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Standardization, Quantification of Total Phenolics, Flavonoids Content and Antibacterial Activity of Yellow Root (*Arcangelisia flava* (L.) Merr.)

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Abstract

Yellow root (*Arcangelisia flava* (L.) Merr.) has been used empirical in West Sumatera, Indonesia for stomach disease, anti-malaria, and tumors. The present study is to characterized non-specific, specific parameters, total phenolics content (TPC), total flavonoids content (TFC), and antibacterial activity from yellow root. Standardization of yellow root extract tests followed to Indonesian Herbal Pharmacopoeia. TPC was determined by Folin-Ciocalteu colorimetric method. TFC was calculated by aluminum chloride colometric assay, antibacterial activity was conducted by the agar diffusion method from extract and fractions against *Propionibacterium acnes* ATTC 1223. The results for non-specific parameters showed the water content of the extract (10.1%), shrinkage drying extract (12%). Specific parameters showed that extract have a specific odor, bitter in taste, brownish-yellow in color, and have a thick physical appearance. Microscopic parameters showed fragments such as fibrous sklerotic cells, parenchymal cells, exocarpic stone cells, stone cells, essential oil cells, and tracheal cells. The value of water-soluble compounds (5.25%), and the value of the ethanol-soluble (19.75%). Thin layer chromatography from extract showed the presence spots of alkaloids, phenolics, flavonoids, and saponins. The yellow root extract contained total phenolic and flavonoid contents of 87.37 mg GAE/g and 73.80 mg QE/g, respectively. At 800 ppm, the ethanolic extract and fractions, with an inhibition zone of 20.53±0.58 mm, approaching that the clindamycin phosphate (21.90±0.40 mm). Overall, these finding demonstrate that the standardized ethanolic extract of Arcangelisia flava (L.) Merr. roor possesses promising antibacterial activity against P.acnes, supporting its potential as a source of bioactive compounds for further development as a natural anti-acne agent. ~~From this research we can obtained that Yellow yellow root extract has a total phenolic content of 87.37 mg GAE/g and flavonoid content of 73.80 mg QE/g. Extract has better antibacterial activity against *Propionibacterium acnes* than its fraction in 800 ppm with diameter inhibition 20.2353± 0.58 mm. Standardization of yellow root extract revealed that all parameters matched the criteria requirements. Extract has high quantities of phenolics and flavonoids. The antibacterial activity from extract was higher from fraction~~

Keywords: *Arcangelisia flava* (L.) Merr; ~~*Arcangelisia flava*~~; flavonoids; phenolics; antibacterial; standardization

Introduction

Indonesia has one of the world's most extensive tropical forest areas. The spread of Indonesian flora and fauna is very broad, and there are some varieties that are endemic, which means that these species can only grow or be discovered in one place. Indonesia's rich ethnic diversity has given rise to a wide range of cultural practices, traditions, and indigenous knowledge, including the use of local biodiversity for the treatment of various ailments (1)~~(1)(1)(1)(1)(1)(1)~~. Yellow root (*Arcangelisia flava* (L.) Merr.) belonging to Menispermaceae family, is one of the therapeutic plants being studied

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in this study. Yellow root is used as a jaundice treatment, anti-inflammation, anti-malaria treatment, and a cancer treatment (1–3)~~(1–3)(1–3)(1–3)(1–3)(1–3)(1–3)~~.

To maintain their qualities, medicinal plants require procedures to prove identity, purity, and quality. Herbal medicine ~~Drug~~ standardization entails confirming its identity as well as determining its quality and purity. Because of advances in crude drug chemical understanding, many methods such as botanical, chemical, spectroscopic, and biological methods are currently employed to estimate active ingredients present in crude medications in addition to physical constants (4)~~(4)(4)(4)(4)(4)(4)~~.

Numerous studies have been conducted on yellow root, particularly pharmacological activity tests, (5)~~(5)~~.~~(Yuliana et al)~~, but standardization of yellow root has not been conducted. ~~Therefore, this study was conducted to determine the specific and nonspecific parameters, chromatographic patterns, and antibacterial activity of yellow root extract against the acne-causing bacteria, *Propionibacterium acnes*.~~

Previous studies have demonstrated various pharmacological properties of *Arcangelisia flava* (L.) Merr., including antimicrobial activity. More specifically, a previous study reported that polarity-based fractions of *A. flava* were able to inhibit the growth of *Propionibacterium acnes*. However, that study mainly focused on antibacterial screening of the fractions and did not integrate the antibacterial findings with comprehensive quality standardization of the plant material and extract, quantitative determination of total phenolic and flavonoid contents, and direct comparison between the crude extract and its fractions (6)~~(23)~~. Therefore, the relationship between the standardized quality characteristics, phytochemical content, and antibacterial activity of *A. flava* root extract against acne-associated bacteria remains insufficiently characterized. This represents an important research gap because standardization is essential to ensure the identity, consistency, and reproducibility of medicinal plant extracts intended for further pharmaceutical development.

Propionibacterium acnes was selected as the test organism because it plays an important role in inflammatory acne through its interaction with the pilosebaceous unit and host inflammatory responses. Moreover, increasing resistance of *P. acnes* to antibiotics commonly used for acne treatment, particularly clindamycin and macrolides, has become an important therapeutic concern (7)~~(24)~~. Recent clinical studies have also demonstrated the ability of *P. acnes* isolates to form biofilms and have reported substantial resistance to commonly used anti-acne antibiotics (8)~~(25)~~. These

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problems reinforce the need to explore plant-derived antibacterial compounds as potential complementary sources of anti-acne agents.

The selection of *A. flava* is also phytochemically relevant. Recent chemical investigations have identified several alkaloidal constituents in this plant, including berberine, jatrorrhizine, and palmatine (9)~~(26)~~. Of particular interest, berberine has recently been demonstrated to exert direct antibacterial activity against *P. acnes*, with reported MIC and MBC values of 6.25–12.5 µg/mL and 12.5–25 µg/mL, respectively. Its antibacterial action was associated with disruption of the bacterial cell wall and membrane and alteration of genes involved in peptidoglycan synthesis (10)~~(27)~~. Accordingly, evaluation of the standardized *A. flava* root extract and its fractions against *P. acnes* provides a rational approach to link the phytochemical characteristics and quality parameters of the extract with its potential biological activity.

~~The purpose of this research was to evaluate both specific and non-specific parameters of herbal therapeutic substances in accordance with the standards of the Indonesian Herbal Pharmacopoeia. Non-specific properties like shrinkage, drying time, and water content were measured. Specific properties are extract identity and organoleptic evaluation, microscopic identification, detection of compounds dissolved in certain solvents, phytochemical screening, and chromatogram profile. This study also analyze the total phenolics content (TPC), total flavonoids content (TFC) and antibacterial activity *Propionibacterium acnes* ATCC 1223 of yellow root~~.

Methods

Materials

Arcangelisia flava radix (yellow root) were collected from Perum Perhutani Pontianak, Southeast Pontianak, Kubu Raya District, West Kalimantan in April 2021. The plant was identified by Plant Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia, with No. No.21/HB/03/2021 which stated that the plant used was *Arcangelisia flava* (L.) Merr. ~~All chemicals were analytical grade and purchased from Merck (Germany), i.e.~~ Mmethanol, ethanol, hydrochloride acid, acetic acid, sodium hydroxide, sodium ferric chloride, magnesium powder, ~~and~~ aluminium chloride and Silica gel F254 purchased from Merck (Germany). Gallic acid, quercetin, caffein was purchased from Sigma Aldrich (USA) ~~and Silica gel F254 were purchased from Merck KGaA (Germany)~~. Propionibacterium ATCC 1223 were obtained from the Microbiology

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Laboratory, Padjadjaran University. Analytical-grade sulfuric acid, barium chloride, and dimethyl sulfoxide were purchased from Sigma-Aldrich (Germany) and bacteriology-grade Mueller–Hinton agar (MHA) from Oxoid (UK).

Determination

The plant was identified by Plant Taxonomy Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia, with No. No.21/HB/03/2021 which stated that the plant used was *Arcangelisia flava* (L.) Merr

Extraction

Yellow root was extracted through maceration using 70% ethanol over a period of 4 x 24 hours, with the solvent being replaced every 24 hours. The extracts were concentrated using a rotary evaporator at 40–50 °C until constant weight is obtained~~reaching a stable weight~~, after which the yield was calculated (5)~~(5)(5)(5)(5)(5)(5)~~

Fractionation

The viscous ethanolic extract (10 g) was mixed with distilled water to obtain a final solution volume of 100 mL. This solution underwent triple liquid–liquid extraction using both n-hexane and ethyl acetate. All extracts were then collected and evaporated (12)~~(7)(6)(6)(6)(6)(6)~~

Shrinkage drying measurement

To determine drying shrinkage, 1 g of extract was introduced into a closed weighing bottle that had previously been conditioned by heating and weighed. The sample was then oven-dried at 105 °C with the bottle's lid removed. Following heating, the bottle was placed in a desiccator to allow it to return to ambient temperature. This cycle was repeated until a constant mass was recorded, with a weight variation of less than 0.0005 g between successive measurements (13)~~(8)(7)(7)(7)(7)(7)~~

Water content

The extract (3 g) is wrapped in aluminium foil and put in a dry round bottom flask. Fifty mL of toluene is added to the flask through a cooler (vertical condenser) and heated carefully for 60 minutes. Toluene is used to rinse the inside of the condenser, and the setup is left undisturbed until distinct toluene and water layers form. The amount of the water layer is then recorded (13)~~(8)(7)(7)(7)(7)(7)~~

Extract identity and organoleptic evaluation of extracts

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Extract identity includes nomenclature description, plant's other names, including the Indonesian name, as well as the plants parts used. The organoleptic properties evaluated include the extract's color, smell, and taste (13)~~(8)(7)(7)(7)(7)(7)~~

Microscopic identification

The powdered yellow root was positioned on a microscope slide and treated with chloral hydrate. After covering it with a cover slip, the preparation was gently heated over a Bunsen burner using tube clamps, avoiding boiling. Once dried, the slide was observed microscopically at 10x magnification (14)~~(9)(8)(8)(8)(8)(8)~~

Identification of compounds that are soluble in specific solvents

Two samples of yellow root extract (1.5 g each) were weighed and designated as Extract 1 and Extract 2. Extract 1 underwent 24-hour maceration in a stoppered flask with water–chloroform mixture (50 mL), whereas Extract 2 was macerated using 50 mL of 96% ethanol. Following filtration, 10.0 mL of each extract was placed in a pre-weighed container and evaporated. The residue was then dried in an oven at 105 °C to achieve a stable weight (13)~~(8)(7)(7)(7)(7)(7)~~

Phytochemical screening

The identification of chemical components in yellow root ethanol extract involved phytochemical testing for the presence of flavonoids, alkaloids, tannins, saponins, steroids, triterpenoids, and glycosides (15)~~(10)(9)(9)(9)(9)(9)~~

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Alkaloid test

On a porcelain cup, 100 mg simplicia and viscous extract placed. After that, samples are dissolved in 5 mL HCl 2 M. The obtained solution was divided into two test tubes. As a blank, three drops of HCl 2 M are added to the first tube. Three drops of Dragendorff reagent are added to the second tube. The Dragendorff reagent will form orange deposits.

Phenolic test

On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL hot water. Filter up the samples, add with a few drops of 10% iron (III) chloride solution. The presence of tannins is indicated by a dark blue or greenish-black color.

Flavonoids test

On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of hot water. Filter up the samples, add 0.05 mg Mg powder and 1 mL concentrated HCl, and vigorously shake. Positive tests produce the colors red, yellow, or orange.

Tannin test

On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of hot water. Filter up the samples, add with 1 mL of 1 % gelatine solution. The presence of tannins is indicated by white precipitate.

Saponin test

On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of hot water. Filter up the samples, followed by 5 ml of water. The test tube is then vigorously shaken for 10 seconds, and one drop of HCl 2 N is added. A positive test forms a stable froth that reaches a height of 1-10 cm and remains stable for at least 10 minutes.

Steroid test

On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of ether. Filter up the samples, added along with two drops of glacial CH_3COOH. The solution is gently shaken and set aside for a few minutes. Blue or green indicates steroids.

Glycosides test

On test tube, 100 mg simplicia and viscous extract placed. Add 5 mL of ethanol following evaporating. The residue added 1 mL of glacial CH_3COOH and 10 drops of concentrated H_2SO_4. Blue or green indicates steroids.

Chromatogram profile

In order to separate different phytochemicals from the ethanol extract of yellow root, the sample was applied manually with a capillary tube onto silica gel GF_{254} plates (15x5 cm, 3 mm thick). The plates were then placed in a mobile phase system based on Table 1 (14)~~(9)(8)(8)(8)(8)(8)~~~~mobile phase system based on~~ Karthika et al (2014)~~Karthika et al (2014)Karthika et al (2014)Karthika et al (2014)Karthika et al (2014)Karthika et al (2014)~~ ~~guidelines (Table 1)~~. After Thin Layer Chromatography (TLC) development, specific visualization reagents—Dragendorff's and 5% ferric chloride—were sprayed onto the plates to detect alkaloids and phenolics, respectively

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(16)~~(11)(10)(10)(10)(10)(10)~~. The developed spot colors were noted, and the Rf values were calculated using formula 1 (16)~~(11)(10)(10)(10)(10)(10)~~:

$$\text{Retention factor (Rf)} = \frac{\text{distance travelled by the solute}}{\text{distance travelled by the solvent}} \quad (1)$$

~~the corresponding Rf values were:~~

~~Retention factor (Rf)= distance travelled by the solute / distance travelled by the solvent~~

Table 1: Determination of phytochemicals yellow root extract with suitable mobile phases through TLC

Compounds	Mobile Phases	Spraying Reagents	Colour of the spot
Alkaloids	Chloroform: Methanol (3:2)	Dragendorff	Orange/ Brown
Polyphenols	Chloroform: Methanol (7: 3)	$FeCl_3$	Blackish blue
Flavonoids	Ethylacetate: Methanol: Water: Glacial acetic acid (1,7: 0,5: 0,5: 0,5)	$FeCl_3$	Grey
Saponins	Kloroform: Metanol (2,2: 0,5)	Vanillin H_2SO_4 reagent	Violet Blue

Total phenolics content (TPC)

To evaluate the total phenolics, the Folin-Ciocalteu technique according to Hanifah et al (2020)~~Chun et al (2003)~~ with modifications (17)~~(12)(11)(11)(11)(11)(11)~~ The test sample (2500 ppm in 70% ethanol) was mixed with 5 mL of the Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and 4 mL of 1M sodium carbonate. After 15 minutes of incubation, the absorbance was recorded at 776.5 nm~~its maximum wavelength~~. The absorbance of samples was calculated on standard curve through linear regression. The total phenolic content was expressed in mg GAE/g using the following formula 2.~~The phenolic concentration was calculated based on a standard curve obtained through linear regression.~~

$$TPC\ (mg\ GAE/g) = \frac{c \times v \times fp}{g} \quad (2)$$

Description:
TPC : Total Phenolic Content (mg GAE/g)
c : concentration of gallic acid compound (mg/L)
v : volume of the sample used (L)
fp : dilution factor
g : weight of the sample (g)

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Total flavonoids content (TFC)

The determination of flavonoids content was done using Chang et al (2020) method with modifications (18)~~(13)(12)(12)(12)(12)~~ The sample was produced at a concentration of 5000 ppm using 70 percent ethanol. An aliquot of 0.5 mL of sample was added to a test tube containing 1.5 mL of 70% ethanol. Subsequently, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water were added. The mixture was incubated at room temperature for 30 minutes. Absorbance was then measured at 435.,5 nm~~the maximum absorbance wavelength~~ using a UV-Vis spectrophotometer. ~~, and the total flavonoid concentration was determined using a quercetin standard curve via linear regression analysis.~~

The absorbance of samples was calculated on standard curve through linear regression. The total flavonoid content was expressed in mg QE/g using the following formula 3.

$$TFC\ (mg\ QE/g) = \frac{c\ x\ v\ x\ fp}{g} \quad (3)$$

Description:
TPC : Total Flavonoid Content (mg QE/g)
c : concentration of quercetin compound (mg/L)
v : volume of the sample used (L)
fp : dilution factor
g : weight of the sample (g)

Preparation of P. acnes Culture

P. acnes ATCC 1223 pure culture was inoculated onto slant MHA medium and incubated for 24 hours at 37 °C. The bacterial colonies were then suspended in 5 mL of sterile 0.9% NaCl solution. The turbidity of the bacterial suspension was standardized to the 0.5 McFarland scale, corresponding to 1.5 × 10^8 CFU/mL. The 0.5 McFarland standard was created by mixing 1.175% barium chloride with 1% sulfuric acid in a volumetric ratio of 0.05 to 9.95 (13)~~(13)(13)(13)(13)(13)~~

Antibacterial Activity Assay

A petri dish containing Mueller Hinton Agar (MHA) was inoculated with 20 µL of the bacterial suspension and left to solidify. A perforator (6 mm diameter) was used to make wells in the agar. For each well, 50 µL of solution was added: ~~25~~10% clindamycin phosphate served as the positive control, 1% DMSO as the negative control, and the experimental groups included plant extract, ~~and~~or water , ethyl acetate, and n-hexane fraction~~s~~ at 800 ppm and 600 ppm concentrations. The culture plates

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were incubated for 18–24 hours at 37 °C, and the size of the inhibition zones was determined with a calliper (13)~~(13)(13)(13)(13)(13)~~

Results and discussion

From 1.5 kg of dried yellow root, a total of 151.86 g of concentrated extract was obtained, resulting in a yield of 10.12%. The appearance of yellow root simplicia and its powdered form is shown in Figure 1.

a **b**

Figure 1: Yellow Root (a); Dried Yellow Root (b)

Maceration is an extraction technique where the sample is soaked in a solvent (menstruum) until it is fully immersed. After extraction, the solution (micelle) is separated from the solid residue (marc) through filtration. Evaporation is then used to remove the micelle from the menstruum (20)~~(15)(14)(14)(14)(14)(14)~~. This technique was chosen to protect thermolabile chemicals while also adhering to Indonesian herbal pharmacopoeia (14)~~(9)(8)~~.11~~1111111111~~ Ethanol was chosen because it is polar, miscible with water, nontoxic at low concentrations, and requires only a modest quantity of heat to concentrate the extract (1)~~(1)(1)(1)(1)(1)(1)~~. Extract yield from this research was 10.12%. In this study, separation funnel method was used to separate the component in the extract. The solvents used for fractionation were *n*-hexane, ethyl acetate, and water. Those fractions were used to analyze antibacterial activity. Yield of each fraction showed in table 2.

Table 2: Yield Fraction from Yellow Root Extract

Fraction	Yield (%)
n-hexane	2.075
Ethyl acetate	5.075
Water	81.025

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The fraction yield value does not reach 100%, this is likely because the natural raw material consists of various structural components and metabolites. Only certain active compounds are dissolved, while the dregs (such as fiber and complex carbohydrates) are left behind or discarded. (21)~~(16).Aqila Alviola Bani, Asni Amin, Abdul Mun'im, Maksum Radji.~~

This study looked at non-specific factors such as shrinkage drying and moisture measurement from simplicia and extract. The drying shrinkage measurement provides insight into the amount of compounds that may be lost throughout the drying procedure. Meanwhile, the water content of simplicia and extract is related to their quality and storage capacity. The water content and shrinkage drying of simplicia do not exceed 10%, but the water content ranges from 5-30% in the thick extract category. So, according to Table 3, both simplicia and extract met the Indonesian Herbal Pharmacopoeia criteria~~.~~

Table 3: The Characterization of Non-Specific Parameters from Yellow Root

Parameters	Results (%)	
	Simplicia	Extract
Shrinkage drying	3.0	12.0
Water content	3.8	10.1

To objectively confirm the identity of a material, a specific parameter such as extract identification and organoleptic evaluation must be conducted. The parameter must be determined in order to provide objective identity from the compound's name and specific identity. The organoleptic properties of yellow root ethanolic extract were evaluated. The yellow root plant has not been included in the Indonesian herbal pharmacopoeia, so the determination of specific and non-specific parameters is only carried out based on observations by researchers.~~-~~The results of the form, colour, smell, and taste tests are shown in Table 4.

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~~The results of the form, colour, smell, and taste tests are shown in Table 4.~~

Table 4: The Characterization of Specific Parameters from Yellow Root Extract

Parameters	Characteristics
Identity	
Scientific name for plants	*Arcangelisia flava* L. (Merr)
Used parts	Radix
Indonesian name plants	Akar kuning (Yellow root)

Organoleptic	
Form	Thick
Color	Brownish-yellow
Smell	Specific
Taste	Bitter

The purpose of assessing dissolved compound levels is to estimate the amount of compounds that can be extracted using particular solvents. Water primarily dissolves polar molecules, while ethanol is effective for solubilizing both semi-polar and non-polar compounds (22)~~(17)(15)(15)(15)(15)~~. Yellow root is more soluble in water than ethanol, as seen in Table 5. According to these observations, the compounds detected in yellow root extract are polar. This result was accordanced with the fractionation results as seen in table 2 previously.

Table 5: Parameters Levels of Dissolved Compounds in Specific Solvents

Parameter	Characteristics
Soluble ethanol compounds	5.25%
Soluble water compounds	19.75%

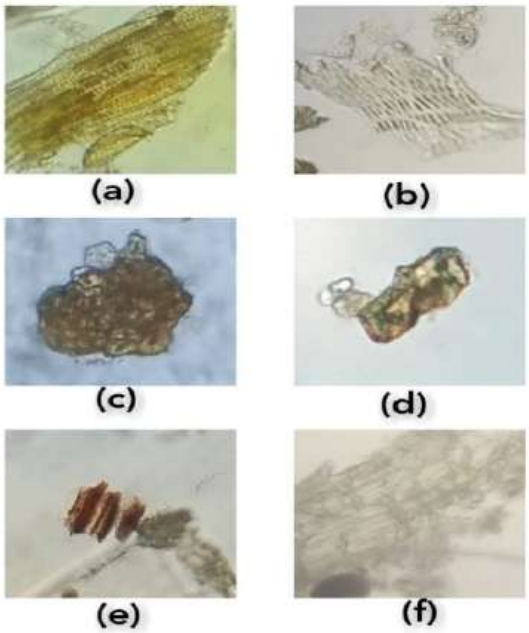

Figure 2: Microscopic from yellow root powder fragments (a) fibrous sklerotic cells; (b) parenchymal cells; (c) exocarpic stone cells; (d) stone cells; (e) essential oil cells; and (f) tracheal cells.

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The purpose of using a microscope to examine yellow root powder is to discover the properties of herb identification fragments. The chloral hydrate solution is meant to remove cell components such as protein and starch, allowing cell identification fragments on the herbs to be clearly visible under a microscope (23)~~(18)(16)(16)(16)(16)(16)~~. Figure 2 showed the presence of fibrous sclerotic cells, parenchymal cells, exocarpic stone cells, stone cells, essential oil cells, and tracheal cells in yellow root.

Table 6 showed the results of the phytochemical screening. Phytochemical screening not only reveals the components of plant extracts and which ones predominate, but it also aids in the search for chemical compounds that can be used in the development of powerful pharmaceuticals. During phytochemical screening, the colour or changes caused by a reaction with a certain response are

observed qualitatively. This screening aimed to detect and characterize secondary metabolite constituents within the simplicia and extracts.

Secondary metabolites are available in medicinal plants, and alkaloids, flavonoids, glycosides, saponins, and terpenoids are among several bioactive substances present (24)~~(19)(17)(17)(17)(17)(17)~~. The chromatographic method is widely used for isolating components from plants. Thin-layer chromatography (TLC) was utilized to distinguish secondary metabolites such as alkaloids, phenolics, flavonoids, and saponins in yellow root ethanolic extract. Various mobile phase formulations were tested (see Table 1), and the samples were spotted onto TLC plates for analysis. Following derivatization with specific spraying reagents, color development was observed, indicating the presence of alkaloids, phenolics, flavonoids, and saponins. These compounds exhibited varying Rf values across different solvent systems in the TLC analysis of ethanolic yellow root extract (Figure 3).

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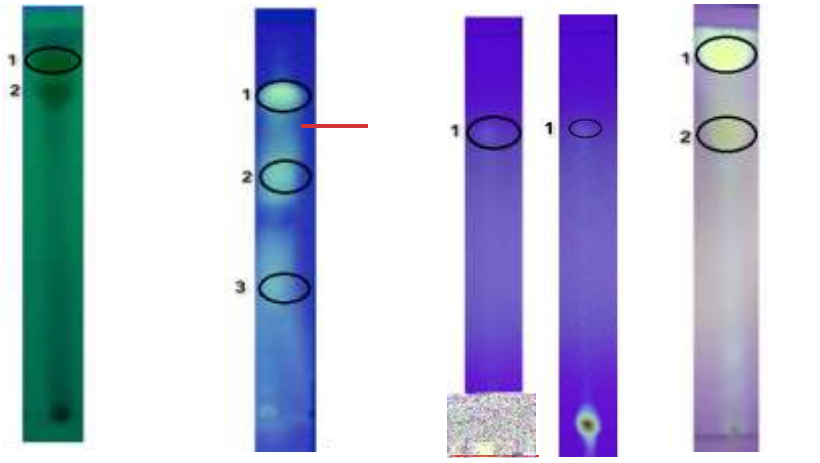
Table 6: Results of Identification Bioactive Compounds from Yellow Root

Phytochemical Test	Simplicia	Extract
Alkaloids	+	+
Phenolics	+	+
Flavonoids	+	+
Tannins	-	-
Saponins	+	+
Steroids	-	-
Glycosides	-	-

*(+) = positive test (exist); (-) = negative test (not exist)



~~**Alkaloids**~~
~~Spot 1: Rf = 0.88~~
~~(Alkaloids 1)~~
~~Spot 2: Rf = 0.78~~
~~(Alkaloids 2)~~

~~**Polyphenols**~~
~~Spot 1: Rf = 0.85~~
~~(Polyphenols 1)~~
~~Spot 2: Rf = 0.57~~
~~(Polyphenols 2)~~
~~Spot 3: Rf = 0.37~~
~~(Polyphenols 3)~~

~~**Flavonoids**~~
~~Spot 1: Rf = 0.75~~
~~(Flavonoids 1)~~

~~**Saponins**~~
~~Spot 1: Rf = 0.80~~
~~(Saponins 1)~~
~~Spot 2: Rf = 0.60~~
~~(Saponins 2)~~

Figure 3: Rf Values of Yellow Root Extract ~~on Various Secondary Metabolites~~

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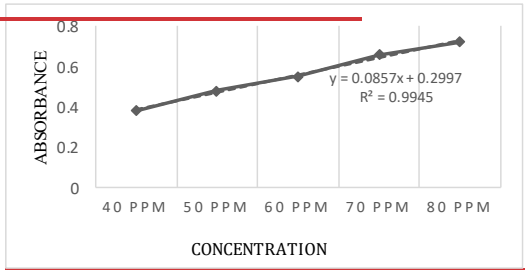
Table 7: Rf Value of Yellow Root Extract on Various Secondary Metabolites

Metabolites	Number of Spot	Rf Value
Alkaloids	1	0.88
	2	0.78
Polyphenols	1	0.85
	2	0.57
	3	0.37
Flavonoids	1	0.75
Saponins	1	0.80
	2	0.60

Total phenolic content (TPC) is frequently determined using the Folin-Ciocalteu assay, a well-established analytical technique. Since high phenolic levels are often linked to strong antioxidant properties, this assay plays a key role in evaluating overall antioxidant potential. The Folin-Ciocalteu reaction involves electron transfer from phenolic compounds and other reductants to phosphomolybdic-phosphotungstic acid complexes, forming a blue complex with peak absorbance (λmax) between 700 - 800 nm. As a result, the absorption spectra of yellow root extract were compared to that of gallic acid, a reference component (25)~~(20)(18)(18)(18)(18)(18)~~. The calibration curve for acid gallic was obtained from the study data, as shown in Figure 4. Using the gallic acid

calibration curve with the linear regression model y = 0.0857x + 0.2997 (R^2 = 0.9945), the total phenolic content (TPC) of yellow root extract was determined to be ~~87.372~~2.280 mg gallic acid equivalents (GAE) per gram of dried extract (Table 7).



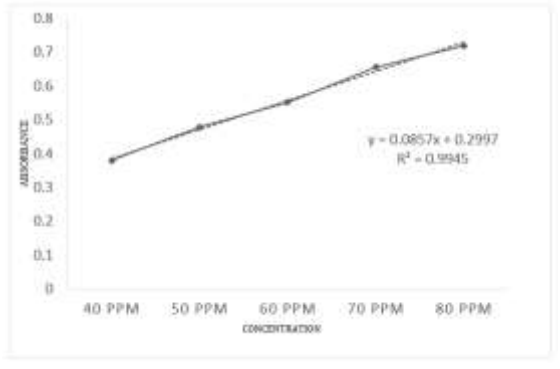


Figure 4: Calibration Curve of Gallic Acid (n=3)

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Table 7: Results of TPC on Yellow Root Extract

Sample	Replication	Concentration (ppm)	Absorbance	Total Phenolics Content (mg GAE/g)	Average of Total Phenolics Content (mg GAE/g)±SD
Extract	1	2500	0.317~~269~~	2.,523~~86.92~~	2.,280~~87.37~~±0.971~~011~~
	2		0.321	3,.107~~87.72~~	
	3		0.308	1.,211~~87.52~~	

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Pls give more explanation of this, as you mentioned in the paragraphs using this linier regresion for determining TPC

Total flavonoid content (TFC) was determined using a colorimetric technique with quercetin as a reference flavonoid component (26)~~(21)(19)(19)(19)(19)(19)~~ TFC in the extracts were calculated from the regression equation of the calibration curve (y=0.0753x+0.0082; R^2=0.997) as shown in Fig. 5 and expressed as mg quercetin equivalents (QE) each gram of sample in dry weight (mg/g). The

absorption of quercetin was measured at a maximum wavelength of 435 nm. The flavonoids concentration of the leaf extract was determined to be ~~73.8~~71.071 mg QE/g, as indicated in Table 8.

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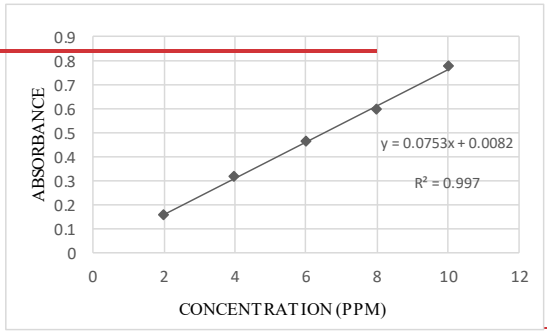

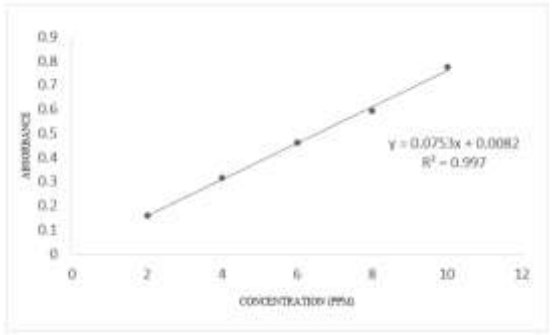

Figure 5: Calibration Curve of Quercetin (n=3)

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Table 8: Results of TFC on Yellow root Extract

Sample	Replication	Concentration (ppm)	Absorbance	Flavonoids content (mg QE/g)	Average Flavonoids content (mg QE/g)±SD
Extract	1	2500	0.438	~~74.08~~71.348	~~73.80~~71.071±0.~~002~~253
	2		0.436	~~73.74~~71.016	
	3		0.435	~~73.56~~70.850	

Clindamycin phosphate, employed in this study as a positive control, is approved by the U.S. Food and Drug Administration for treating conditions such as sepsis, intra-abdominal and lower respiratory tract infections, gynecological infections, and infections of the skin, bones, and joints. The antibiotic may exhibit bacteriostatic or bactericidal properties, contingent upon the organism, infection location, and concentration The predominant bacteria in the pilosebaceous unit of normal human skin is *P.*

acnes, a Gram-positive anaerobic bacterium. *P. acnes* is linked to several disorders, including skin diseases, and is the most frequent source of opportunistic infections, such acne vulgaris (13)~~(13)(13)(13)(13)(13)(13)~~.

~~The inhibition zones for *P. acnes* were significantly affected by the concentration of both extract and fraction (p-value = 1.02×10^{-4}). A positive correlation was noted between concentration and inhibition diameter, as presented in Table 9. The differences in antibacterial activity may be due to variations in the secondary metabolites of yellow root contained within the extract and fraction, influencing their effectiveness against *P. acnes* (27)(22)(20)(20)(20)(20)(20).~~

The antibacterial activity results demonstrated a clear concentration-dependent trend for the ethanolic extract and all fractions of yellow root. At 800 ppm, the ethanolic extract produced the largest inhibition zone (20.53 ± 0.58 mm), followed by the water fraction (18.60 ± 0.20 mm), ethyl acetate fraction (15.93 ± 0.35 mm), and n-hexane fraction (14.83 ± 0.50 mm). At 600 ppm, the same order of antibacterial activity was observed, with inhibition zones of 18.90 ± 0.62, 17.16 ± 0.66, 14.46 ± 0.66, and 13.26 ± 0.05 mm, respectively. These findings indicate that increasing the sample concentration increased the amount of antibacterial constituents available to interact with the bacterial cells, resulting in a larger growth-inhibition zone.

Interestingly, the crude ethanolic extract exhibited greater antibacterial activity than each individual fraction. Although fractionation is frequently performed to enrich compounds according to their polarity, it does not necessarily result in stronger biological activity. The higher activity of the crude extract observed in this study may indicate additive or synergistic interactions among several classes of secondary metabolites that remain together in the unfractionated extract. In contrast, liquid–liquid partitioning may separate complementary bioactive compounds into different fractions or reduce the concentration of individual active constituents through incomplete recovery during the fractionation process. Therefore, the antibacterial effect of *A. flava* may not depend on a single fraction or compound but rather on the combined contribution of multiple constituents.

Among the fractions, the water fraction showed the strongest activity, reaching an inhibition zone of 18.60 ± 0.20 mm at 800 ppm. This finding suggests that relatively polar constituents may contribute substantially to the antibacterial effect of the extract. This interpretation is consistent with the phytochemical screening of the present study, which demonstrated the presence of alkaloids, phenolics, flavonoids, and saponins in the ethanolic extract. Nevertheless, because individual

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Some fraction usually exhibit large inhibition zone than crude extract. However the data in this table show the opposite value.

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compounds were not isolated and quantitatively correlated with antibacterial activity in the present investigation, the activity cannot be attributed conclusively to a specific compound.

The antibacterial activity may partly be associated with protoberberine-type alkaloids reported in *A. flava*, particularly berberine (9)~~(26)~~. Recent experimental evidence demonstrated that berberine inhibited several *P. acnes* strains, with MIC and MBC values ranging from 6.25–12.5 µg/mL and 12.5–25 µg/mL, respectively (10)~~(27)~~. Berberine treatment caused morphological alterations of *P. acnes*, increased leakage of intracellular components, and affected genes involved in peptidoglycan biosynthesis, suggesting disruption of bacterial cell-wall and membrane integrity as possible antibacterial mechanisms (10)~~(27)~~. These findings provide a plausible mechanistic basis for the antibacterial activity observed for the yellow root extract, although further chemical profiling and activity-guided isolation are required to determine which constituents contribute most strongly to the observed effect.

The ethanolic extract at 800 ppm produced an inhibition zone of 20.53 ± 0.58 mm, which was close to that produced by the clindamycin phosphate positive control (21.90 ± 0.40 mm). However, this comparison should be interpreted cautiously because inhibition-zone diameter is influenced not only by intrinsic antibacterial potency but also by the concentration, molecular size, solubility, and diffusion characteristics of compounds in the agar medium. Therefore, the present results establish the antibacterial potential of the extract but do not demonstrate equivalence to clindamycin. Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and identification of the major active compounds would be required to more accurately characterize its antibacterial potency and mechanism.

Table 9: Antibacterial Activity of Extract and Fractions against *P. acnes* (n = 3)

Sample	Inhibition Zone (mm)±SD	
	Concentration 800 (ppm)	Concentration 600 (ppm)
Ethanol extract	20.53±0.58	18.90±0.62
n-hexane fraction	14.83±0.50	13.26±0.05
Ethyl Acetate fraction	15.93±0.35	14.46±0.66
Water fraction	18.60±0.20	17.16±0.66
Clindamycin phosphate (10%)	21.90±0.40	21.36±0.80

Conclusions

~~From this research we can obtained that yellow root extract has a total~~ This study demonstrated that yellow root extract contained a total phenolic content of 87.37 mg GAE/g and a total flavonoid

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content of 73.80 mg QE/g. The extract exhibited stronger antibacterial activity against Propionibacterium acnes than its fractions, producing an inhibition zone diameter of 20.23 ± 0.58 mm at a concentration of 800 ppm. The standardization results indicated that all evaluated parameters of the yellow root extract met the established quality requirements. The extract contained high levels of phenolic and flavonoid compounds, and its antibacterial activity was greater than that of the corresponding fractions.

phenolic ~~content of 87.37 mg GAE/g and flavonoid content of 73.80 mg QE/g. Extract has better antibacterial activity against *Propionibacterium acnes* than its fraction in 800 ppm with diameter inhibition 20.23± 0.58 mm~~

~~Standardization of yellow root extract revealed that all parameters matched the criteria requirements. Extract has high quantities of phenolics and flavonoids. The antibacterial activity from extract was higher from fraction.~~

Acknowledgement

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Conflict of interest

Authors declare no conflict of interests

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