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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. 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 20.23± 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

***Keywords**: 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). Yellow root (*Arcangelisia flava* (L.) Merr.) belonging to Menispermaceae family, is one of the therapeutic plants being studied in this study. Yellow root is used as a jaundice treatment, anti-inflammation, anti-malaria treatment, and a cancer treatment (1–3).

To maintain their qualities, medicinal plants require procedures to prove identity, purity, and quality. 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).

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

Arcangelesia flava radix (yellow root) were collected from Perum Perhutani Pontianak, Southeast Pontianak, Kubu Raya District, West Kalimantan in April 2021. All chemicals were analytical grade and purchased from Merck (Germany), i.e. methanol, ethanol, hydrochloride acid, acetic acid, sodium hydroxide, sodium ferric chloride, magnesium powder, and aluminium chloride. Gallic acid, quercetin, caffein was purchased from Sigma Aldrich (USA) and Silica gel F_{254} were purchased from Merck KGaA (Germany). Propionibacterium ATCC 1223 were obtained from the Microbiology 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 reaching a stable weight, after which the yield was calculated (5)

Fractionation

The 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 (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 (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 (7)

Extract identity and organoleptic evaluation of extracts

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 (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 (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 (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 (9)

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 GF254 plates (15x5 cm, 3 mm thick). The plates were then placed in a mobile phase system based on Table 1 (8). 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 (10). The developed spot colors were noted, and the Rf values were calculated using formula 1 (10):

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

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

No	Compounds	Mobile Phases	Spraying Reagents	Colour of the spot
1	Alkaloids	Chloroform: Methanol (3:2)	Dragendorff	Orange/ Brown
2	Polyphenols	Chloroform: Methanol (7: 3)	$FeCl_3$	Blackish blue
3	Flavonoids	Ethyl acetate: Methanol: Water: Glacial acetic acid (1,7: 0,5: 0,5: 0,5)	$FeCl_3$	Grey
4	Saponins	Chloroform: Methanol (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 Chun et al (2003) with modifications (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 its maximum wavelength. The phenolic concentration was calculated based on a standard curve obtained through linear regression.

Total flavonoids content (TFC)

The determination of flavonoids content was done using Chang et al (2020) method with modifications (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 using a UV-Vis spectrophotometer, and the total flavonoid concentration was determined using a quercetin standard curve via linear regression analysis.

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)

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% clindamycin phosphate served as the positive control, 1% DMSO as the negative control, and the experimental groups included plant extract or fraction at 800 ppm and 600 ppm concentrations. The culture plates were incubated for 18–24 hours at 37 °C, and the size of the inhibition zones was determined with a calliper (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 (menstrum) 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 (14). This technique was chosen to protect thermolabile chemicals while also adhering to Indonesian herbal pharmacopoeia.11 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). 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

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 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 number 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 (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 accordance 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%

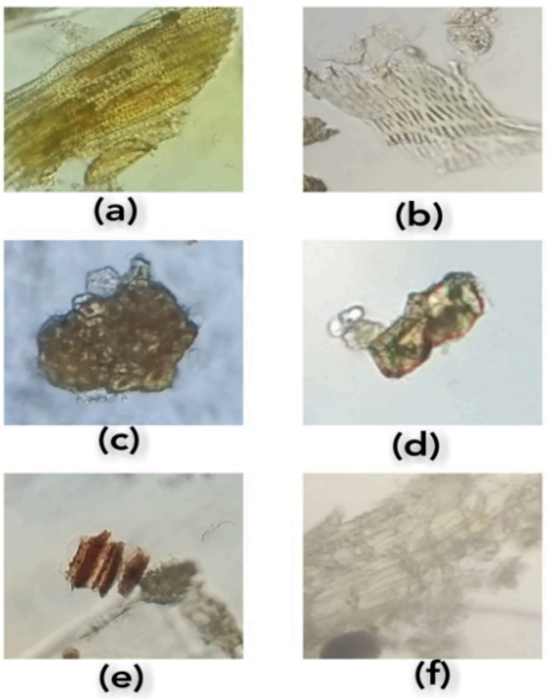


Figure 2: Microscopic from yellow root powder fragments (a) fibrous sklerotic cells; (b) parenchymal cells; (c) exocarpic stone cells; (d) stone cells; (e) essential oil cells; and (f) tracheal cells.

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 (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 (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).

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)

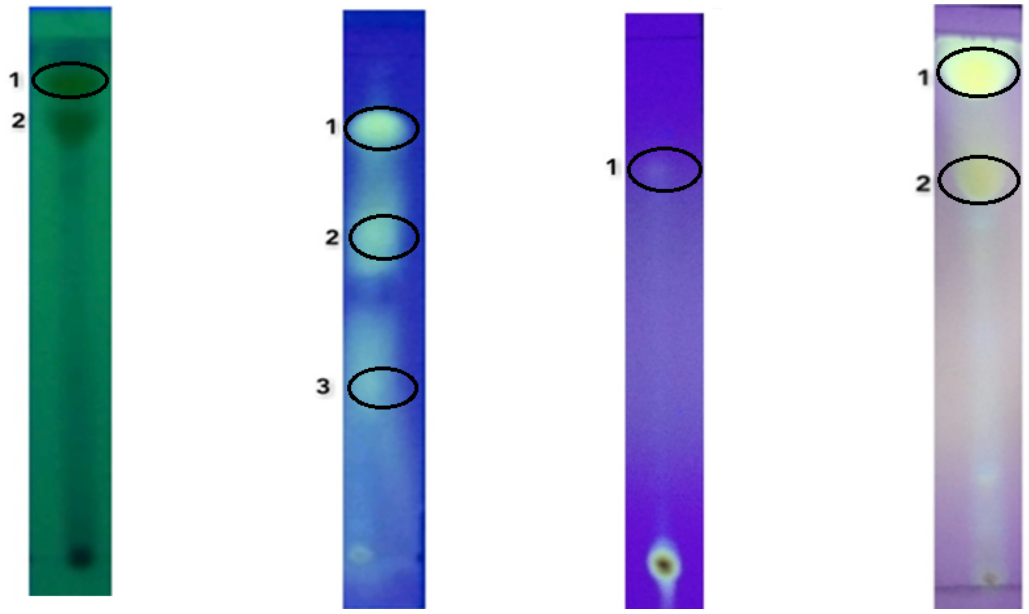


Figure 3: Chromatogram of Yellow Root Extract

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 (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 87.37 mg gallic acid equivalents (GAE) per gram of dried extract (Table 7).

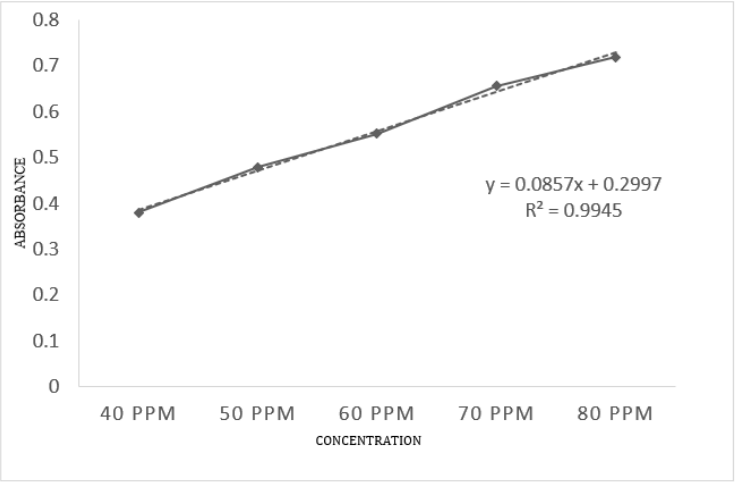


Figure 4: Calibration Curve of Gallic Acid (n=3)

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		0.269	86.92	
	2	2500	0.321	87.72	87.37±0.011
	3		0.308	87.52	

Total flavonoid content **(**TFC) was determined using a colorimetric technique with quercetin as a reference flavonoid component (19) TFC in the extracts were calculated from the regression equation of the calibration curve (y=0.0753x+0.082; 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 mg QE/g, as indicated in Table 8.

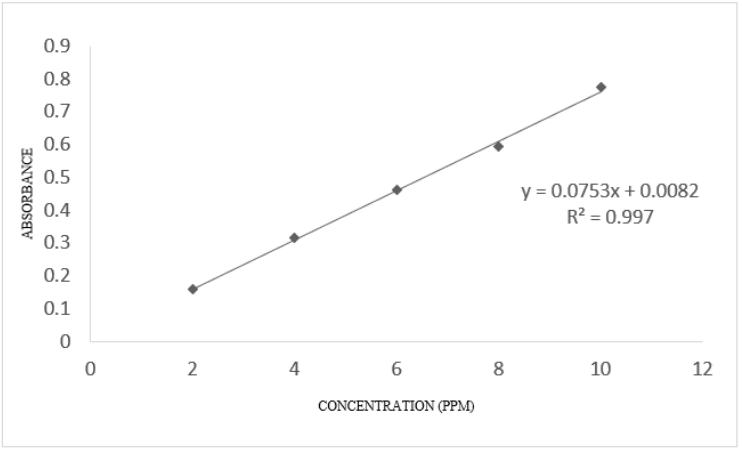


Figure 5: Calibration Curve of Quercetin (n=3)

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		0.438	74.08	
	2	2500	0.436	73.74	73.80±0.002
	3		0.435	73.56	

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).

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* (20).

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

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.

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Conflict of interest

Authors declare no conflict of interests

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