Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(9):99-104
(http://derpharmachemica.com/archive.html)

Isolation of scopoletin from subang-subang plants (*Spilanthes paniculata* Wall. ex Dc.)

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ABSTRACT

Isolation of scopoletin from ethyl acetate extract of Subang-Subang (Spilanthes paniculata Wall. Ex. DC.) was successfully established respectively by column and preparative thin layer chomatography. Ethyl acetate extract was separated by column chromatography using silica gel as stationary phase. Several organic solvent such as n-Hexane, ethyl acetate and methanol were used as mobile phase and the isolated compound was purified by preparative thin layer chromatography using n-Hexane : ethyl acetate (1:1) as the eluent. Isolated compound obtained was 11 mg of white solid form. Purification test of isolated compound which was effectued by TLC (Thin Layer Chromatography) using 10% NaOH gave blue colour fluorecence and the UV-Vis spectrum shown a weak absorption peak at 260 nm and 357 nm. Isolated compound which was characterized by FT-IR spectroscopic method indicated respectively the -OH , C-H, C=O, and C=C (aromatic) stretching at 3435 cm^{-1}, 2917 cm^{-1}, 1654 cm^{-1}, 1535 cm^{-1}, and only at 1462 cm^{-1} which was shown the C-H bending. Absorption bands at wave numbers 1241 cm^{-1} and 1165 cm^{-1} indicated the presence of ester groups. Based on the results of characterization, the isolated compound was classified into scopoletin compound. Bioactivity test as antibacterial of ethyl acetate extract and scopoletin compound by disk diffusion method had shown that the ethyl acetate extract and scopoletin compound are inactive against Neisseria sp bacteria.

Keywords*:* Subang-Subang, *Spilanthes paniculata* Wall. Ex DC , *Scopoletin*, antibacterial

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INTRODUCTION

Subang-Subang plant (*Spilanthes paniculata* Wall. Ex DC)) is a weed plant in asteraceae family [1]. There are some bioactivity tests were found to the Subang-Subang plant extracts such as antimicrobial antioxidant and cytotoxicity activities [2]. The plant has some applications in pharmaceuticals as an anti tooth ache formulation for pain relief [3], swelling and gum infections [3], periodontosis [4] and in mouthwashes [5]. A number of constituents had been isolated from the *Spilanthes paniculata Wall. Ex DC* for example, spilanthol, isobutylamides [6,7] and triterpenoids [8]. There has been reported that Subang-Subang also has coumarin compounds [9]. Coumarin compound prenyletin in before has been isolated from the ethyl acetate extract of Subang-Subang plant [10]. Coumarin and its derivatives are biologically very active. It was found that the enhanced activities are dependent on the coumarin nucleus. Biological significance of these compounds include anti-bacterial, anti-thrombotic and vasodilatory, anti-mutagenic, lipoxygenase and cyclooxygenase inhibition, scavenging of reactive oxygen species, and anti-tumourigenic [11].

From the phytochemical profile test, ethyl acetate extract of the plant is positive containing coumarin. As far as literature study that has been done, there are still only few of reports about coumarin compound characterisation and its activity as antimicrobial agent from Subang-Subang plant (*Spilanthes paniculata* Wall. Ex DC)) especially to bacteria *Neisseria sp*. Preference of the bacteria is based on the application of this plant as traditional medicine for toothache so the bacteria *Neisseria sp* is used because it is a kind of mouth bacteria [12]. Major oral microorganisms

include *Streptococcus pneumoniae, Staphylococcus aureas, Streptococcus mitis* and *Neisseria subflava* in the gingival crevice, *Streptococcus oralis, Rothia mucilaginosa* and *Kingella oralis* on teeth surface, *Streptococcus infantis, Streptococcus pseudopneumoniae, Actinomyces viscosus* on tongue surface [13].

MATERIALS AND METHODS

1. Chemical agent, tools and instruments

The chemical agents that have been used in this research are Subang-Subang plant (*Spilanthes paniculata* Wall. Ex DC)), technical solvent that has been distillated which is hexane, ethyl acetate and methanol. The stationary phase that has been used in column chomatography is silica gel (0,063-0,200 nm). Stationary phase for thin layer chromatography analysis used (silica gel 60 F254), filter paper (Whatman No. 40) and aluminium foil. The chemical that are used in coumarin test are node marker NaOH 1%. The chemicals that are used in antimicrobial test are bacteria bred from pure bacteria *Neisseria sp* which is taken from microbiology laboratory of medicinal faculty of Andalas University, medium Blood Agar Plate (BAP) which is taken from microbiology laboratory UPTD. Laboratory of Dinas Kesehatan West Sumatera Province, and filter paper (Whatman No. 40) as disk. DMSO as positive control solvent and positive control amoxicillin tablet 500 mg produced by PT Dankos Farma.

Tools that are used are distillation instruments, spectrophotometer *Ultraviolet Visible* (Thermo scientific serial evolution 201), spectrophotometer *Fourier Transform Infrared* (Thermo Scientific Nicolet iS10), column chomatography, UV lamp (λ 254 and 365 nm), autoclave, incubator, *laminar air flow*, petri dish, and ose needle.

2. Research procedure

2.1 Preparation of sample and compound isolation

Subang-Subang plant is taken from Andalas University region, Limau Manis, Padang, West Sumatera. Plant identification is done in Andalas University herbarium. Subang-Subang plant (*Spilanthes paniculata* Wall. Ex DC) is extracted by maceration (immersion), sample in fine powder form (± 1.050 g) is poured into macerator (three dark bottles each containing x 350 g of sample), then hexane solvent is added until all the samples are immersed and solvent volume ± 5 cm above sample layer. Sample is macerated by hexane solvent for four days and shaken by shaking the bottle each day to perfect the extraction process Subang-Subang then filtered. Maceration is done repeatedly for 5 times until the macerate almost has no colour. Each macerat solvent is then evaporated using rotary evaporator in temperature 40^0C and gathered then concentrated hexane extract that received is scaled. Next, the residue of the macerate with hexane is remacerated with ethyl acetate with same procedure so that ethyl acetate extract is received. Then the residue of ethyl acetate maceration is re-macerated again with methanol with same procedure so that methanol extract is again receiced[4]. After three extracts are gotten that are from Subang-Subang plant, ethyl acetate extract is chosen to isolate the coumarin compound. This is based on phytochemical test of extract that showing ethyl acetate extract containing coumarin.

Coumarin in ethyl acetate extract was extracted through separation by column chomatography with eluent SGP system (Step Gradien Polarity) with stationary phase of silica gel and mobile phase of hexane, ethyl acetate and methanol. Towards the elucidation products, TLC (Thin Layer Chroamography) test is redone, node with Rf value and same node mark is combined into 1 sub-fraction containing coumarin is purified by TLC preparative. From the product of TLC preparative, TLC test was done by addition a specific 1% NaOH reactant, bright blue fluorescence node indicated the existence of coumarin.

Purification test is done through TLC test using some eluent polarity ratio and some node marker agents that are specific for other secondary metabolites. Pure compound will show single node mark after repeated elucidation with varied eluent polarity gradient and does not change when node marker agents are added. Then compound classification and identification is done to know what is the composition of the isolated compounds. If there is bright blue fluorescence marker after addition of NaOH 1%, the compound is positively containing coumarin.

2.2. Characteristics

Isolated compounds is characterized using UV and FT-IR spectroscopy.

2.3. Antibacterial activity

Bred bactery that has been prepared in medium BAP that has already solidified. Filter paper Whatmann No. 40 (d = 5 mm) is immersed in ethyl acetate extract with concentration variation 1000 mg/L, 500 mg/L, 250 mg/L, 125 mg/L and 0 mg/L then put into petridish containing bacteria growth medium. For isolated compounds, concentration variation that are used are 1000 mg/L, 500 mg/L, 250 mg/L, 125 mg/L snd 0 mg/L. Amoxicillin is used as positive control. Growth medium is incubated for 1x24 hour in temperature 37^0C. After incubation, halo zone observation is done.

RESULTS AND DISCUSSION

1. Sample preparation and compound isolation

1.1 Phytochemical profile

Phytochemical test result from ethyl acetate extract of Subang-Subang plant can be seen in table 1.

Table 1: **Phytochemical test result from ethyl acetate extract of Subang-Subang plant (*Spilanthes paniculata* Wall. ex DC.)**

Secondary metabolites	Test result
Alkaloid	-
Flavonoid	-
Fenolik	-
Steroid	+
Triterpenoid	+
Coumarin	+
Saponin	-

1.2 Isolated compounds analysis

Isolated compounds that are achieved were 11 mg white solids. Result of TLC test of isolated compounds give single node blue fluorescence with varied eluent, the results are listed in table 2.

Table 2: Result of TLC test of isolated compounds give single node blue fluorescence with variation of eluent

No.	Eluent	Rf
1.	hexana : ethyl acetate (5:5)	0,57
2.	hexana : ethyl acetate (4:6)	0,64
3.	hexana : ethyl acetate (2:8)	0,83

Result of coumarin purity test of isolated compounds with varied node marker agents for other secondary metabolites are listed in table 3.

Table 3: Result of coumarin purity test of isolated compounds with varied node marker agents

No	Marker agents	Result
1	UV Lamp 254 nm	There is no node blue fluorescence
2	UV Lamp 365 nm	One node blue fluorescence
3	UV Lamp 365 nm + NaOH 1%	One node blue fluorescence increase bright
4	UV Lamp 365 nm + Sitroborat	There is no change
5	Uap I_2	One node brown
6	LB (*Liebermann-Burchard)*	There is no change

2. Characterization

UV spectrum of isolated compound (figure 1) shows 3 absorption peaks which are in wavelengths 202,8 nm, 305,4 nm and 410,2 nm. In wavelength absorption 410,2 nm, it is indicated that there are $n \rightarrow \pi^*$ transitions. This transition is lactone ring transition of coumarin structure. Meanwhile the absorptions for wavelengths 202,8 and 305,4 indicating that there is some $\pi \rightarrow \pi^*$ transitions. This transition is happens in conjugated double bonds in benzene structure in coumarin. Coumarin compound prenyletin has been isolated from the ethyl acetate extract of Subang-Subang plant. UV spectrum of prenyletin (figure 2) shows 2 absorption peaks which are in wavelengths 208,9 nm and 345,36 nm [10].Spectrum that is achieved compared to the standard coumarin spectrum has been reported, that can be observed in figure 3. Based on the standard spectrum, it can be observed that spectrum pattern of isolated compounds has similarity with spectrum pattern of scopoletin whereas this scolopetin has absorptions in wavelengths 245 nm, 277 nm, and 391 nm. This isolated compound has absorptions in wavelengths 202,8 nm, 305,4 nm and 410,2 nm so that can be assumed that isolated compounds are coumarin compounds that are classified in scolopetin class.

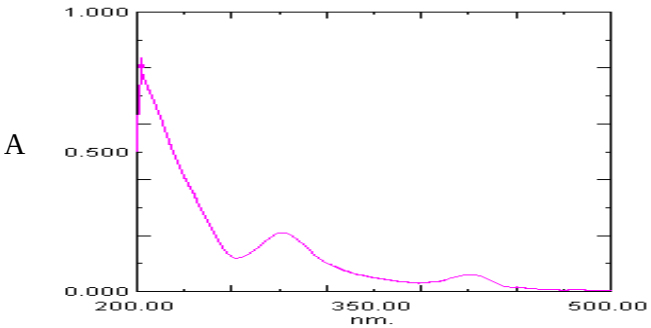

Figure 1: **UV spectrum of isolated coumpound**

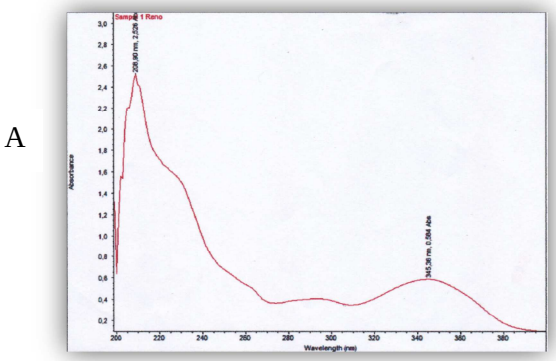

Figure 2: UV spectrum of prenyletin [10]

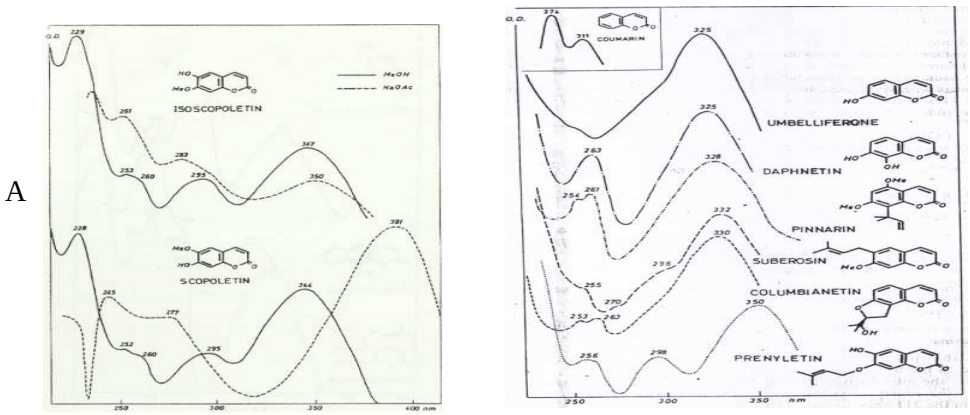

Figure 3: Spectrum that is achieved compared to the standard coumarin spectrum has been reported [14]

From FTIR spectrum (figure 4) there is -OH stretching in wavelength 3435 cm^{-1}. Then there is absorption band 2917 cm^{-1} that indicating the existence of C-H stretching, wavelength 1654 cm^{-1} indicating C=O stretching and absorption band in 1535 cm^{-1} indicating aromatic C=C stretching. Absorption band in 1462 cm^{-1} indicating C-H bending. Absorption bands in wavelengths 1241 cm^{-1} and 1165 cm^{-1} indicating that there are ester group in this compound, this hypothesis is enforced by that isolated compounds are coumarin compounds that have hydroxyl groups (scopoletin).

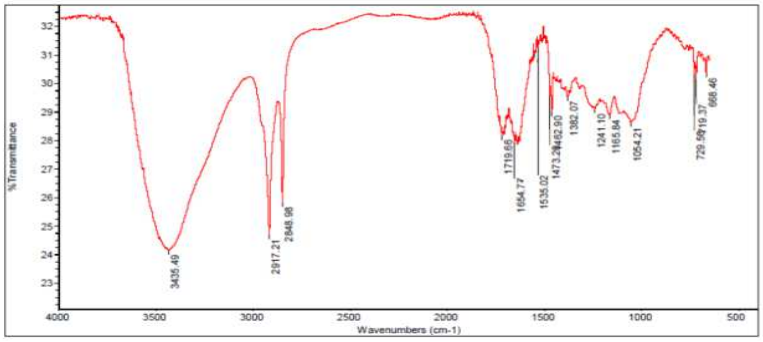

Figure 4: FTIR spectrum of isolated compounds

3. Antibacterial activity

Antibacterial activity is seen from observation result from halo zone which is shown by the existence of halo zone around paper disk. Observation result is listed in table 4 and 5.

Table. 4: **Antibacterial test result from ethyl acetate extract Subang-Subang plant towards bacteria *Neisseria sp***

Bacteria	Diameter of halo zone (mm)					Positive control
	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1.000 mg/L	
Bacteria *Neisseria sp*	-	-	-	-	-	31 mm

Table 5 : Result of antibacterial activity test of isolated compounds toward bacteria *Neisseria sp*

Bacteria	Diameter of halo zone (mm)					Positive control
	0 mg/L	125 mg/L	250 mg/L	500 mg/L	1.000 mg/L	
Bacteria *Neisseria sp*	-	0,3	0,4	0,5	0,6	27,3

From the bacteria growth inhibition zone data and based on category of compound performance in inhibiting bacteria growth, it can be concluded that ethyl acetate extract of Subang-Subang plant in concentration 1000 mg/L to 125 mg/L is inactive as antibacterial agent towards bacteria *Neisseria sp* that is shown by the nonexistence of halo zone around paper disk. Meanwhile the category of compound performance in inhibiting bacteria growth it can be concluded that coumarin compound from isolated products in 1000 mg/L to 125 mg/L is inactive as antibacterial agent towards bactery *Neisseria sp* that us shown by the miniscule amount of average value of halo zone around paper disk. These show that coumarin compound from isolated compounds (scolopetin) are not active compounds that are acting as antibacterial agents in Subang-Subang plant.

CONCLUSION

From this research it can be concluded that isolated compounds are classified as coumarin compounds that are known by giving positive result using NaOH 1% as node marker agents and there are specific absorptions of coumarin functional groups in FT-IR spectrum. Based on UV spectrum data, isolated compounds are identical with coumarin scopoletin. From the bacteria growth inhibition zone data and based on compounds performance toward inhibiting microbes growth it can be concluded that coumarin compounds from isolated compounds are inactive as antibacterial agents toward bacteria *Neisseria sp.*

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