Phytochemical profiles and potential health benefits of *Helicteres hirsuta* Lour.

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1. INTRODUCTION

Helicteres hirsuta Lour. (*H. hirsuta*) has been used traditionally as a medicinal plant in some Asian countries (Thailand, Laos and Vietnam) for many years.

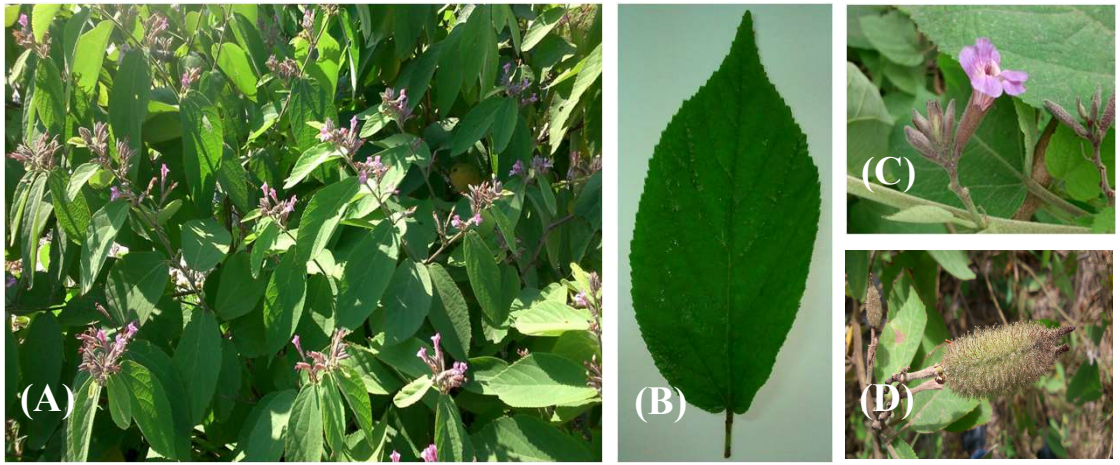


Figure 1. *H. hirsuta* plant (A), leaf (B), flower (C) and fruit (D) (Pham, 2014)

- **Recent findings:**

H. hirsuta

Extracted

Isolated

Lignans, phenolics, flavonoids, saponins

Possess antioxidant capacity

Exhibit antimicrobial activity

Kill various types cancer cells

⇒ This plant contains key bioactive compounds, which are linked with health benefits.

- **Several studies have been conducted to**:

+ extract,

+ isolate,

+ identify the key bioactive compounds from *H. hirsuta*, and

+ investigate its beneficial health effects.

- Therefore, **we aim to**:

+ review its traditional use,

+ summarise the extraction and isolation techniques on recovery of its bioactive compounds, and

+ discuss its potential use as therapeutic agents for prevention of different diseases.

2. TRADITIONAL USE

Table 1. Traditional use of *H. hirsuta* in some countries

Part used	Disease treatment	Preparation	Mode of administration	Country	References
Root	Malaria and diabetes mellitus	The root is decocted.	Oral intake	Thailand	Chuakul et al. (2002)
Root	Uterus pain	A handful (about 200 g) of the root mixed and boiled with the vine of Kheua sai tan.	Oral intake	Laos	Libman et al. (2006)
Whole plant	Furuncle	+ The whole plant is crushed with water or rice wine. + The whole plant is sun dried, then roasted in a hot pan and boiled with water to obtain the decoction.	+ The mixture is applied externally to the affected area. + Oral intake	Vietnam	Vo (2012)
Root	Pain relief	+ The root is ground and mixed with rice wine. + The root is boiled.	+ The mixture is applied externally to the affected area. + Oral intake	Vietnam	Vo (2012)
Root and leaf	Dysentery, measles, flu	The material is sun dried, then roasted in a hot pan and boiled with water to obtain the decoction.	Oral intake	Vietnam	Vo (2012)

3. BIOACTIVE COMPOUNDS DERIVED FROM *H. HIRSUTA*

Table 2. Bioactive compounds and antioxidant capacity of the powdered extracts from *H. hirsuta* leaf and stem (Pham, 2017a)

	Bioactive compounds			Antioxidant capacity (mg TE/g)			
	TPC (mg GAE/g)	TFC (mg CE/g)	Saponins (mg ESE/g)	DPPH*	ABTS*	FRAP	CUPRAC
Leaf powdered extract	192.6 ± 17.2	215.2 ± 29.3	808.0 ± 70.3	358.5 ± 31.2	564.3 ± 42.6	441.1 ± 42.2	747.8 ± 63.6
Stem powdered extract	212.6 ± 19.6	280.1 ± 27.7	347.5 ± 44.6	397.44 ± 37.8	588.6 ± 48.9	542.7 ± 32.1	867.3 ± 75.8

The values are expressed as mean ± standard deviation (n = 3).
TPC – total phenolic content, TFC – total flavonoid content, GAE – gallic acid equivalents, CE – catechin equivalents, ESE – escin equivalents, TE – trolox equivalents, FRAP – ferric reducing antioxidant power, CUPRAC – cupric ion reducing antioxidant capacity, *scavenging capacity.

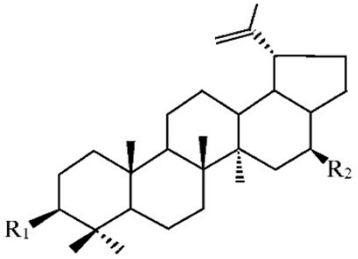
3. BIOACTIVE COMPOUNDS DERIVED FROM *H. HIRSUTA (CONT'D)*

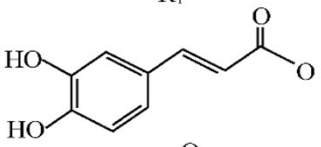
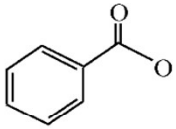
Table 3. Identified bioactive compounds in *H. hirsuta*

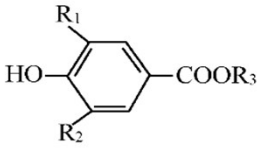
Collected place	Plant parts	Isolated compounds	Quantity	References
West Java, Indonesia	Stem	(±)-Pinoresinol	0.00063%	Chin et al. (2006)
		(±)-Medioresinol	0.00025%	
		(±)-Syringaresinol	0.00039%	
		(−)-Boehmenan	0.00016%	
		(−)-Boehmenan H	0.00006%	
		(±)-trans-Dihydrodiconiferyl alcohol	0.00051%	
Khanh Hoa, Vietnam	Stem	Rutin	2.83 mg/g PE	Pham et al. (2017a)
Binh Phuoc Province, Vietnam	Root	3-O-trans-caffeoylbetulinic acid	2.67 mg/kg DS*	Quang et al. (2018)
		3β-benzoylbetulinic acid	0.89 mg/kg DS*	
		Betulinic acid methyl ester	1.11 mg/kg DS*	
		Betulinic acid	4.22 mg/kg DS*	
		Lupeol	0.89 mg/kg DS*	
		4-hydroxybenzoic acid	2.22 mg/kg DS*	
		3,4-dihydroxybenzoic acid methyl ester	0.89 mg/kg DS*	
		4-hydroxy-3,5-dimethoxybenzoic acid	1.78 mg/kg DS*	
		Stigmasterol	1.11 mg/kg DS*	
	Stem and leaf	5,8-dihydroxy-7,4′-dimethoxyflavone	0.80 mg/kg DS*	
		Isoscutellarein 4′-methyl ether 8-O-β-D-glucopyranoside	1.00 mg/kg DS*	
		Methyl caffeate	0.50 mg/kg DS*	
Binh Duong Province, Vietnam	Aerial parts	3β-O-acetylbetulinic acid	10.2 mg/kg DS*	Nguyen et al. (2019)
		Simiarenol	3.4 mg/kg DS*	
		4,4′-sulfinylbis(2(tert-butyl)-5-methylphenol)	4.5 mg/kg DS*	
		7-O-methylisoscutellarein	5.2 mg/kg DS*	
		7,4′-di-O-methylisoscutellarein	2.5 mg/kg DS*	
		Stigmasterol	NR	
		β-sitosterol	NR	

NR: Not Reported.
PE: Powdered Extract.
DS: Dried Sample.
** Recalculated based on the data reported in the respective reference.*

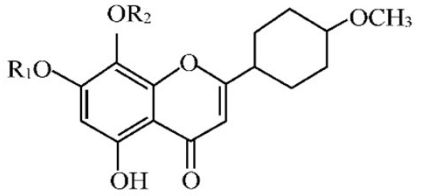
3. BIOACTIVE COMPOUNDS DERIVED FROM *H. HIRSUTA (CONT'D)*

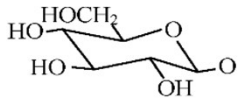


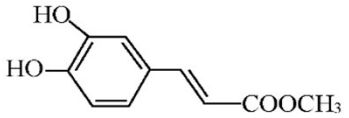
	R_1	R_2
1		COOH
2		COOH
3	OH	$COOCH_3$
4	OH	COOH
5	OH	CH_3



	R_1	R_2	R_3
6	H	H	H
7	H	OH	CH_3
8	OCH_3	OCH_3	H



	R_1	R_2
9	CH_3	H
10	H	



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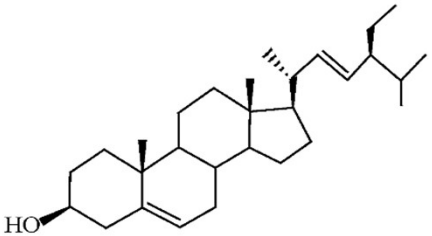

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Figure 2. Chemical structures of compounds 1-12 from *H. hirsuta* leaf, stem and root

3-*O-trans*-caffeoylbetulinic acid (1),
3β-benzoylbetulinic acid (2),
betulinic acid methyl ester (3),
betulinic acid (4),
lupeol (5),
4-hydroxybenzoic acid (6),
3,4-dihydroxybenzoic acid methyl ester (7),
4-hydroxy-3,5-dimethoxybenzoic acid (8),
5,8-dihydroxy-7,4′-dimethoxyflavone *(9)*
isoscutellarein *4′*-methyl ether *8-O-β-D*-glucopyranoside (10),
methyl caffeate (11), and
stigmasterol (12).
Modified from Quang et al. (2018)

3. BIOACTIVE COMPOUNDS DERIVED FROM *H. HIRSUTA (CONT'D)*

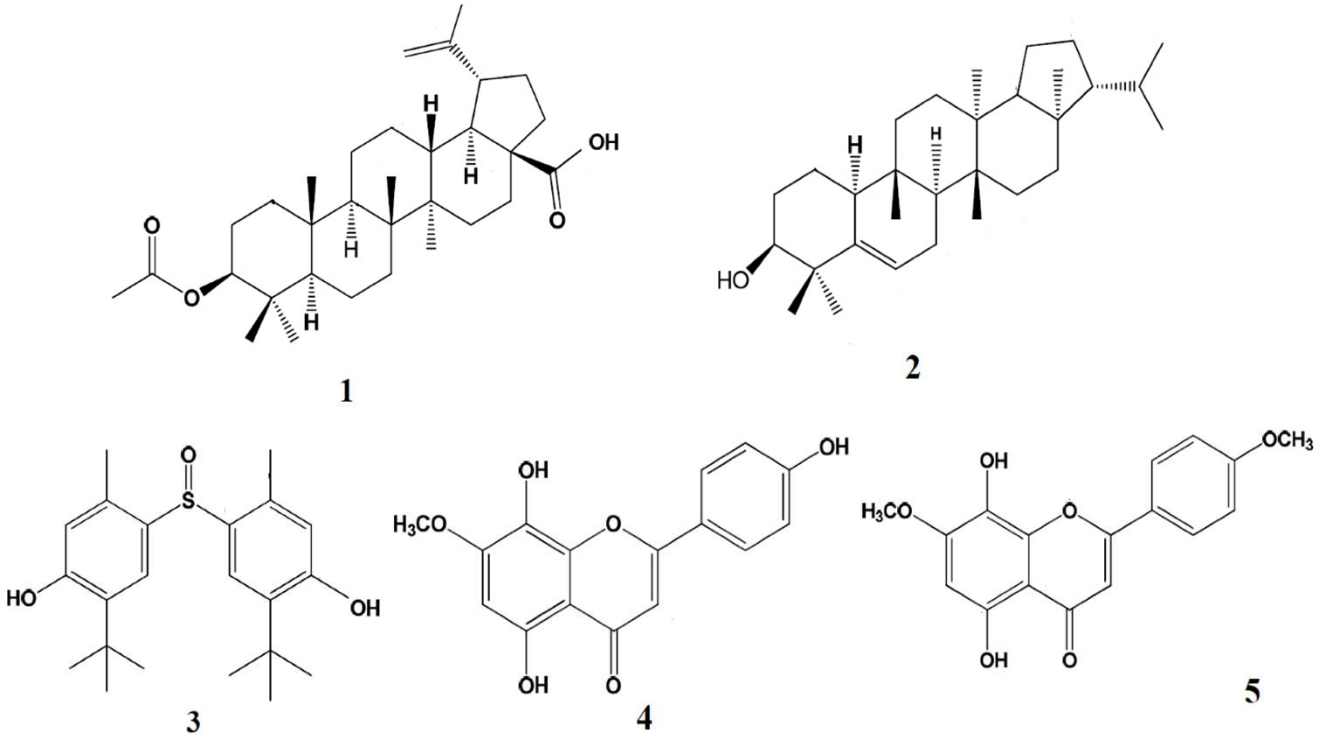

Figure 3. Chemical structures of isolated compounds 1-5 from aerial parts of *H. hirsuta*
3β-*O*-acetylbetulinic acid (1), simiarenol (2), 4,4'-sulfinylbis(2(*tert*-butyl)-5-methylphenol) (3), 7-*O*-methylisoscutellarein (4) and 7,4'-di-*O*-methylisoscutellarein (5).
Modified from Nguyen et al. [8]

4. RECOVERY BIOACTIVE COMPOUNDS FROM *H. HIRSUTA*

Table 4. Procedures of extraction and isolation of bioactive compounds from *H. hirsuta*

Plant material	Compounds isolated	Procedures	References
Stem	(±)-Pinoresinol, (±)-Medioresinol, (±)-Syringaresinol, (−)-Boehmenan, (−)-Boehmenan H, and (±)-Trans-dihydrodiconiferyl alcohol	- Drying and grinding the plant material, - Extracting dried sample with methanol overnight at room temperature and evaporating to dryness, - Redissolving with a mixture of MeOH-H_2O, then separating with hexane and $CHCl_3$, - Washing $CHCl_3$ with 1% saline solution, - Fractionating the $CHCl_3$ fraction by chromatography over a silica gel column, using gradient mixtures of CH_2Cl_2 and MeOH, - Purifying individual compounds by column chromatography and preparative TLC.	Chin et al. (2006)
Stem and leaf	• 5,8-Dihydroxy-7,4′-dimethoxyflavone; • Isoscutellarein 4′-methyl ether 8-*O*-*β*-*D*-glucopyranoside; and • Methyl caffeate	- The dried stem and leaf were extracted with methanol, then the crude extract was partitioned using *n*-hexane, ethyl acetate and *n*-butanol. - The butanol extract was purified by silica gel column chromatography, eluting with $CHCl_3$/MeOH/H_2O to give **5,8-dihydroxy-7,4′-dimethoxyflavone**. - **Isoscutellarein 4′-methyl ether 8-*O*-*β*-*D*-glucopyranoside** and **methyl caffeate** were purified from EtOAc extract by repeated silica gel column using hexane/EtOAc gradient from 0-100% EtOAc.	Quang et al. (2018)

Table 4. Procedures of extraction and isolation of bioactive compounds from *H. hirsuta* (Cont'd)

Plant material	Compounds isolated	Procedures	References
Root	• Betulinic acid methyl ester; • Stigmasterol; betulinic acid; • 3-O-*trans*-caffeoylbetulinic acid; • 3β-benzoylbetulinic acid; • 3,4-dihydroxybenzoic acid methyl ester; • 4-hydroxybenzoic acid • Lupeol; and • 4-Hydroxy-3,5-dimethoxybenzoic acid	- Dried roots were extracted with methanol, then concentrated under vacuum to obtain a residue. - Separating the residue by different solvents to obtain five sub-extracts including *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol extracts. - The hexane extract was applied to silica gel column, hexane/EtOAc gradient from 10/1 to 100% to give ten fractions. Fraction 3 was chromatographed on silica gel, hexane/acetone (10/1) to give compounds **betulinic acid methyl ester** and **stigmasterol**. - The dichloromethane extract was chromatographed on silica gel column, using hexane- chlorofrom to give ten sub-fractions. Sub-fraction 8 was further purified by silica gel column, using a solvent system hexane-EtOAc (1.5/1) to give **betulinic acid**. - The EtOAc extract was chromatographed on silica gel column, using hexane-EtOAc (1:1) gradient to EtOAc (100%) to afford eight fractions (Fr1- 8). + Fraction 1 and 2 were purified by prep. HPLC using hexane-EtOAc (1/1) to yield **3-O-*trans*-caffeoylbetulinic acid**, **3β-benzoylbetulinic acid** and **3,4-dihydroxybenzoic acid methyl ester**. + **4-hydroxybenzoic acid** was obtained from fraction 3 by prep. HPLC eluting with hexane-EtOAc (1/1.5). + **Lupeol** and **4-hydroxy-3,5-dimethoxybenzoic acid** were purified from fraction 7 by silica gel column chromatography using hexane-EtOAc (1/2) and followed by prep. HPLC with hexane-EtOAc (1/1.5).	Quang et al. (2018)

Table 4. Procedures of extraction and isolation of bioactive compounds from *H. hirsuta* (Cont'd)

Plant material	Compounds isolated	Procedures	References
Aerial parts	• Stigmasterol; • β-Sitosterol; • 3β-*O*-Acetylbetulinic acid; • 4,4′-Sulfinylbis(2(*tert*-butyl)-5-methylphenol); • 7,4′-di-*O*-Methylisoscutellarein; • 7-*O*-Methylisoscutellarein;	- The powdered sample was extracted by reflux using *n*-hexane, CH_2Cl_2, EtOAc and MeOH for 2h at boiling point of each solvent to obtain four extracts H1, D1, E1 and M, respectively. - The MeOH extract (M) was dissolved in water and partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH to obtain another four extracts H_M, D_M, E_M and B_M, respectively. + H1 extract was subjected to silica gel column chromatography. The elution was monitored by TLC and fractions of similar TLC fingerprint were combined to obtain 21 fractions (R.H1-R.H21). Fraction R.H10 produced crystals which were recrystallized to yield the mixture of **stigmasterol** and **β-sitosterol**. Fraction R.H16 was washed and recrystallized in MeOH to yield **3β-*O*-acetylbetulinic acid**. + D1 extract was subjected to silica gel column chromatograply to obtain 24 fractions (R.D1-R.D24). Fractions R.D21 and R.D23 were combined and re-chromatographed by a further silica gel to obtain 7 subfractions (R.D21.1-R.D21.7). Subfraction R.D21.3 was separated using a semi-preparative HPLC-DAD system to obtain **4,4′-sulfinylbis(2(*tert*-butyl)-5-methylphenol)**. + D_M extract was subjected to silica gel column chromatograply to obtain 7 subfractions (D_M.1-D_M.7). D_M.1 and D_M.2 were washed and recrystallized in MeOH to obtain **7,4′-di-*O*-methylisoscutellarein** and **7-*O*-methylisoscutellarein**, respectively. The supernatant of D_M.2 was recrystallized, dried and then purified using the same semi-preparative HPLC system as used for R.D21.3 to further obtain **7-*O*-methylisoscutellarein**.	Nguyen et al. (2019)

Table 4. Procedures of extraction and isolation of bioactive compounds from *H. hirsuta* (Cont'd)

Plant material	Compounds isolated	Procedures	References
Aerial parts	• Simiarenol	- The powdered sample was extracted under reflux using aqueous ethanol 70% as the solvent at boiling point for 24 h to obtain EtOH extract. - The EtOH extract was dissolved in distilled water and partitioned with *n*-hexane, CH_2Cl_2, EtOAc and *n*-BuOH to obtain 4 extracts H2, D2, E2 and B, respectively. H2 extract was subjected to silica gel column chromatography to obtain 17 subfractions (EH.1-EH.17). Subfraction EH.2 was purified by silica gel column chromatography to obtain four subfractions (EH.1.1-EH.2.4). Subfraction EH.2.4 was washed and recrystallized in *n*-hexane to obtain **simiarenol**.	Nguyen et al. (2019)

TLC: Thin layer chromatographic. EtOAc: Ethyl acetate. MeOH: Methanol. EtOH: Ethanol. n-BuOH: n-Butanol.
HPLC: High performance/pressure liquid chromatography.

5. THE POTENTIAL HEALTH BENEFITS OF *H. HIRSUTA*

5.1. Potential use as novel anticancer agents

- **(±)-Pinoresinol, (−)-boehmenan** and **(−)-boehmenan H** isolated from *H. hirsuta* stem possessed cytotoxic activity against:
  + Human lung carcinoma Lu1 (IC_{50} values of 0.8, 10.4 and 5.3 µg/mL, respectively)
  + Hormone-dependent human prostate carcinoma LNCaP (IC_{50} values of 0.5, 9.5 and 7.7 µg/mL, respectively), and
  + Human breast carcinoma MCF-7 cell lines (IC_{50} values of 1.7, 10.0 and 10.2 µg/mL, respectively) (Chin et al., 2006)

5.1. Potential use as novel anticancer agents (Cont'd)

- **3-O-*trans*-caffeoylbetulinic acid, betulinic acid methyl ester, betulinic acid** and **lupeol** isolated from the root, and **5,8-dihydroxy-7,4′dimethoxyflavone** isolated from the leaf and stem revealed their moderate cytotoxic activity against five cancer cell lines (Hela, HepG2, SK-LU-1, AGS and SK-MEL-2) with IC_{50} values of 46.03–80.17 μg/mL (Quang et al., 2018).

- **3β-O-acetylbetulinic acid** and **4,4'-sulfinylbis(2(*tert*-butyl)-5-methylphenol)** isolated from aerial parts of *H. hirsuta* possessed cytotoxic acitivity on leukemia CCRF-CEM and colon HCT116 cancer cell lines with the IC_{50} value range of 14.6–31.5 μM (Nguyen et al., 2019)

5.1. Potential use as novel anticancer agents (Cont'd)

- Rutin found in *H. hirsuta* stem (Pham et al., 2017d) possessed anticancer effects against various cancer cell lines *in vitro* and *in vivo* through cell cycle arrest and/or induction of apoptosis (Araújo et al., 2011; Chen et al., 2013; Karakurt, 2016; Lin et al., 2012).
- The extracts and saponin-enriched fractions from *H. hirsuta* leaf and stem effectively inhibited 11 cancer cell lines (Pham et al., 2018).
- Fractions derived from *H. hirsuta* stem possessed strong anti-pancreatic cancer activity *in vitro*. The active fractions caused cell cycle arrest at S phase and promoted apoptosis in MIAPaCa-2 PC cells (Pham et al., 2020).

5.1. Potential use as novel anticancer agents (Cont'd)

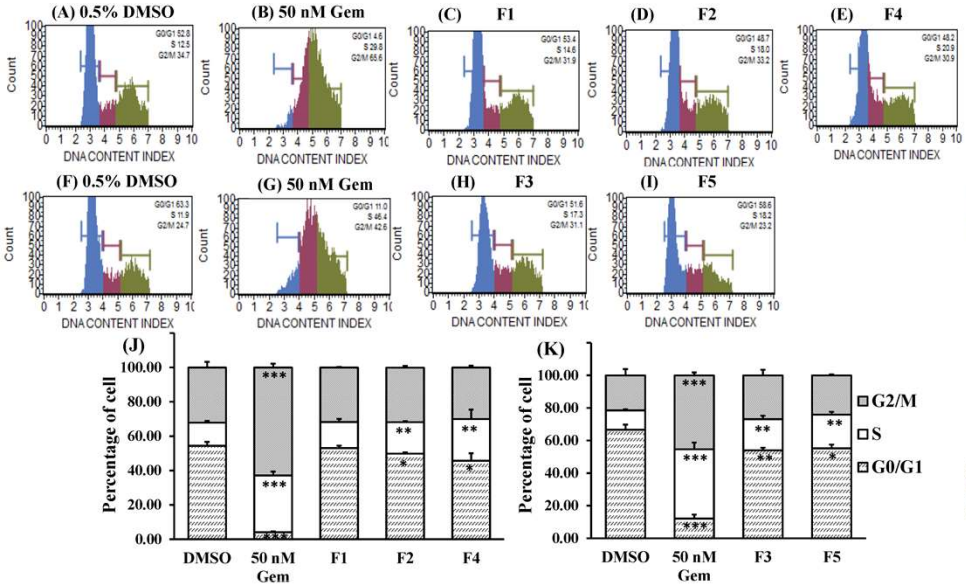

Figure 4. Cell cycle analysis of MIA PaCa-2 cells using the Muse™ Cell Cycle Kit after 48 h of treatment (Pham, 2020)

(A), (F) Untreated control; (B), (G) Gemcitabine: 50 nM; (C), (D), (E), (H) and (I): Fractions derived from *H. hirsuta* stem at 10 µg/mL. Graphs (J) and (K) show the cell cycle percentages of control and treated MIA PaCa-2 cells. (*), (**) and (***) indicate the values that are significantly different from the untreated control ($p<0.05$, $p<0.01$ and $p<0.001$, respectively).

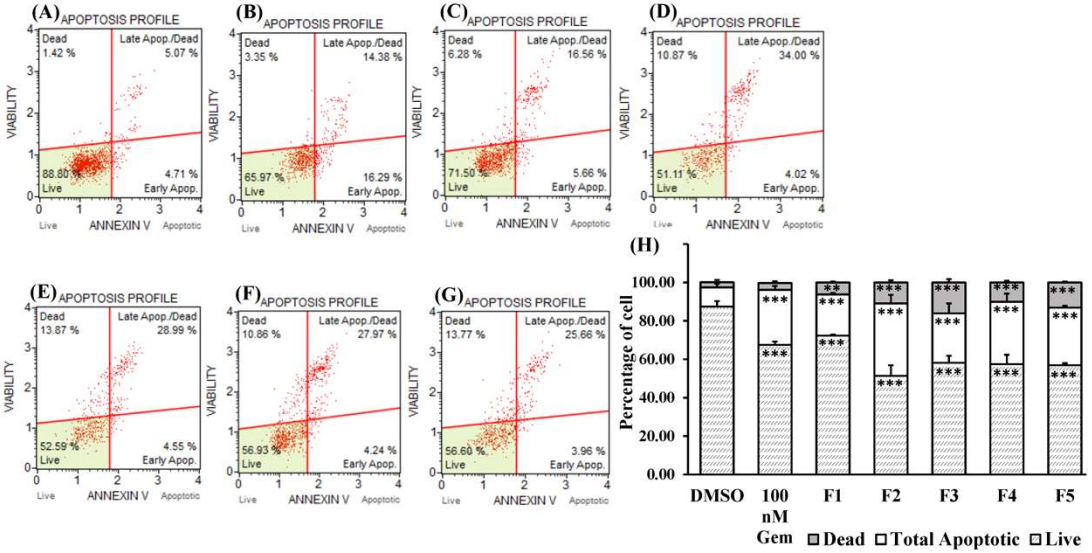

Figure 5. Apoptosis profiles of MIA PaCa-2 cells as studied by the Muse™ Annexin V & Dead Cell Kit after 48 h of treatment (Pham, 2020)

(A) Untreated control; (B) Gemcitabine: 100 nM; (C), (D), (E), (F) and (G): Fractions derived from *H. hirsuta* stem at 10 µg/mL. Graphs (H) show the live, total apoptotic and dead cell percentages of control and treated MIA PaCa-2 cells. Significant differences were observed between the untreated control and treatments. (**) and (***) indicate the values that are $p<0.01$ and $p<0.001$, respectively.

These findings reveal that *H. hirsuta* could be a potential source for investigation of anticancer therapeutic agents.

5.2. Potential use as antidiabetic agents

- Many complications of diabetes such as retinopathy and atherosclerotic vascular disease have been linked to oxidative stress.

  ⇒ Antioxidants can play an important role in prevention of diabetes (Andrade-Cetto and Heinrich, 2005).

- Medicinal plants have been known as a rich source of antioxidants,
- Many studies have linked medicinal plants and diabetes.

  ⇒ **Medicinal plants are potential sources for diabetes prevention and treatment.**

5.2. Potential use as antidiabetic agents (Cont'd)

For example:

Trinh et al. (2016) screened 18 medicinal plants traditionally used as antidiabetic medicine in Vietnam, and found 11 species could effectively exhibit activities of α-glucosidase and α-amylase, two key enzymes responsible for serum glucose regulation.

Franco et al. (2018) found *Bauhinia forficata*, *Syzygium cumini*, *Matricaria recutita* and *Echinodorus grandiflorus* exhibited strong antioxidant, anti-glycation capacities, inhibitory activities against α-amylase, α-glucosidase and lipase.

5.2. Potential use as antidiabetic agents (Cont'd)

- There is limited investigation on the antidiabetic effect of *H. hirsuta*, BUT:

  + The historical use of *H. hirsuta* root for diabetes mellitus treatment in Thailand (Chuakul et al., 2002), and

  + The high level of bioactive compounds with strong antioxidant capacity present in this plant material (Pham et al., 2015; Pham et al., 2017a; Pham et al., 2017b)

  ⇒ indicate that it would be a potent source for exploring agents used in prevention and treatment of diabetes.

Future studies are recommended to study the link of *H. hirsuta* with prevention and/or treatment of diabetes.

5.3. Potential use as anti-obesity agents

- H. hirsuta contains a range of bioactive compounds, such as betulinic acid, pinoresinol, syringaresinol and their derivatives, which have been reported to possess anti-obesity activity.

For example:

+ Betulinic acid exhibited anti-obesity activity by directly inhibiting pancreatic lipase (IC_{50} value of 21.10 μM)

+ Syringaresinol-4-*O*-*β*-D-glucoside effectively regulated lipogenesis and glucose consumption *in vitro* (Wang et al., 2017)

Future studies are needed to explore potential use of *H. hirsuta* for prevention and/or treatment of obesity.

5.4. Potential use against malaria

- Herbal plants have been used as folk medicines against malaria in many countries:

+ In Ethiopia, the local people use the leaf of *Aloe macrocarpa* Todaro plant for malaria treatment,

Then, Tewabe and Assefa (2018) found aloin and aloinoside in the leaf of this plant exhibited potent antimalarial activity in a dose-dependent manner.

+ In Namibia, the extract of *Mundulea sericea* traditionally used for antimalaria was found to possess strong *in vitro* anti-plasmodial activity with low IC_{50} values of 3.279 – 9.440 µg/mL (Nafuka and Mumbengegwi, 2013)

5.4. Potential use against malaria (Cont'd)

- *H. hirsuta* root has been traditionally used to treat malaria (Chuakul et al., 2002).
- Moreover, **betulinic acid** and **its derivatives** in the root of *H. hirsuta* were found to play an important role in malaria treatment (Innocente et al., 2012; Quang et al., 2018; Silva et al., 2015),

⇒ Supporting the traditional use of *H. hirsuta* against malaria.

More *in vivo* and clinical studies are needed to explore the use of *H. hirsuta* for antimalaria.

Conclusion

- *H. hirsuta* has been widely used as a medicinal plant to treat a variety of diseases in some Asian countries.
- The extracts and isolated compounds from this plant possessed strong antioxidant and anticancer *in vitro*, offering a promising source of active compounds for health problems.
- Further studies of the mechanism of bioactive constituents from this plant may provide more evidence and well understanding of their mechanism of action for health benefits.
- More *in vivo* tests associated with clinical trials need to be implemented to validate and confirm their effects, supporting the development of nutraceutical and pharmaceutical products from this plant material.

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Thank you!