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Phytochemical Studies of Fractions and Compounds Present in Vernonanthura Patens with Antifungal Bioactivity and Potential as Antineoplastic

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1. Introduction

Phytochemical research is closely related to the needs of finding new and effective pharmaceuticals. Searching for plant substances that are capable forbeing used to develop new therapeutic drugs against catastrophic recognized illnesses such as cancer, diabetes and AIDS is one of the main topics that researchers around the world have been focusing.

The wonderful plant diversity of South America and more specifically from the Amazon region has around 30-50% of the worlds biodiversity therafor it is an important source for this type of study. Beside the significant undiscovered resources from these regions, ancestral knowledge of indigenous peoples is another relevant and complementary source for biodiscovery programs. Traditional healers guard centuries of accumulated knowledge about natural medicinal resources of this region. These ancient "physicians" hold the key to discovering new drugs that could benefit millions of people around the world. The Amazon forest has contributed dozens of substances to western medicine. Among the best known are the "curare"; a key component of modern anesthetics and quinine, the first contribution of "natural medicine" to treat malaria[1].

Study of new plant species and the structural elucidation of its bioactive molecules are the most important aims of phytochemical research which is in constant technological development.

[1] Fundación Icaro. La medicina tradicional de los pueblos indígenas amazónicos: Descubriendo la Amazonía europea. Disponible en el sitio: http://www.fundacion-icaros.org/index.php/component/content/article/8-descubriendo-la-amazonia-europea

Initial phytochemical screening and further isolation, purification and identification of molecules structure have made a major breakthrough with the development of new methods of chromatography and spectroscopy. The establishment of new and more effective bioassays is also one of the essential aspects that support biodiscovery programs today.

This chapter contains the main results on the phytochemical study of *Vernonanthura patens* leaves which according to ancestral knowledge, have been used to treat different diseases in humans.

2. Botanical classification, general characteristics and ethnobotanical knowledge on *Vernonanthura patens*

Vernonanthura patens is a wild plant broadly distributed throughout America. It grows from 0 to 2200 meters above sea level in the Ecuadorian coastal region. Folk medicine uses its leaves cooked to combat malaria, postpartum treatment and for healing infected wounds of animals by washing with a plant mixture which includes *V. patens* leaves (Blair, 2005).

It is also used against headaches, to clean and heal wounds (Kvist *et al.,* 2006); treatment of leishmaniasis (Gachet *et al*., 2010); preparation of antivenom (Tene *et al*., 2007) and as a poultice of leaves to combat athlete's foot (Valadeau *et al*., 2009). Its usefulness for treating certain types of cancer has also been referred by indigenous healers. There is however there are few chemical studies about this species

2.1 *Vernonanthura patens* (Kunth) H. Rob. botanical classification and general characteristics

Species *V. patens* belongs to the *Asteraceae* family, quoting 60 synonyms and one basionym (*Vernonia patens* Kunth) (ARS-GRIN, 2009). Referred to *Vernonia patens* HBK in the list of lignocellulose species investigated in Ecuador, it is a source of raw material for pulping and papermaking (Acuña, 2000). It is also commercially important in the beekeeping industry, and is ranked as one of the most important honeybee plants from Tundo, Olmedo and Loja (Camacho, 2001) for its excellent production and availability of nectar and pollen (Ramirez *et al*., 2001).

In the Ecuadorian province of Zamora it is one of four ecologically important species belonging to the typical families of disturbed forests that are been regenerated (Camacho, 2001; REMACH, 2004). It is now registered as representative tree species of secondary forests in Ecuadorian coastal zone (Aguirre, 2001).

The species has the following synonyms (Blair, 2005):

Cacalia patens (Kunth), Kuntze
C. aschenborniana (Schauer) Kuntze
C. baccharoides (Kunth) Kuntze
C. haenkeana (DC.) Kuntze
C. lanceolaris (DC.) Kuntze
C. suaveolens (H.B.K.) Kuntze
Vernonanthura patens (Kunth) H. Rob
Vernonia ascherbotniana Schauer
V. lanceolaris D.C.

V. micradenia DC.
V. monsonensis Hieron
V. pacchensis Benth
V. salamana Gleason
V. suaveolens Kunt
V. treberbaneri Hieron

2.1.1 Taxonomy

Taxon	*Vernonanthura patens* (Kunth) H. Rob
Genus	*Vernonanthura*
Family	*Asteraceae* (alt. Compositae)
Number	415138
Synonyms	*Vernonia patens* Kunth (basionym)
Place of publication	Phytologia 73:72, 1992
Verified name	02-Jun-2008 by systematic botanists of ARS. Last update: 02-Jun-2008 (ARS-GRIN, 2009)

2.1.2 Vernacular names

Table 1 presents a list of vernacular names that are assigned to *V. patens* according to the countries it is grown.

Country/Location		Vernacular name	References
Colombia	Tulua, Valle del Cauca-	Yasmiande, varejón	Blair, 2005
	Valle del Cauca	Pebetero	Terreros *et al*, proyecto ECOFONDO-ACDI 2004-2009
Costa Rica		Cusuco	Chavarría *et al*., 1998
		Tuete, tuete blanco	Rodríguez, 2005
Ecuador	Prov. Loja and El Oro	Laritaco	Tobías, 1996
	Prov. Guayas	Chilco Blanco	León, 2006
	Quinindé, Bilsa, Viche, Esmeraldas, Muisne and Salina Prov. Esmeralda	Chilca	REMACH, 2004
El Salvador	Chalatenango	Sukunang	PROMABOS a, 2006
Guatemala		Xuqunán Xuquinái	PROMABOS, 2006
Panamá		Salvia blanca, Sanalego	Diéguez et al, 2006

Table 1. List of vernacular names assigned to *V. patens*

2.1.3 Geographical distribution

V. patens is native from America and can be found in Belize, Costa Rica, Brazil, Venezuela, Panama, Bolivia, Mexico and Ecuador according to the data reported by Missouri Botanical Garden[2].

[2] Tropicos.org. Missouri Botanical Garden. 23 Jun 2011.http://www.tropicos.org/Name/2740044.

2.1.4 Habitat

V.patens grows wild in the inter-Andean forest located in the south of Ecuador; its maximum height is 3-6 meters and its altitudinal distribution is between 0 and 2000 meters above sea level (Tobías, 1996; León, 2006). This species has been identified in the vegetal community of dry forests at the south-west of Ecuador[3].

This species is sometimes grown or kept in farms after its spontaneous appearance. Generally it can be found near the forest trail and on the edge of the rivers. Flowering and fruiting occurs between May and October.

2.1.5 Botanical information

V. patens (Figure 1), is a small branched shrub, growins up to six meters high with furrowed stems and ferruginous trichomes. Alternate leaves are petiolate, narrowly lanceolate, petiole tomentose with ferruginous trichomes, 4-11mm long; the leaves are entirely or weakly serrate, rounded base with a sharp or acuminate apex leaves are 7-15 cm long and 1.3 - 1.2 cm wide, the adaxial surface is bright and the abaxial is pubescent or puberulent, subcoriaceous, penninerved. Inflorescence is paniculate, terminal, extended branched with the endings scorpioid, provided with leaves and bracts, capitates sessile and very shortly pedicellate, with numerous bell-shaped flowers, 8 mm long, 4-5 sets bracts imbricated, tomentose and of dark brown color, corolla glabrous, about 5 mm long, weakly pubescent achenes, pappus hairs-layered irregular shaped edges that are about 7 mm long. A detailed description of the botanical characteristics of this species has been published by Blair (2005).

Fig. 1. *Vernonanthura patens* (laritaco). It grows wild in different Ecuadorian areas belonging to the provinces of Loja, El Oro, Guayas, Manabí and Los Ríos**.**

2.2 Ethnomedical information

In Ecuador the inhabitants of the south-west of Loja and the Marcabelí region of El Oro province recognize both its healing power and analgesic action. They use the leaves of *V. patens* to wash wounds and to relieve headaches. It is also employed as anti-inflammatory to soothe coughs and against certain types of cancers. In addition, a veterinary practice is described as it can heal infected wounds by washing with a mixture of plants that includes leaves from this species (Blair, 2005)**.** Other interesting uses have been also reported.

[3] http://www.darwinnet.org/index.php?option=com_content&view=article&id=153%3Aarticulos-cientificos-y-reportes-&catid=25%3Acontenido&Itemid=1

Gacheta *et al*., (2010) informed its usefulness for leishmanianis treatment; Tene *et al* (2007) indicating its use in the preparation of antivenon and the use of "laritaco" leaves in poultices to combat athlete's foot is referred by Valadeau *et al.*, (2009).

Different uses of *V. patens* have been registered in other South American countries. In the Bolivian community of Tacama, the juice of the plant stem is applied against conjunctivitis (Tacana, 1999) and in Colombia the watery brews of the aerial parts mixed with "panela"[4], white wine and rosemary are used against malaria. It is also used to relieve pain due to labor and to purge (Blair, 2005).

2.3 Biological and chemical activity

There are very few biological and chemical studies of the specie *V. patens*. The only results published so far refer to the antimalarial activity against *Plasmodium falciparum*, Itg2 strain (Blair, 2005) ,anti-*Leishmania* activity (Valadeau *et al.,* 2009) of the leaves of this species and no antiprotozoal activity against different strains of Leishmania (Fournet, 1994). On the chemical composition of the species, reports lack of sesquiterpene lactones and sesquiterpenes present in the aerial parts (Mabry, 1975; Jakupovic, 1986). There are some references on genus *Vernonanthura* that show the presence of diterpenes compounds (Portillo *et al.,* 2005; Valadeau *et al.,* 2009), flavonoids (Borkosky *et al*., 2009; Mendonça *et al.,* 2009), triterpenes (Tolstikova *et al.,* 2006, Gallo *et al.,* 2009), saponins (Borkosky *et al.,* 2009) and sesquiterpene lactones. In addition, different biological activities have been described assuming that certain chemical groups could be responsible for the therapeutic properties attributed to species of this genus (Pollora *et al.,* 2003, 2004; Portillo *et al.,* 2005; Bardon *et al.,* 2007).

These were the main factors that led to the Laboratorio Bioproductos Centro de Investigaciones Biotecnológicas del Ecuador to undertake a chemical-pharmacological study of *Vernonanthura patens* leaves from plants growing in Ecuadorian areas. Such investigations are part of the Biodiscovery Program developed by this center.

3. Phytochemical screening

As an initial step of thephytochemical screening research allows to determine qualitatively the main groups of chemical constituents present in a plant. This screening can guide the subsequent extraction and / or fractionation of extracts for the isolation of groups of interest. The phytochemical screening routine is performed by extraction with suitable solvents of increasing polarity and the application of color reactions (Miranda & Cuellar, 2001).

These reactions are characterized by their selectivity to types or groups of compounds, their simplicity, short time consuming and capacity to detect small amount of compounds using a minimum requirement of laboratory equipment. The results are recorded by the presence (+) or absence (-) of the color reactions.

[4] "Panela" is a unrefined sugarcane product obtained from the boiling and evaporation of sugarcane juice. It contains sucrose and fructose and is a typical product of Latin America, but can be finding in certain Asian countries.

The general outline of steps followed for performing the phytochemical screening of *V. patens'* leaves is presented in Figure 2, while the analysis of the extracts obtained at different polarities is schematically shown in Figure 3. This methodology has been referred previously (Miranda & Cuellar, 2000; Manzano *et al.*, 2009).

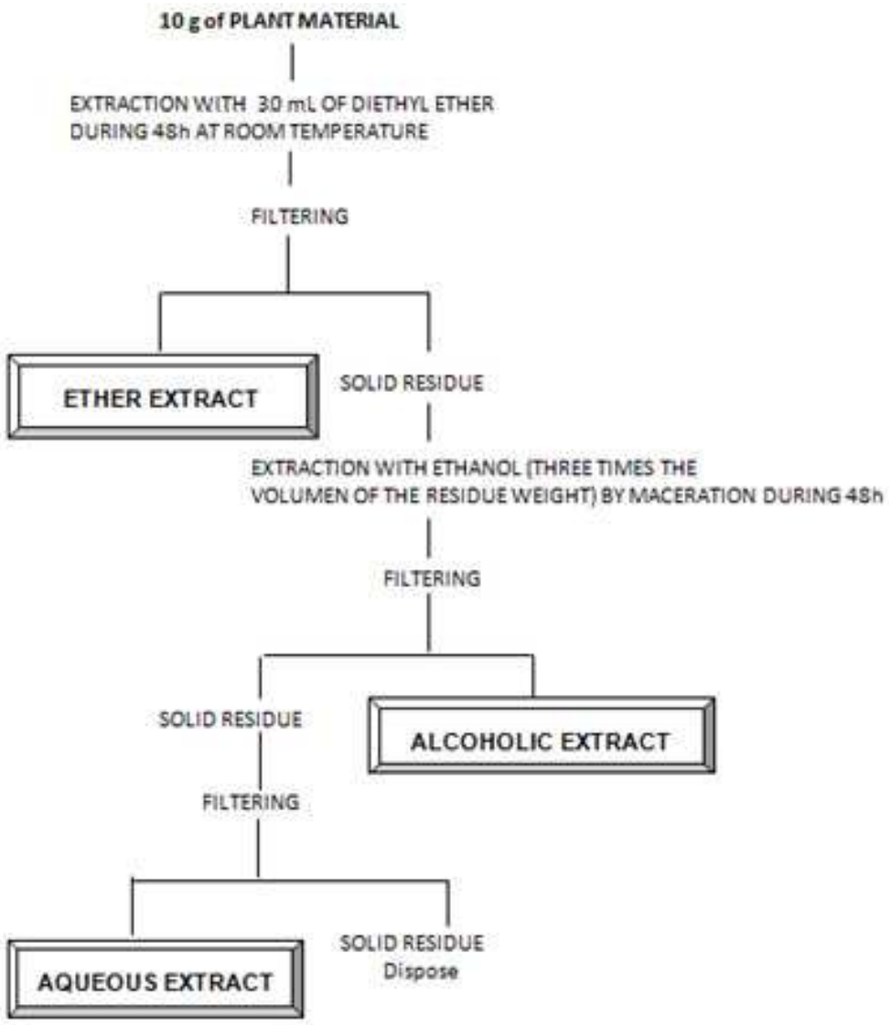


Fig. 2. General procedure used for performing the phytochemical screening of *V. patens* leaves.

The plant material of adult leaves of *Vernonanthura patens* (laritaco) were used from plants at the vegetative state which were growing in the citadels "July 25", "Imbabura" and "June 24" and all belonget to the Canton Marcabelí, province El Oro, Ecuador. Leaves were collected at early morning at different dates during the months of December to February in 2009 and 2010.

Botanical identification was performed and voucher specimens of the herbs were prepared and deposited at the National Herbarium of Ecuador (QCNE) and a duplicated sample (CIBE37) was kept as herbal witness in the laboratory of the CIBE-ESPOL Bioproducts. Prior

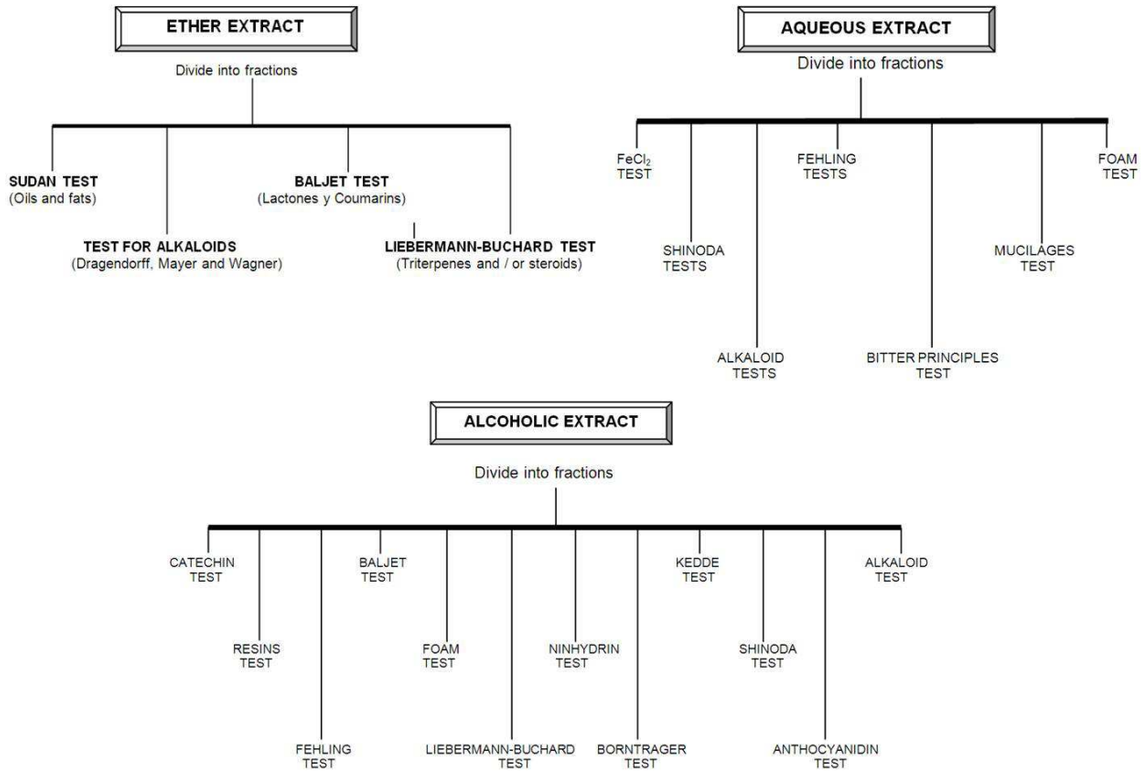


Fig. 3. Chemical reactions carried out in each type of V. *patens'* leaf extracts obtained from using solvents of different polarity.

consent was obtained and authorized by the corresponding agencies of the government. The fieldwork and data collection were conducted in accordance with the institutional, national and international principles and guidelines for using and conserving plant biodiversity.

For conducting the phytochemical screening, extraction and fractionation, leaves samples were dried using an automatic dryer (45 °C, 8 hours) and then pulverized in a blender and screened. The fraction that remained in the sieve of 2 mm in diameter was collected and kept in polyethylene bags of low density at 24 ˚C.

The result of phytochemical screening is presented in Table 2. This reveals moderate to low concentration of essentials oils, alkaloids, reducing compounds, phenols, tannins, flavonoids, quinones, saponins, triterpenes and steroids. Some of these chemical compounds have been associated to antibacterial, antifungal, antiprotozoal and citotoxicity properties and thus have a potential therapeutic use (Nweze *et al.*, 2004; Reuben *et al.*, 2008; Vital *et al.*, 2010).

4. Plant extracts, fractions and compounds

The dry plant material (67 g of leaves of *V. patens*) was subjected to successive extractions with HPLC grade methanol by maceration in a closed container and in the absence of light. The extraction time was eight days and was conducted until total depletion of plant material; agitator and a rotary evaporator were used for solvent recovery.

Chemical groups	Essays	Extracts		
		Ether	Alcoholic	Aqueous
Essential oils, fatty compounds	Sudan	+		
Alkaloids	Dragendorff Mayer		+	+
Aminoacids	Ninhidrine		-	-
Antocianidine	Antocianidine		-	-
Cardiotonic	Kedde		-	-
Reducing compounds	Fehling		-	+
Phenols and tannins	Ferric chloride		+	+
Flavonoids	Shinoda		-	+
Lactones	Baljet		-	-
Mucilages	Mucilages		-	-
Bitter principles	Bitter principles		-	-
Quinones	Börntrager		+	-
Resins	Resins		-	-
Saponins	Foam		+	+
Triterpenes and steroids	Lieberman-Buchard	+	+	-

Table 2. Chemical groups detected in *V. patens* leaves through the phytochemical screening.

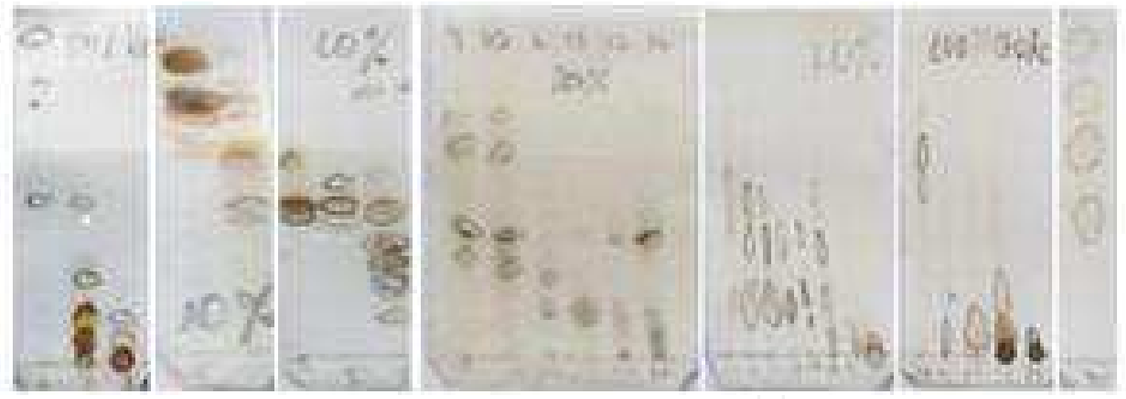
The extract was evaporated to dryness, yielding 7g (10.44%) of methanol extract. The methanol residue was subjected to fractionation by successive column chromatography (CC) packed with activated silica from 60 to 200 mesh; elution was performed with solvents of increasing polarity using mixtures of hexane and ethyl acetate (10, 9:1 , 8:2, 3:7, 10) (Table 3). The extracts were analyzed by thin layer chromatography (TLC) on 60 F254 silica gel cromatofolios (Merck) with fluorescent indicator and a solvent system hexane / ethyl acetate (9:1). Plates were observed under UV light at 254 and 366 nm wavelengths.

Solvent	Proportion (%)
Hexane	100
Hexane/ethyl acetate	90:10
Hexane/ethyl acetate	80:20
Hexane/ethyl acetate	30:70
Ethyl acetate	100
Ethyl acetate/methanol	70:30

Table 3. Solvents and proportions used in the chromatographic column fractionation of *V. patens*.

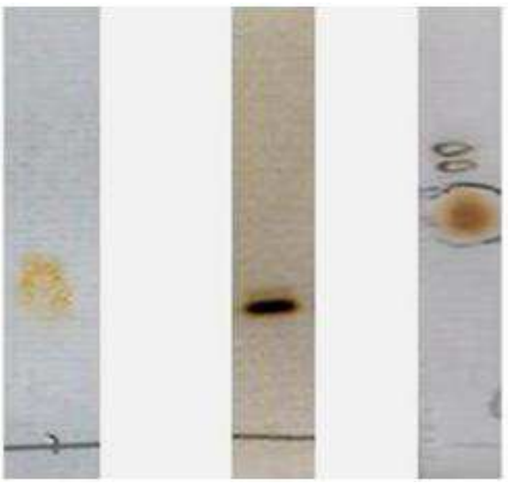
Six fractions were obtained (Figure 6): Fr 1 hexane (79mg), Fr 2 Hex / EtOAc 90:10 (1370mg), Fr 3 Hex / EtOAc 80:20 (0.60 mg), Fr 4 Hex / EtOAc 30:70 (0.41mg), Fr 5 EtOAc (0.21 mg), fraction 6 EtOAc / MeOH 70:30 (1760m g) and three pure compounds of the EtOAc fraction 10 and 20% (Figure 7): 57 mg of the compound [1] , 20 mg of the compound [2] and 90 mg of the compound [3].

The isolated fractions with different solvents from methanol extract of leaves of *V. patens* by column chromatography, have not been referred to this species, resulting in a high mass in the hexane fraction (79mg) compared with other extracted fractions. Nevertheless, methanol, ethyl acetate and hexane extracts from other plant species had showed a relevant antimicrobial activity (Ramya *et al.*, 2008).



Fraction 1 Hexane	Fraction 2 Hex/EtoAc: 90:10	Fraction 3 Hex/EtoAc: 80:20	Fraction 4 Hex/EtoAc: 30:70	Fraction 5 EtoAc 100%	Fraction 6 EtoAc/MeOH 70:30

Fig. 6. Isolated fractions from methanol extract of *V. patens*



Compound 1 Compound 2 Compound 3

Fig. 7. Chromatographic plate (TLC) showing the three pure compounds isolated from *V. patens*. Pure compounds were isolated from Fr 2 Hex / EtOAc 90:10 (1370mg).

5. Bioassays

Assays for screening the bioactivity of natural products has had an impressive history of development and is one of the keys for discovering new natural bioactive compounds.

In this study, a qualitative preliminary evaluation of the antifungal capacity of fractions and pure compounds isolated were conducted in order to select the most active. Those selected were re-evaluated to quantify their ability to inhibit fungal growth.

The diffusion method (Avello *et al.,* 2009) in potato dextrose agar (PDA) was used to determine the antifungal activity of fractions and pure compounds isolated from *V. patens* leaves at 100 and 200 μg mL^{-1}. Dilutions were made with dimethylsulfoxide (DMSO) 10%.

Strains of *Fusarium oxysporum* and *Penicillium notatum*, isolated from infected *Pinus radiata* and *Citrus sinense* fruits and maintained in the Collection of Fungi at University of Concepcion were used.

Holes of 5 mm Ø were made in the agar with a sterile cork borer and filled with 20 µL of each concentration of fractions and pure compounds. DMSO 10% was used as negative control in each plate. A disc (5 mm Ø) of already grown fungus was placed in the center of Petri dishes and incubated at 22 °C. Evaluations were made during two weeks.

Experimental design was completely randomized and each assay was performed in triplicate. Descriptive statistics of the experimental data was made in order to represent and point out its most important features.

Most relevant antifungal activity was observed in fraction 1 (100% hexane) and pure compounds 1 and 3 at the both concentrations tested.

The hexane fraction inhibited the growth of both fungal species tested. Highest inhibition exerted against *Penicillium notatum* (80.2%) and *Fusarium oxysporum* (81.5%) occurred when using 200 µg mL^{-1} of this fraction. Statistical differences ($P \leq 0,05$) with negative controls indicated that DMSO did not influence the results of biological evaluation.

Pure compounds showed selective inhibition properties and a certain concentration dependence in its antifungal activity. Compound 1 showed a rate of inhibition of 50 and 90% (100 and 200 µg mL^{1} respectively) against *Penicillium notatum* while compound 3 was capable to inhibit 80 and 100% of the *Fusarium oxysporum* growth for each assayed concentrations.

Screening for antifungal activity of fractions and pure compounds of *V. patens* has been conducted for the first time. The potential of these results is relevant.

6. Structural identification and quantitative analysis of the fractions and isolated compounds

6.1 Chemical characterization of the fraction with antifungal activity

The isolated fraction with antifungal activity were analyzed for structural identification by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890A gas chromatograph with an Agilent 5975 detector (Avondale, PA.USA) equipped with a column HP-5MS of 5m long (0.25 mm in diameter and 0.25 cm inside diameter). Helium was used as the carrier gas; the analytical conditions were: initial temperature: 100 ° C (increasing 8 ° C per minute to a final temperature of 250 ° C); inlet temperature and mass detector: 250 °C and 300 °C respectively. The mass detector was used in scan mode ("scan") with a range of 100 to 400 amu.

According to this technique and the analytical conditions described, this chromatogram was obtained and is as shown in Figure 8.

Using the library computer and taking into consideration those compounds that exceeded the 90% of confidence, structures of 33 components could be assigned (Table 4).

The compounds identified are mostly hydrocarbons, a logical result given the solvent used. There was a relative abundance of possible bicyclical sesquiterpenos (peaks 1-5) and of the acyclic triterpeno squalene (peak 30). For the sesquiterpenos exist antecedents of antimicrobial activity (Gregori *et al .*, 2005) and for the escualeno reports of activity antioxidant, antitumor

and antimicrobial activities, in addition to its beneficial effect for preventing cardiovascular diseases by reducing cholesterol and triglycerides (Garcia *et al.,* 2010).

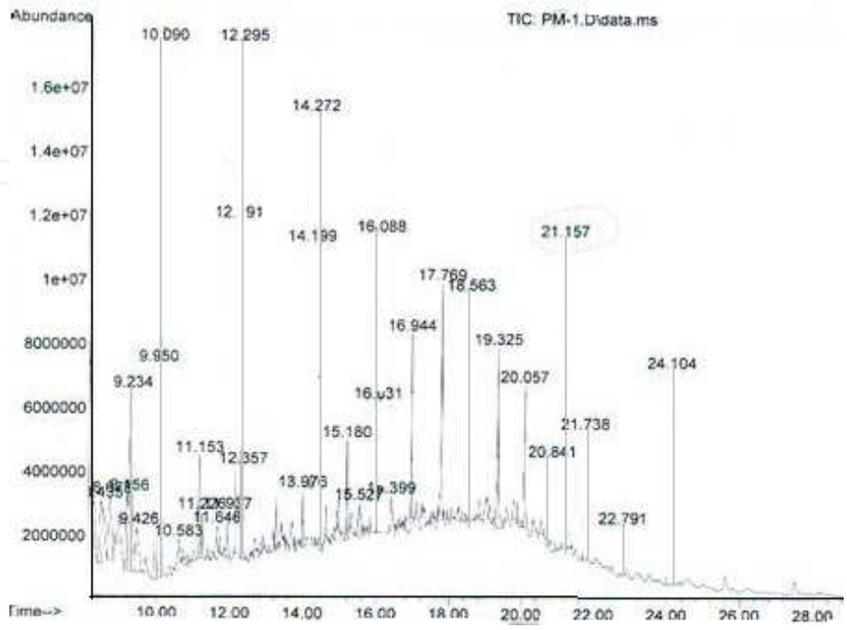


Fig. 8. Analytical gas chromatogram of the hexane fraction of *Vernonanthura patens*.

For this reason, it is possible to hypothesis that antifungal activity of *V. patens* against *F. oxysporum* and *P. notatum* which has been determined could be directed related to the squalene presence despite not being the main component of the fraction tested. The remaining compounds, individually or collectively, could also be involved in the bioactivity demonstrated. The results described here have not been reported previously for *V. patens*.

6.2 Structural identification of isolated compounds

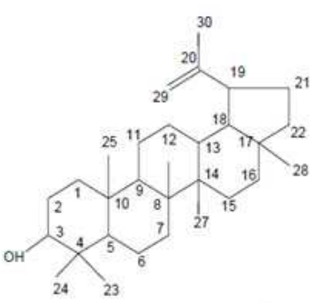
The structures of the three compounds isolated from the hexane soluble fraction by column chromatography were identified by their spectroscopic patterns as compared with references. These pure compounds were identified as Lupeol (compound 1), Acetyl Lupeol (compound 2) and Epi Lupeol (compound 3) (Figure 9).

Spectroscopy was performed in the Laboratory of Organic Chemistry at the University of Lund. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker DRX500 spectrometer with an inverse multinuclear 5 mm probe head equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$, and the solvent signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (*J*) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimized for $^{1}J_{CH}$=145 Hz and $^{n}J_{CH}$=10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker XWIN-NMR software (rev. 010101).

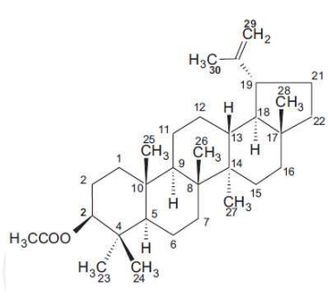
The results that are shown in this chapter are unpublished and have not been previously registered for the species *V. patens*. Even though, the elucidated structures of the pure compounds have been found in other vegetal species, and recognize their diverse biological activity which includes antineoplastic action against certain types of cancer (Gallo & Sarachine, 2009).

Peak	Time retention	Name
1	8.435	α-caryophyllene (sesquiterpene)
2	8.678	Napthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl) - (1 α., 4a. α, 8a. α). (bicyclic sesquiterpene)
3	9.156	Naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl) (bicyclic sesquiterpene)
4	9.234	Naphthalene, 1, 2, 3, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl) (bicyclic sesquiterpene)
5	9.426	Naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl) - [1S-(1. α, 4a. β., 8a. α)] (bicyclic sesquiterpene)
6	9.950	2 - tetradecene (E) -
7	10.090	Hexadecane
8	10.583	2, 6, 10, - trimethyl-pentadecane,
9	11.153	2,6,11-trimetil-dodecano,
10	11.226	2,6,11-trimethyl-dodecane,
11	11.646	Tritetracontano
12	11.937	Heptadecane, 3-methyl-
13	12.191	3 - octadecane, (E) -
14	12.295	Heptadecane
15	12.357	4-methyl-heptadecane,
16	13.976	Octadecane
17	14.199	(E) -3 - eicosane,
18	14.272	Eicosane
19	15.180	Heneicosano
20	15.527	Octadeciloxy –2-Ethanol
21	16.031	Docosenoic
22	16.088	2 - Bromo dodecane
23	16.399	1 - bromo-octadecane
24	16.944	1-iodo-Hexadecane
25	17.769	Tetracosanoic
26	18.563	11-decyl-tetracosanoic
27	19.325	1-chloro-Heptadecosano,
28	20.057	5,14-dibutyl-octadecane
29	20.841	Nonadecane
30	21.157	Squalene
31	21.738	Eicosane
32	22.791	9-octyl-Heptadecane
33	24.104	Hentriacontane

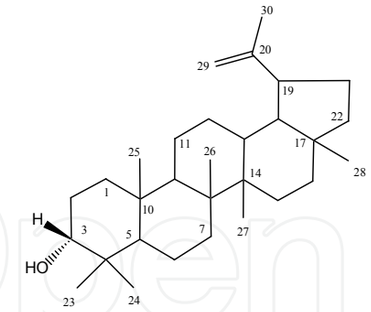
Table 4. Identified compounds in hexane fraction of *V. patens* with antifungal activity.



Lupeol



Acetyl Lupeol



Epi Lupeol

Fig. 9. Structure of compounds identified in *V. patens*.

7. Concluding remarks

Phytochemical screening of *V. patens* has showed the presence of essentials oils, alkaloids, reducing compounds, phenols, tannins, flavonoids, quinones, saponins, triterpenes and steroids, of which some have been previously associated to important biological activities.

Fractions and pure compounds of this species were screened for the first time for antifungal activity. Hexane fraction and two pure compounds further identified as Lupeol and Epilupeol, were active against two important fungal pathogens at high rate (80-100%). Hexane fraction reduced the growth of *Fusarium oxysporum* in 80% and Epilupeol completely inhibited the *Fusarium oxysporum* growth.

Thirty-three chemical compounds in the hexane fraction from *V. patens* leaves were determined, Of which must are hydrocarbons. Antifungal activity of this fraction can be related to presence of squalene and/or combined activity of others identified compounds. Further research must be done for determining specific bioactivity of identified compounds.

Chemical structures of three isolated compounds were elucidated, corresponding to Lupeol, Acetyl Lupeol and Epi Lupeol. These compounds are recognized for their significant and diverse biological activities, including antimicrobial and antineoplastic actions.

Results of this study show that *V. patens* can be considered as important potential candidate for further chemical and biological researches and justify its inclusion in the biodiscovery program of CIBE.

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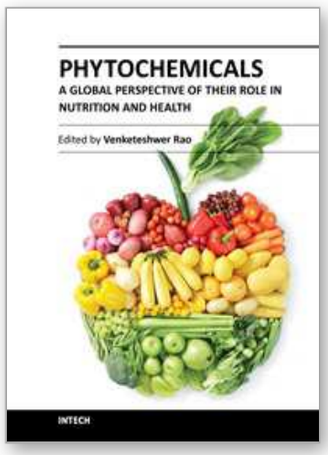

Phytochemicals - A Global Perspective of Their Role in Nutrition and Health

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Phytochemicals are biologically active compounds present in plants used for food and medicine. A great deal of interest has been generated recently in the isolation, characterization and biological activity of these phytochemicals. This book is in response to the need for more current and global scope of phytochemicals. It contains chapters written by internationally recognized authors. The topics covered in the book range from their occurrence, chemical and physical characteristics, analytical procedures, biological activity, safety and industrial applications. The book has been planned to meet the needs of the researchers, health professionals, government regulatory agencies and industries. This book will serve as a standard reference book in this important and fast growing area of phytochemicals, human nutrition and health.

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