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Original Article

ESTABLISHMENT OF *PSEUDERANTHEMUM PALATIFERUM* (NEES) RADLK CALLUS CULTURE AND SCREENING OF SECONDARY METABOLITE PRODUCTION

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ABSTRACT

Objective: This study aims to establish callus culture of *Pseuderanthemum palatiferum* and to investigate the production of secondary metabolites from callus extracts.

Methods: Callus tissues were initiated using explants from *in vitro*, aseptically-grown plants. The effects of medium salt base (Murashige & Skoog; MS or Gamborg B5; GB5) and plant growth regulators (2,4-dichlorophenoxyacetic acid; 2,4-D, naphthaleneacetic acid; NAA, benzyl aminopurine; BAP) on the initiation of callus tissues were investigated. The growth of callus culture was studied, and an optimized medium was determined. The production of secondary metabolites in callus was investigated, in comparison with *P. palatiferum* leaf, on ethanolic extracts using test reagents and thin-layer chromatography (TLC).

Results: The condition suitable for initiation of callus from leaf explant was MS salt bases, supplemented with 5.37 µM NAA, which yielded friable callus within 2 w. After transfer, best growth was observed in MS medium supplemented with 5.37 µM NAA and 0.44 µM BAP, after 4 w. Chemical screening and TLC analysis of callus extracts showed presences of some secondary metabolites similar to that of the leaf extract, together with additional phytochemicals not originally found in *P. palatiferum* plant.

Conclusion: Callus culture was successfully established. With optimum culture conditions, this *in vitro* culture and can serve as another method to obtain medicinally-useful secondary compounds from *P. palatiferum*.

Keywords: *Pseuderanthemum palatiferum*, plant tissue culture, callus, secondary metabolites, thin-layer chromatography.

INTRODUCTION

Pseuderanthemum palatiferum (Nees) Radlk (Acanthaceae), or H wan-Ngoc, is a traditional Vietnamese medicinal plant with reputed activities for the treatment of many illnesses, including hypertension, diarrhea, rheumatoid, inflammatory bowel diseases, dysentery, constipation, influenza or cold, hepatitis, tumor, and nephropathy [1]. This Acanthaceous plant, known in Thai as Phay a Wa Norn, has received much attention and is popularly used as a medicinal herb [2]. Leaves of *P. palatiferum* were reported to contain several secondary metabolites, including β–sitosterol, stigmasterol, kaempferol, apigenin, salicylic acid, triterpenoid saponin, phytol, as well as primary active metabolites such as a proteinase enzyme pseuderantin. The plant was also rich in essential amino acids such as lysine, methionine, threonine, and minerals such as calcium, potassium, magnesium and iron [3].

The acquisition of secondary metabolites from natural or field-cultivated plants by extraction depends greatly on the growing environment, which significantly affects the quality and quantity of chemical compounds. Plant tissue culture technology offers an alternative route for both plant propagation and secondary metabolite production. The method allows the growth of plant materials that otherwise would be difficult to grow in nature because of geographic, climatic, or seasonal restrictions. From the plant materials, the production of secondary metabolites can also be manipulated *in vitro*, which can lead to overproduction of compounds of interest, or the production of chemicals not originally found in native plants.

Plant tissue culture has been studied in several members of the Acanthaceae family for the purpose of secondary metabolite production, including *Andrographis paniculata* [4], *Adhatoda vasica* [5], and *Rhinacanthus nasutus* (L.) Kurz [6]. The genus *Pseuderanthemum* consisted of more than 40 species, several of which were studied for useful phytochemicals. One example included *Pseuderanthemum carruthersii var. atropurpureum* [7], a plant used in Vietnam to heal wounds. The leaf extract of this plant was found to contain some fatty compounds, iridoids, phenylethanoids, and flavonoids. The root also composed of lignans and triterpenes [8]. Another related species, *Pseuderanthemum bicolor*, was used in the Philippines to cure diseases. Some of the compounds from the root extract were identified as lupeol, botulin, and a mixture of stigmasterol and sitosterol. Antimicrobial activity was also reported from this plant [9].

Despite the increasingly available information on phytochemicals, biological activity, and physiological studies, the report on plant tissue culture of the plant genus *Pseuderanthemum* was very limited. To date, only a callus initiation study on *P. acuminatissimum* has been published [10]. Callus induced from young leaf explants on Murashige and Skoog (MS) medium, supplemented with 4.52 µM 2,4-dichlorophenoxyacetic acid (2,4-D), was reported to yield the highest fresh and dry callus weight. The production of secondary metabolites from the cultured callus was not investigated.

Because of the significance of *P. palatiferum* from the phytochemicals bioactivity perspectives, it is of interest to attempt an *in vitro* culturing of *P. palatiferum* and to assess its ability to produce secondary metabolites. In this study, conditions suitable for callus initiation of *P. palatiferum* were investigated, callus culture was established. The effects of medium salt bases, and the types and amounts of plant growth regulators on the initiation and growth of cultured tissues were studied. The production of secondary metabolites was also investigated from the extracts of these tissue cultures.

MATERIALS AND METHODS

Plant materials

Fresh shoots and leaves of *P. palatiferum* were collected from the medicinal plant garden of Faculty of Pharmacy, Chiang Mai University. Leaf explants were dried at 50 °C and grinded to powder for extraction. Fresh shoot explants were surface-sterilized and used for establishment of aseptic plants for tissue culture use.

Preparation of aseptic plants

Shoot explants of *P. palatiferum* were washed thoroughly with distilled water, then surface-sterilized with 2% v/v sodium hypochlorite solution for 15 min. After rinsing three times with sterile distilled water, the explants were cut to 1.5 cm size and placed on one-quarter strength (1/4X) MS medium, supplemented with sucrose 20 g/l, agar, with no plant growth regulator (PGR) and pH adjusted to 5.8. The cultures were maintained under a 16/8 light/dark cycle at 25±1°C until leaves and roots developed. The obtained sterile plants were later used for callus initiation.

Establishment of callus culture

Leaves and stems were excised from the aseptically-grown plants and placed on MS medium containing sucrose 20 g/l, agar, supplemented with different types and concentrations of PGRs, and pH adjusted to pH 5.8. The tested PGRs included naphthalene acetic acid (NAA) 5.37 µM, 2,4-D 4.52 µM, NAA 5.37 µM+2,4-D 4.52 µM, NAA 5.37 µM+ benzyl aminopurine (BAP) 0.44 µM, 2,4-D 4.52 µM+BAP 0.44 µM, and NAA 5.37 µM+2,4-D 4.52 µM+BAP 0.44 µM) The cultures were incubated under a 16/8 light/dark cycle at 25±1°C. The formations of callus and/or other changes were observed during a 4-week period. Each tested group consisted of 30 samples.

Growth determination of callus

Callus established from aseptic plants was subcultured onto MS or Gam borg B5 (GB5) media. Each medium contained the salt base, sucrose 20 g/l, agar, and supplemented with different PGRs, with the pH adjusted to 5.8. The fresh and dried weights of callus culture were determined, and used to calculate the growth index (GI) as follows;

$$\text{Fresh growth index (FGI)} = \frac{\text{Final Fresh Weight–Initial Fresh Weight}}{\text{Initial Fresh Weight}}$$

Extraction

Leaves of *P. palatiferum* plant and callus tissues were collected, oven-dried, and grinded to powder. The powder (10 g) was then extracted by maceration with ethanol 95% 100 ml (3X) at room temperature for 24 h. Extracts were filtered through Whatman filter paper No.1, and dried under vacuum using a rotary evaporator.

Phytochemical screening tests

Preliminary tests for screening groups of secondary metabolites, including flavonoids (Shinoda's test), tannins, saponins (Froth test), terpenoids (Salkowski's test), anthraquinones (Borntrager's test), coumarins, and alkaloids, were carried out using standard, published methods [11].

Comparison of chemical compositions in extracts by thin-layer chromatography (TLC)

The thin-layer chromatographic (TLC) fingerprint was used to compare chemical compositions between extracts of tissue cultures and the leaf extract. Crude extracts, prepared to concentrations of 10 mg/ml, were spotted on TLC silica gel $G60F_{254}$ aluminum sheet. TLC sheets were developed in two developing solvent systems (DVS) of toluene: ethyl acetate: formic acid 5.0: 4.5: 0.5 (DVS-1) and toluene: chloroform: ethanol 10.0: 2.0: 0.5 (DVS-2). After development, TLC plates were evaluated under UV lights (254 nm and 365 nm), and sprayed with ani aldehyde-sulfuric acid (AS) reagent for detection of spots of different phytochemicals. Retention factor (Rf) of each spot was calculated as follows;

$$*\text{Rf} = \frac{\text{distance from starting line to middle of spot}}{\text{distance from starting line to solvent front}}$$

RESULTS AND DISCUSSION

The explants (terminal bud, lateral bud) of *P. palatiferum* can be grown on MS 1/4X media with no PGR during4 w. In the first week, the explant showed no significant change (fig.1A). After two weeks, plantlets that composed of a primary root, lateral root, leaves, and shoot tip were observed (fig.1B). Plantlets continued to grow in week 3 (fig. 1C), and reached3-4 cm height, with 4-5 leaves within 4 w (fig. 1D).

Leaves placed on all tested media developed callus within 4 w, but with different rates and characteristics (table 1). Callus formation on MS media supplemented with NAA 5.37 µM, 2,4-D 4.52 µM, and NAA 5.37 µM+2,4-D 4.52 µMall appeared after 2 w (fig. 2A-C, table 1), while on other media, callus appeared on 3rd week of culture (fig. 2D-F). MS medium supplemented with NAA 5.37 µM produced white, friable callus at the wound of leaf explant in the 2nd week. The callus grew bigger and covered most of the leaf explant in the 4th week of culture (fig. 2A). Despite being a weaker auxin than 2,4-D, NAA was reported to promote better callus induction in several plants, including two plant species of the Acanthaceae–*Justicia gendarussa* Burm. f. [12] and *Andrographis paniculata* (Burm. F) [13]–and also in *Pluchealanceolata* Oliver & Hiern [14]. MS medium supplemented with 2,4-D 4.52 µM produced light brown, friable callus covering the leaf surface. The callus grew very slowly as the color became darker in the 3rd week and appeared to stop growing by the end of the 4th weeks (fig. 2B). This was likely a result of the oxidation of phenolic compounds, released from the callus tissues, which caused reduced growth and eventual cell death [15]. 2, 4-D has been reported to cause similar browning in callus induction of *Asystasiagangetica* (L) T. Anderson [16]. MS medium supplemented with NAA 5.37 µM+2, 4-D 4.52 µM induced light yellow, friable callus that grew to become yellow in the 3rd and 4th weeks (fig. 2C). MS media supplemented with NAA 5.37 µM+BAP 0.44 µM, 2,4-D 4.52 µM+BAP 0.44 µMand NAA 5.37 µM+2,4-D 4.52 µM+BAP 0.44 µM started inducing callus on the surface of the explant in the 3rd week. The growths of callus on these media, however, were much lower compared to the first three media. MS medium supplemented with NAA 5.37 µM was chosen for study the effect of PGRs and type of medium on the growth of callus.

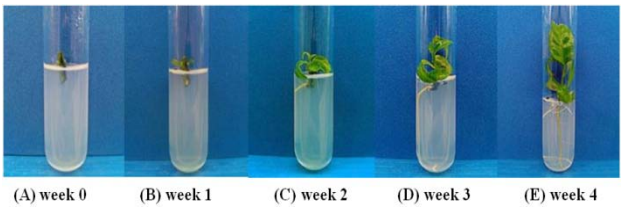

Fig. 1: Development of sterile *P. palatiferum* plants from explant duringa 4-week period

Growth of callus cultures

The growth index of *P. palatiferum* callus, cultured on MS or GB5 media with different PGRs for 4 w, are compiled in table 2. Essentially, the callus growth pattern followed the typical 3 phases: the lag phase, the exponential (log) phase, and the linear phase. Callus tissues formed and grew slowly in the lag phase. Then a rapid growth took place until the growth reached a maximum plateau. This was followed by a stable and/or slight, gradual decrease of growth in linear phase.

As shown in table 2, the optimum medium for the growth of callus was found to be MS medium supplemented with NAA 5.37 µMand BAP 0.44 µM. The medium suitably sustained the growth of white, friable callus, resulting in the highest GI of 6.29±0.66 on Day 24, while the GIs of others were in the range of 0.63-3.06. When MS was used as saltbase, the media with a PGR combination of an auxin (NAA or 2, 4-D) and a cytokinin (BAP) yielded better growth than those containing an auxin alone. Similar results were found in previous reports on *A. gangetica* [16], *Artemisia absinthium* L. [17], *P. lanceolata* [14], and *Centella asiatica* [18]. In contrast, GB5-based media yielded better callus growth when an auxin was used solely as a PGR. GB5 medium supplemented with NAA 5.37 µM promoted the highest callus growth with a GI of 3.09 on day 28.

Phytochemical screening test

Leaf and callus extracts of *P. palatiferum* were prepared by solvent extraction and were tested for the presence of secondary compounds by phytochemical screening tests. The results are compiled in table 3. The leaf extract yielded positive results for

tannins, saponins, and terpenoids, but flavonoids, which were found in the leaves according to a previous study [2], were not detected. It was possible that the amount of flavonoids in the leaf sample was lower than the detection limit. Similarly, screening of callus extracts showed the presence of tannins, saponins, and terpenoid as found in the plant leaves. Callus cultures of many plants of the Acanthaceae are capable of producing secondary metabolites that are accumulated in mother plants. Examples included the callus of *Barlerialupulina* L indl which produced cardiac glycosides, flavonoids, phenolic compounds, tannins, and terpenoids [19], while the callus derived from *Asystasiagangetica* was reported to contain saponins, tannins, flavonoids, phytosterols, terpenoids, and anthraquinones [20]. In addition, flavonoids, coumarins and alkaloids were detected in the callus extracts. Novel compounds not originally detected in mother plants are known to accumulate in plant tissue cultures [21], including callus culture [22].

Table 1: Effect of plant growth regulators on the initiation of callus on MS media during a 4-week period

PGRs (μM)			Week 1		Week 2		Week 3		Week4	
NAA	**2,4-D**	**BAP**	**Appearance of callus**	**Response (tubes)**	**Appearance of callus**	**Response (tubes)**	**Appearance of callus**	**Response (tubes)**	**Appearance of callus**	**Response (tubes)**
5.37	-	-	no change	-	white, friable callus at the wound of leaf explant	30	more white, friable callus at the wound of leaf explant	30	white, friable callus at the wound, covering most of leaf explant	30
-	4.52	-	no change	-	brown, friable callus covering leaf surface	28	dark brown, friable, covering leaf surface	30	dark brown, friable callus covering leaf surface	30
5.37	-	0.44	no change	-	no change	-	white, friable, at wound of leaf explant	8	more white, friable callus at wound of leaf explant	17
-	4.52	0.44	no change	-	no change	-	brown, friable callus at wound of leaf explant	26	more brown, friable callus at wound of leaf explant	30
5.37	4.52	-	no change	-	white to yellow, friable callus covering leaf surface	30	more white to yellow, friable callus covering leaf surface; size increased	30	white to deep yellow, friable callus covering leaf surface; size increased	30
5.37	4.52	0.44	no change	-	no change	-	white to brown, friable, at wound of leaf explant	17	white to brown friable callus, size increased	30

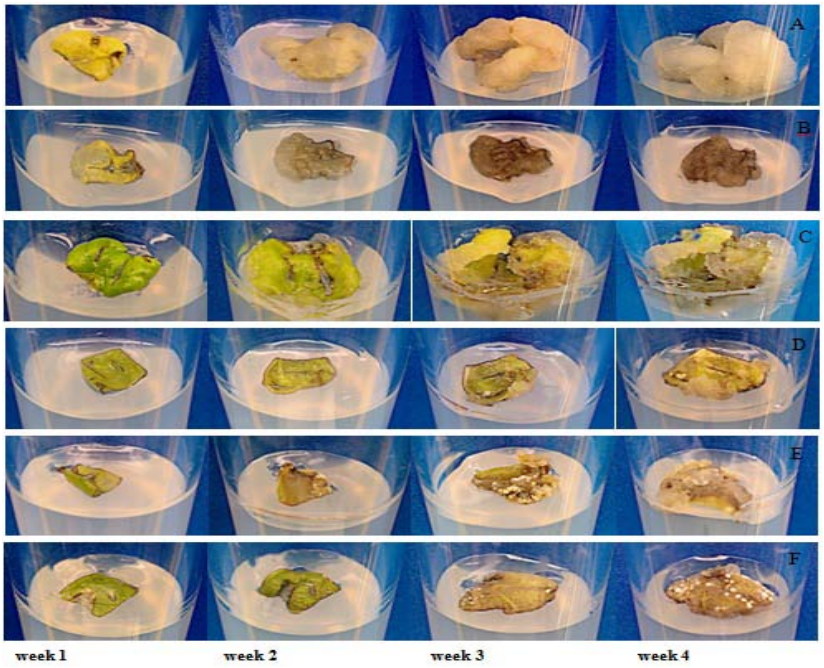

Fig. 2: *P. palatiferum* callus formation from leaf explant on (A) MS+NAA 5.37 μM; (B) MS+2,4-D 4.52μM; (C) MS+NAA 5.37 μM+2,4-D 4.52μM; (D) MS+NAA 5.37 μM+BAP 0.44μM; (E) MS+2,4-D 4.52μM+BAP 0.44μM; (F) MS+NAA 5.37 μM+2,4-D 4.52μM+BAP 0.44μM during a 4-week culture period

Table 2: Growth indices (GI) of callus cultures maintained on MS or GB5 media with different types and amounts of PGRs

Medium	PGRs (µM)			Growth index (±SD)								
	NAA	2,4-D	BAP	D_0	D_3	D_7	D_{10}	D_{14}	D_{17}	D_{21}	D_{24}	D_{28}
MS	5.37	-	-	0	0.12±0.06	0.18±0.03	0.44±0.06	0.67±0.14	2.03±0.16	3.24±0.35	3.06±0.05	3.07±0.13
	-	4.52	-	0	0.24±0.02	0.46±0.01	0.80±0.05	0.53±0.04	1.11±0.12	1.01±0.30	0.65±0.03	0.59±0.01
	5.37	4.52	-	0	0.36±0.09	0.19±0.17	0.30±0.17	0.35±0.11	2.19±0.27	3.26±0.85	0.99±0.29	1.01±0.46
	5.37	-	0.44	0	0.21±0.01	0.97±0.08	1.18±0.02	1.85±0.41	3.84±0.34	5.67±0.74	6.29±0.66	6.09±0.42
	-	4.52	0.44	0	0.19±0.06	0.30±0.12	0.47±0.15	1.07±0.32	1.26±0.40	1.45±0.16	1.68±0.15	1.44±0.23
	5.37	4.52	0.44	0	0.19±0.09	0.50±0.10	0.56±0.17	0.58±0.16	0.98±0.31	1.31±0.35	1.28±0.03	1.27±0.22
GB5	5.37	-	-	0	0.22±0.02	0.82±0.06	1.31±0.07	1.29±0.27	1.88±0.03	2.51±0.23	2.87±0.13	3.09±0.86
	-	4.52	-	0	0.25±0.05	0.54±0.08	0.60±0.03	1.08±0.07	0.76±0.04	0.72±0.12	0.63±0.32	0.83±0.12
	5.37	4.52	-	0	0.17±0.03	0.19±0.08	0.34±0.09	1.03±0.16	1.39±0.34	0.63±0.22	1.06±0.04	0.89±0.13
	5.37	-	0.44	0	0.13±0.03	0.37±0.22	0.32±0.10	0.52±0.18	1.24±0.12	1.32±0.33	1.78±0.09	1.75±0.06
	-	4.52	0.44	0	0.08±0.07	0.13±0.05	0.19±0.04	0.34±0.07	0.47±0.06	0.40±0.10	0.64±0.20	0.64±0.07
	5.37	4.52	0.44	0	0.26±0.08	0.49±0.19	0.63±0.21	0.66±0.31	0.99±0.14	0.99±0.34	1.03±0.17	1.29±0.16

Table 3: Phytochemical screening of *P. palatiferum* leaf and callus extracts

Tests	Results of samples												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Flavonoids													
-Shinoda's test	-	+	+	+	-	-	+	-	+	-	-	-	+
Tannins													
-Ferric chloride	+	+	+	+	+	+	-	+	-	+	+	+	+
-1% gelatin	+	+	+	+	+	+	+	+	+	+	+	+	+
-Gelatin salt	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins													
-Froth test	+	+	+	+	+	+	-	+	+	+	-	+	-
Terpenoids													
-Salkowski's test	+	-	-	+	+	-	+	-	+	-	-	+	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-	-
-Borntrager's test													
Coumarins													
-NaOH-paper	-	+	+	+	-	-	-	-	-	-	-	-	-
Alkaloids													
-Dragendorff's	-	+	+	+	+	+	+	+	+	+	+	+	+
-Mayer's	-	+	+	+	+	+	+	+	+	+	+	+	+
-Marme's	-	+	+	+	+	+	+	+	+	-	-	-	-
-Wagner's	-	+	+	+	+	-	+	-	+	-	-	-	-
-Valser's	-	+	+	+	+	+	+	+	+	-	-	+	-

Note:+present,-absent

Samples

1	*P. palatiferum* leaf extract	*7*	Callus from GB5+2,4-D 4.52µM
2	Callus from MS+NAA 5.37 µM	*8*	Callus from MS+2,4-D 4.52 µM +BAP 0.44 µM
3	Callus from GB5+NAA 5.37 µM	9	Callus from GB5+2,4-D 4.52µM+BAP 0.44µM
4	Callus from MS+NAA 5.37 µM+BAP 0.44µM	*10*	Callus from MS+NAA 5.37 µM+2,4-D 4.52µM
5	Callus from GB5+NAA 5.37 µM+BAP 0.44µM	*11*	Callus from GB5+NAA 5.37 µM+2,4-D 4.52µM
6	Callus from MS+2,4-D 4.52µM	*12*	Callus from MS+NAA 5.37 µM+2,4-D 4.52µM+BAP 0.44µM
		13	Callus from GB5+NAA 5.37 µM+2,4-D 4.52µM+BAP 0.44µM

Comparison of chemical compositions by chromatographic fingerprint

TLC chromatograms from DVS-1 revealed several chemical compositions in *P. palatiferum* leaf extract and some callus extracts (fig. 3). Under UV 254 nm, bands were detected at Rf 0.41, 0.50, 0.56, 0.71. Spots of callus culture extracts could be detected in some media, including MS+NAA+BAP (Rf 0.15, 0.39, 0.48 and 0.69), GB5+2,4-D (Rf 0.10, 0.19, 0.48 and 0.69) and GB5+2,4-D+BAP (Rf 0.10, 0.19, 0.48, 0.59 and 0.69). A green fluorescent spot could be observed at Rf 0.63 under UV 365 nm in *P. palatiferum* leaf extract, and also in callus culture extracts from GB5+2,4-D+BAP. Blue fluorescent spots were detected in the callus extracts from MS+NAA+BAP, at the Rf values of 0.13, 0.23, 0.25 and 0.40. The fluorescent spots can be attributed to the presence of phenolic compounds, including flavonoids [23]. After spraying with AS reagent, dark-blue spots were clearly detected at Rf 0.61, 0.68 and 0.81 in both the *P. palatiferum* leaf extract and callus culture extracts. Other spots at different Rf values were also detected in both the callus culture extract and the leaf extract. (table 4)

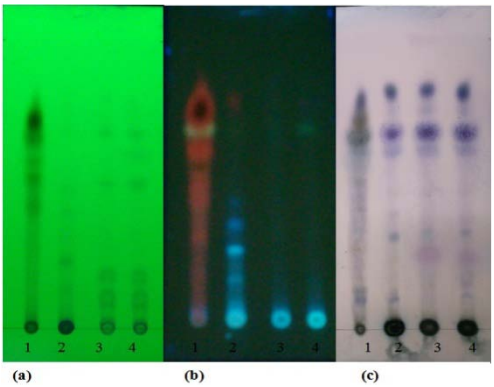

Fig. 3: TLC chromatogram of chemical composition in *P. palatiferum* leaf extract and callus culture extracts. DVS 1 (toluene: ethyl acetate: formic acid 5.0: 4.5:0.5), detection made under (a) UV 254 nm (b) UV 365 nm (c) AS reagent

Samples

1.	*P. palatiferum* leaf extract	2.	Callus from GB5+2,4-D 4.52µ**M**
3.	Callus from MS+NAA 5.37 µ**M**+BAP 0.44µ**M**	4.	Callus from GB5+2,4-D 4.52µ**M**+BAP 0.44µ**M**

Table 4: Rf values of chemical compositions in *P. palatiferum* leaf and callus culture extracts. Detection on TLC chromatogram developed using DVS-1 (toluene 5.0: ethyl acetate 4.5: formic acid 0.5) under UV 254, UV 365, and after AS spraying

Samples		Rf value*		
		UV 254 (nm)	UV 365 (nm)	AS spraying reagent
1.	*P. palatiferum* leaf extract	0.41,0.50,0.56,0.71	0.63	0.10, 0.14, 0.46, 0.50, 0.56, 0.61, 0.68, 0.81
2.	Callus extract from MS+NAA 5.37 µ**M**+BAP 0.44µ**M**	0.15, 0.39, 0.48, 0.69	0.13, 0.23, 0.25, 0.40	0.10, 0.24, 0.31, 0.39, 0.46, 0.61, 0.68, 0.81
3.	Callus extract from GB5+2,4-D 4.52µ**M**	0.10, 0.19, 0.48, 0.69	-	0.24, 0.31, 0.61, 0.68, 0.81
4.	Callus extract from GB5+2,4-D 4.52µ**M**+BAP 0.44µ**M**	0.10, 0.19, 0.48, 0.59, 0.69	0.63	0.10, 0.31, 0.61, 0.68, 0.81

TLC chromatogram developed using DVS-2 clearly showed spots in *P. palatiferum* leaf extract and some callus culture extracts (fig. 4). Under UV 254 nm, Rf values of *P. palatiferum* were 0.20, 0.26, 0.33, 0.38, 0.43, 0.48, and 0.60. Spots of callus culture extracts could be detected in MS+NAA+BAP (Rf 0.60), GB5+2,4-D (Rf 0.60) and GB5+2,4-D+BAP (Rf 0.21, 0.60, and 0.76) media. Strong blue fluorescence spots were detected under UV 365 nm at the very low Rf, suggesting that the compounds were polar. Additional weak fluorescent spots can be seen in callus extracts from MS+NAA+BAP (Lane 2; Rf 0.19), and GB5+2, 4-D+BAP (Lane 4; Rf 0.56). After spraying with AS reagent, dark-blue violet spots with Rf of 0.30, 0.40, 0.53, and 0.83 were observed in *P. palatiferum* leaf extract. These spots were previously defined as terpenoid compounds [24]. Callus extracts showed similar profiles, except the media MS+NAA+BAP for which the band at Rf 0.30 was not detected. Bands at Rf 0.19, 0.46 and 0.70 can only be detected in *P. palatiferum* leaf extract (table 5). Light exposure plays an important role not only in the growth but also the secondary metabolite production of callus, as indicated by the absence of secondary metabolite production in *Psoraleacorylifolia* when cultured in the dark [25]. In addition, the types and amounts of PGRs were known to affect the biosynthesis pathways and the accumulation of secondary metabolites [26], and were also responsible for the production of novel compounds [27].

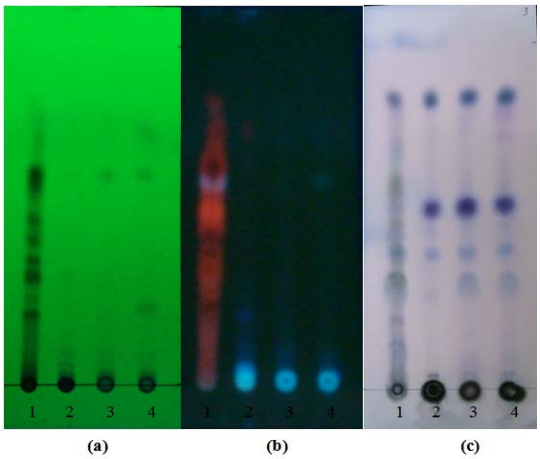

Fig. 4: TLC chromatogram of chemical composition in *P. palatiferum* leaf extract and callus culture extracts. DVS 2(toluene: chloroform: ethanol 10.0:2.0:0.5), detection made under (a) UV 254 nm (b) UV 365 nm (c) AS reagent

Samples

1.	*P. palatiferum* leaf extract	2.	Callus from GB5+2,4-D 4.52 µ**M**
3.	Callus from MS+NAA 5.37 µ**M**+BAP 0.44 µ**M**	4.	Callus from GB5+2,4-D 4.52 µ**M**+BAP 0.44 µ**M**

Table 5: Rf values of chemical compositions in *P. palatiferum* leaf and callus culture extracts. Detection on TLC chromatogram developed using DVS-2 (toluene 10.0: chloroform 2.0: ethanol 0.5) under UV 254, UV 365, and after AS spraying

Samples	Rf value*		
	UV 254 (nm)	UV 365 (nm)	AS spraying reagent
1. *P. palatiferum* leaf extract	0.20, 0.26, 0.33, 0.38, 0.43, 0.48, 0.60	0.56	0.19, 0.30, 0.40, 0.46, 0.53, 0.70, 0.83
2. Callus extract from MS+NAA 5.37 µ**M** +BAP 0.44 µ**M**	0.60	0.19	0.40, 0.53, 0.83
3. Callus extract from GB5+2,4-D 4.52 µ**M**	0.60	-	0.30, 0.40, 0.53, 0.83
4. Callus extract from GB5+2,4-D 4.52 µ**M**+BAP 0.44 µ**M**	0.21, 0.60, 0.76	0.56	0.30, 0.40, 0.53, 0.83

CONCLUSION

Callus culture of *Pseuderanthemum palatiferum* (Nees) Radlk (Acanthaceae) was successfully established for the first time from *in vitro*, aseptically-grown plants. The effects of medium salt base and plant growth regulators on the initiation of callus tissues were investigated and were shown to affect significantly both the formation and the growth of callus tissues. The condition suitable for initiation of callus from leaf explant was MS salt bases, supplemented with 5.37 µM NAA, which yielded friable callus within 2 w, while the best callus growth was observed in MS medium supplemented with 5.37 µM NAA and 0.44 µM BAP, after 4 w. TLC analysis of ethanolic extracts showed that callus tissues retained the ability to accumulate secondary metabolites similar to that of the leaf extract while also produced additional phytochemicals not originally found in *P. palatiferum* plant. Callus culture can be used as another method to obtain the plant materials, as well as the chemical compounds for the applications in pharmaceuticals and cosmetics.

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CONFLICT OF INTERESTS

Declared none

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