatography, Spectrophotometer UV-Visible, Spectroscopy Infrared, Coumarin.

## Introduction

*Homalomena rostrata* Griff Is a plant that grows wild on mountains, river, lakeshores, or planted as medicinal plants and ornamental plants in places that are somewhat protected. Traditionally rhizomes can be used to overcome rheumatism and aches, rheumatic pain after birth, increase sexual activity in men. The existence of these biological activities results from the activity of the secondary metabolites contained in the rhizome. It is known that the content of secondary metabolites contained in the rhizome of nampu is terpenoids, flavonoids, tannins and polyphenols (1). Each secondary metabolite compound has its function in the plant itself and its benefits as an alternative treatment. Like flavonoids, it is reported to have antiallergic, antiviral, antimicrobial, prevent carcinogenic, antioxidant, detoxification and protect against liver damage. Some saponins are then reported to overcome hypercholesterolemia, hyperglycemia, antioxidants, anticancer, anti-inflammatory, and weight loss. Some tannins can be used as antiviral, antitumor and anti-bacterial (2), and many other biological activities still need further research.

With various benefits empirically and seen from each of these compounds' many biological activities, several researchers began to research the rhizome of nampu, according to research results obtained from rhizome phytochemical screening, flavonoids, tannins, quinones, polyphenols, saponins, and mono-sesquiterpenes. Isolates from the n-hexane fraction are thought to be steroid compounds (3). The pharmacological activity of ethanol rhizome extract affects increasing sexual activity and the number of spermatozoa of Wistar rats (4). Ethanol extract, n-hexane fraction, ethyl acetate fraction and water fraction had LC50 values of 97.15 µg / mL, 104.29 µg / mL, 155.47 µg / mL, and 158.01 µg / mL. Thin Layer Chromatography (TLC) results showed that in ethanol extract and n-hexane fraction, there were suspected flavonoid and steroid compounds (Mayang Sari, 2015). (5)

One of the Secondary metabolites in plants are coumarin group coumarin compounds, and their derivatives are many have biological activities such as blood anticoagulants, antibiotics and some show effect inhibiting activity carcinogenic. Coumarin is also an essential ingredient in making perfumes and as a fluorescent material in the textile industry and paper (6). Coumarin is abundant in plants Angiosperms and not infrequently on Gymnosperms and low-level plants. Generally found in the Rutaceae family, Leguminoceae, Umbelliferae and Graminae. Kumaran is found in almost every part herbs ranging from roots, stems leaves to flowers and fruit (7).

## Experimental

### Standardization of Raw Material

### Characteristics of Raw Material

Determination of the characteristics of raw material carried out includes specific and nonspecific parameters. Specific parameters are, as well as determining the levels of water-soluble extracts and soluble concentrations of ethanol. In contrast, nonspecific parameters determine total ash content, water-soluble ash content, acid insoluble ash content, and water content determination by distillation method.

### Phytochemical Screening

The phytochemical screening phase of the nampu includes examining alkaloids, flavonoids, quinones, tannins, polyphenols, saponins, steroids and triterpenes, monoterpenoids and sesquiterpenoids.

### Extraction and Fractionation

The reflux method was carried out using 70% ethanol solvent with 250 g of raw material powder. After being immersed in the solvent, the mixture of simplicity and solvent was refluxed for 1 hour and carried out three times. Each solvent immersion is replaced with a new one.

The process is continued with liquid-liquid extraction (fractionation) using solvents with different polarity levels. The extract was mixed with 100 mL of distilled water. This water extract was then extracted liquid-liquid with n-hexane as much as equal so that the n-hexane fraction and water fraction were obtained. Then the water fraction was extracted liquid-liquid again with ethyl acetate to produce ethyl acetate fraction. Each fraction was concentrated with a rotary evaporator and evaporated above the water bath to obtain the water fraction, n-hexane and concentrated ethyl acetate.

### Isolation

The fraction was dissolved, then it was sprayed on GF254 silica gel with a size of 7x1 cm and then developed with several single developers, then continued using a developer mixture according to the one obtained from the process using a single mobile phase, then the developer mixture was saturated in a closed vessel for 10 minutes. The plate is then inserted into the vessel to elute with the mobile phase, which corresponds to the upper limit specified in the silica plate. The TLC examination profile was carried out under U.V. light with 254 nm and 365 nm.

Thin Layer Chromatography Preparative (TLCP) plates are made with an upper borderline of 1 cm and below 1.5 cm using a plastic spoon. Before eluting the TLCP plate is activated by the oven for 15 minutes. Plates that have been given a fraction are eluted into the chamber to the upper end of the limit. After finishing the elution plate, it is left for a moment, the plate results are seen under U.V. lamp 365 nm and UV 254 nm. Areas that have ribbons are marked using a plastic spoon, and the band area is scraped. Silica powder is then dissolved with a suitable solvent then filtered, concentrated by evaporating. TLCP results in KLT with the appropriate Mobil Phase. The results of the plates are seen under the U.V. lamp 254 nm and 365 nm.

### Purity of isolates Test

The chamber containing the appropriate mobile phase, saturated for 30 minutes. Made 7x7 cm plate size with right and bottom 1 cm, left and above 0.5 cm. The plate results are seen under the 365 nm U.V. lamp. The TLC plate is reinserted into the chamber, containing the 2nd mobile phase (the position is opposite to the first mobile phase direction). The plate results are seen under the 365 nm U.V. lamp. Observed the spot formed. The chamber

containing the appropriate mobile phase is saturated for 30 minutes. Silica plate size 7x1 cm. Isolate is sprayed on the plate then eluted. The elution results are seen under U.V. light 254 and 365 nm. Then it is sprayed with 10% $H_2SO_4$ patches. The process is repeated with different mobile phase.

### 2.5 Isolate Identification

Identification of isolate structure using Uv-Visible spectrophotometer instrument to determine wavelength. Isolate dissolved with methanol analysis pro. Check the absorbance and check the generated spectrum. The sample is reconcentrated in the vial, then the maximum wavelength is observed. Then the absorbance and the resulting spectrum are recorded.

Identification with an Infrared spectrophotometer to determine the functional group. Preparation on the sample. KBr is crushed, then put into a tool and measured the wavenumber. KBr was crushed then mixed with samples and measured in Infrared spectrophotometry.

## 3. Result and Discussion

### 3.1 Standardization of Raw Material

### 3.11 Characteristics of Raw Material

Examination of ash content was shown to determine the physiological and non-physiological ash contents of raw material 0.83, a water-soluble ash content of 3.86 % w/w ± 0.06 which shows that there is physiological ash content such as alkali metal and alkaline earth in the raw material. In comparison, acid insoluble ash content is 1.24% w/w ± 0.14, indicating that non-physiological ash such as silica is contained in the raw material. Determination of the content of water-soluble extracts and soluble levels of ethanol aims to provide an overview of the number of compounds dissolved in water solvents and ethanol solvents. Determination of the content of water-soluble extract gives a value of 20.43 % w / w ± 0.16 and the determination of the content of soluble extracts of ethanol gives a value of 18.74 % w / w ± 0.15, this indicates that the compounds contained in raw material are more soluble in water. Determination of water content was carried out by azeotropic distillation method with toluene solvent, which had previously been saturated with water for 24 hours. Saturation with water aims to prevent water absorption by toluene when used to determine the actual water content in raw material, the water content of raw material was 4% w/v, it shows that the rhizome raw material is suitable for use in research, because it has fulfilled the stipulated requirements, the water content in the raw material is not more than 10% w/v. Results can be seen in Table I.

**Table I. Characteristics of Raw Material**

| Characteristic Type | Result |
|---|---|
| Ash content : | |
| a. Total ash content | 6,05 % w/w ± 0,83 |
| b. Water soluble ash content | 3,86 % w/w ± 0,06 |
| c. Acid insoluble ash content | 1,24 % w/w ± 0,14 |
| Determination of extract content: | |
| a. levels of water soluble extracts | 20,43 % w/w ± 0,16 |
| b. soluble concentrations of ethanol | 18,74 % w/w ± 0,15 |
| Water content | 4 % v/w ± 0 |

### Phytochemical Screening

Phytochemical screening is intended to determine the content of compounds in the rhizome's simplicity (*Homalomene rostrata* Griff.) So that it can be associated with efficacy or pharmacological activity. The groups of compounds found in the rhizome's simplicity are flavonoids, tannins, polyphenols, quinones, monoterpenoids and sesquiterpenoids, and steroids and triterpenoids. Phytochemical screening of ethanol extract was carried out. The groups of compounds in the rhizome's ethanol extract were flavonoids, tannins, polyphenols, quinones, monoterpenoids and sesquiterpenoids, and steroids and triterpenoids. Phytochemical screening of the water fraction was carried out, the class of compounds contained in the rhizome water fraction included flavonoids, tannins, polyphenols, monoterpenoids and sesquiterpenoids, and steroids and triterpenoids. The flavonoid group test was positive with the formation of a yellow ring in the amyl alcohol layer after being reacted with 2N Mg and HCl powder.

The addition of Mg powder serves as a catalyst. The orange colour is formed due to a reduction reaction on the carbonyl group to an alcohol group forming a coloured hydroxy compound and the colour formed will then be attracted by amyl alcohol. The tannin class test was positive when precipitates were formed after the addition of 1% gelatin reagent due to the tannin's specific nature, which precipitates the protein. The polyphenol group test is positive because it forms a blackish-blue colour when adding iron (III) chloride reagent. This change occurs because the oxidation reaction of the hydroxy group in the structure of iron (III) chloride polyphenols forms $Fe(OH)_3$ so that the colour changes become darker because $Fe^{3+}$ is a strong oxidizer (8). Quinone class test was positive because the yellowish-brown colour was formed after the addition of potassium hydroxide. The presence of quinone compounds is indicated by its ability to form coloured salts. This coloured salt is formed between hydroquinone and a robust alkaline solution (NaOH or KOH). Monoterpenoid and sesquiterpenoid class tests were tested positive when purple formed after addition of sulfuric vanillin reagent. Steroid and triterpenoid assays were tested positive because they formed green after adding Liebermann-Bourchad reagent which would react steroids via acetylation reaction to produce acetyl steroid complexes. Results can be seen in Table 2

The method used for the extraction process is a reflux method using 70% ethanol solvent. The choice of the reflux method is made because the sample used is rough-textured. The extract obtained was concentrated with a rotary evaporator to obtain a thick extract. Also determined the weight of 1% extract obtained results of 0.76 w/v ± 0.02. The thick extract obtained was 153.97 g so that the extract yield obtained was 30.79% w/w.

**Table 2. Phytochemical Screening**

| Class of Compound | Result | | |
|---|---|---|---|
| | Raw material | Ethanol Extract | Water Fraction |
| Alkaloid | - | - | - |
| Flavonoid | + | + | + |
| Tanin | + | + | + |
| Polyphenol | + | + | + |
| Kinnon | + | + | + |
| Saponin | - | - | - |
| Monoterpenoid-seskuiterpenoid | + | + | + |
| Steroid-triterpenoid | + | + | + |

Further separation of the extract was carried out based on its polarity with the liquid-liquid extraction method using non-polar n-hexane solvents, the withdrawal of semipolar compounds using ethyl acetate solvent, and the more polar compounds in the water fraction. The purpose of liquid-liquid extraction is to separate compounds based on comparing the distribution of compounds in two solvents that are not intermixed, and the separation ends after equilibrium occurs. The water fraction obtained was then hydrolyzed using 2N hydrochloric acid: water (1: 1) by heating for 30 minutes, then extracting liquid-liquid with chloroform (1: 1). This hydrolysis aims to separate the glycone from the aglycone so that the aglycone becomes free and easy to isolate.

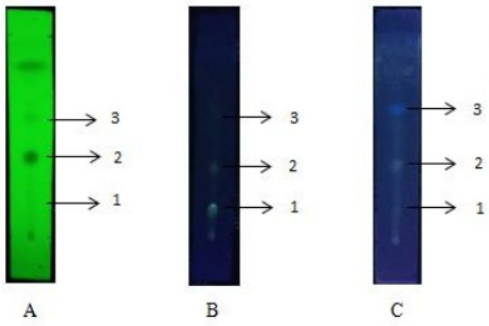



Figure 3. Thin layer chromatography pattern monitoring TLCP results
A : Observed under 254 nm Uv Light before spraying the visible spot 5% KOH with Rf 0.8
B : Observed under 365 nm UV Light before being prayed with visible spots 5% KOH with Rf 0.8

C : Observed under 254 nm UV light after sprayed with visible spots 5% KOH with Rf 0.8

D : observed under UV 365 nm light after being sprayed with visible spots 5% KOH with Rf 0.8

**Isolation**

The isolation stages consisted of an analysis of thin-layer chromatography, compound propagation by preparative thin-layer chromatography, and purification stages of isolates by thin-layer chromatography with three different polarity levels and two-dimensional thin-layer chromatography.

The chloroform fraction from hydrolysis was monstored by thin-layer chromatography (TLC) using chloroform: ethyl acetate ( 6: 4) mobile phase and the results showed a yellow fluorescent spot at Rf 0.13; 0.35 and 0.62 under U.V. light 365 nm and dimming at UV 254 nm. The spraying of 5% KOH spots showed a blue spot at Rf 0.62. Results can be seen in Figure 1.

Thin Layer Chromatography Preparative (TLCP) is used for the separation of target compounds. The results TLCP with silica $GF_{254}$ stationary phase and chloroform: ethyl acetate (6: 4) mobile phase showed one band having yellow fluorescent at the bottom and black at the top at UV 365 nm and damping at UV 254 nm. Band two fluorescing black at UV 365 nm and damping in UV 254 nm, band 3 fluoresce blue on U.V. light 365 nm and damping in U.V. light 254 nm. Band 4 fluoresces black on UV 365 nm and damping in UV 254 nm. Results can be seen in Figure 2

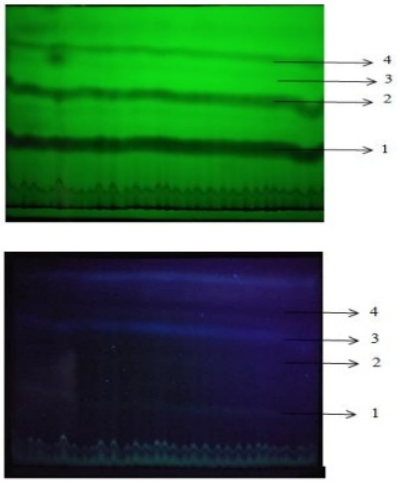



**Figure 2.** Preparative thin-layer chromatography pattern of chloroform fraction *Homalomena rostrata* Griff.

Mobile phase: silica gel GF254
Stationary phase : chloroform: ethyl acetate (6: 4)

Monitoring of TLCP results on bands 1,2,3 and 4 was carried out by TLC using the mobile phase of chloroform: ethyl acetate (6: 4). Which shows the spot is only band 2 with the results showing the reduction in Rf 0.8 under Uv 254 nm and after spraying the appearance of 5% KOH spots showed the Uv 365 nm blue fluorescence results showed that the spot was suspected to be coumarin group. Results can be seen in Figure 3.

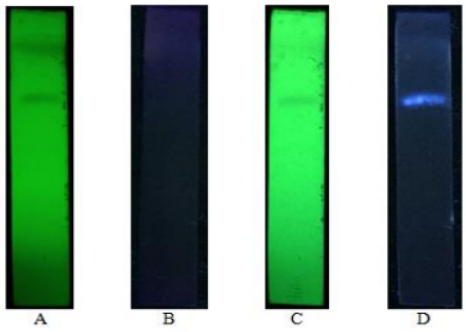



Figure 3. Thin-layer chromatography pattern monitoring TLCP results

A: Observed under 254 nm Uv Light before spraying the visible spot 5% KOH with Rf 0.8

B: Observed under 365 nm U.V. Light before being prayed with visible spots 5% KOH with Rf 0.8

C: Observed under 254 nm U.V. light after sprayed with visible spots 5% KOH with Rf 0.8

D: observed under UV 365 nm light after being sprayed with visible spots 5% KOH with Rf 0.8

Freshly made coumarin in alkaline solutions or stored in a dark place do not show fluorescence. However, if the solution is irradiated with ultraviolet light, it will give blue fluorescence quickly. The same can be done by leaving it in the sun for a long time. In this process, phototransformation occurs from the form of cis-hydroxynamic (III) acid, which does not fluoresce to fluorescence in the form of trans-hydroxynamic (IV) acid **(9).**

Kumaran and its derivatives are highly reactive compounds. The presence of a methyl group at position C-4 or C-6 makes the coumarin nucleus more reactive and can cause the coumarin nucleus to undergo halogenation and condensation reactions with aldehydes. Carbon-6 in the aromatic ring can undergo electrophilic attacks, such as sulfonation or Friedel-Craft acylation reactions. A methyl substituent in the coumarin nucleus reacts differently, depending on the position of the attack. For example, a methyl group bound to C-6 or C-4 is more reactive than a methyl group in the C-3 or C-5 position **(10).** Changes to the basic structure of coumarin are known to affect their biological activity. For example, coumarin with hydroxy groups can be used as anti-inflammatory agents. Submarine synthesis by adding various pharmacophoric groups at positions C-3, C-4 and C-7 intensively active as antimicrobial, anti-HIV, anticancer, antioxidant, and anticoagulant. Coumarin-3-sulfonamides and carboxamides have been reported to have toxic effects on cancer cells in. Substitution at position C-4 with carboxymethyl, arylaminomethyl, and dichloroacetamidomethyl groups shows potential as antimicrobial and anti-inflammatory **(11).**

The purity test is the TLC method with three mobile phases. The test isolates were developed using three different mobile phase, the first mobile phase used was n-hexane: ethyl acetate (6: 4) produced a spot with an Rf value of 0.33, the second mobile phase toluene: ethyl acetate (6: 4) produced spots with Rf

**Isolat Characterization**

Identification of isolates was carried out using UV-Vis spectrophotometry and infrared spectrophotometry. Identification by UV-Vis spectrophotometry was carried out at a wavelength of 240-400 nm using methanol analysis. The results of isolate examination have a maximum wavelength of 278.8 nm and 213.2 nm. Results can be seen in Figure 5.

Identification by infrared spectrophotometry, this analysis is used to see the functional groups of a compound in the range 400-4000 $cm^{-1}$. The infrared spectrum of a molecule is the result of a transition between different levels of vibration energy. Infrared spectrophotometric examination of isolates showed the O-H strain at wavenumbers 3388.93 and 3419.79 ($cm^{-1}$) (3750 - 300 $cm^{-1}$), stretching aromatic C-H at wavenumbers 2922.16; 2953.02; 3115.04 ($cm^{-1}$) (3300-2900 $cm^{-1}$), stretch C = O in wave number 1672.28 ; 1735.93; 1867.09 ($cm^{-1}$) (1900-1650 $cm^{-1}$), stretch C = C aromatic at wavenumbers 1585.49 ($cm^{-1}$) (1675-1500 $cm^{-1}$). Results can be seen in Figure 6.

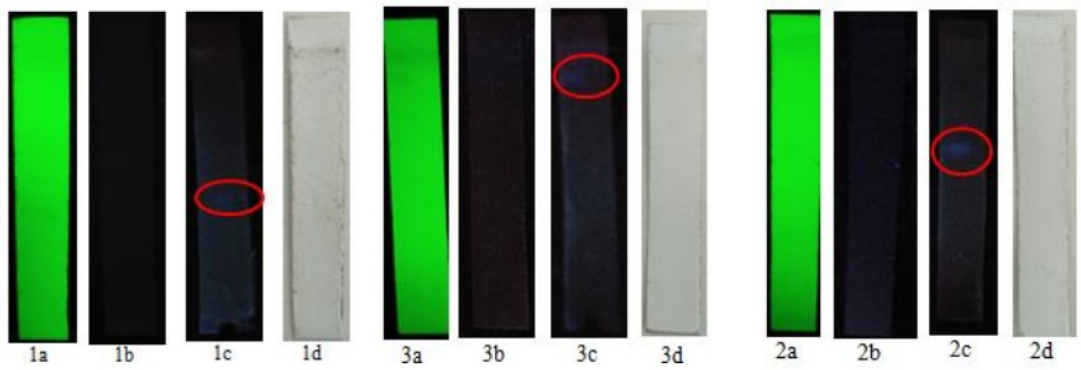



**Figure 4.** Thin Layer Chromatography Pattern isolates with Three different Mobil Phase

1: Mobile phase of n-hexane: ethyl acetate (6:4) with Rf 0.33
2: Mobile phase of toluene: ethyl acetate (6: 4) with Rf 0.55
3: Mobile phase of methanol: ethyl acetate (1:9) with Rf 0.86

a: Under UV 254 nm
b: Under UV 365 nm
c: Under UV 365 nm after spraying 10% $H_2SO_4$ spotting appearance
d: after spraying 10% $H_2SO_4$ visually

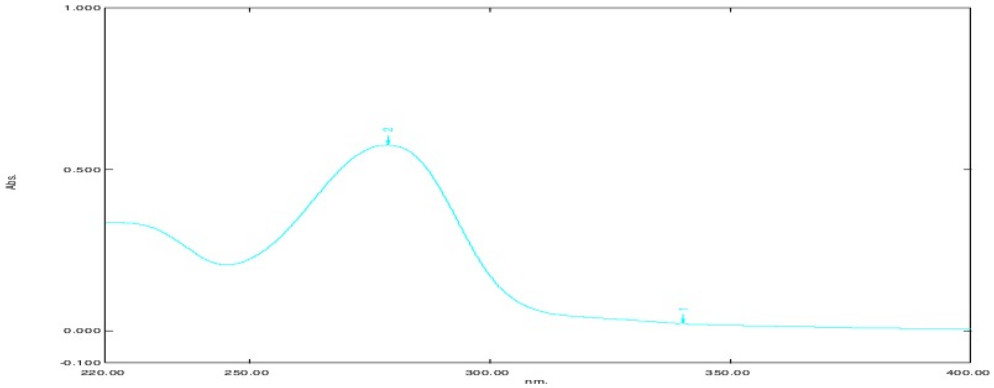



**Figure 5.** The spectrum of isolates in methanol solvent analysis

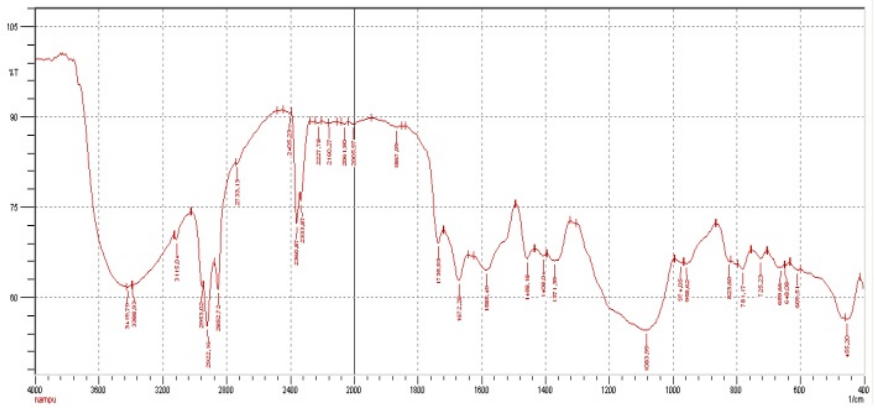



**Figure 6.** Infra red Spektroscopy of isolate

## Conclusion

Which shows the isolates under Uv 254 nm and after spraying the appearance of 5% KOH spots showed the Uv 365 nm blue fluorescence results showed that the spot was suspected to be coumarin group.


## Acknowledgement

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