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Phytochemical investigation of ethanolic extract of *Pericampylus glaucus* leaves from Malaysia by GC-MS analytical technique

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Abstract : The aim of the present research work was to determine the Phyto-constituents in fraction collected from ethanolic extract of *Pericampylus glaucus* leaf using the GC and Mass spectrometry. The Phyto-chemical screening of fraction from ethanolic extract of *Pericampylus glaucus* leaves were carried out with the help of Perkin-Elmer GC followed by Mass Spectrometry.

The GC-MS chromatogram of *Pericampylus glaucus* leaves fraction that was collected from ethanolic extract followed bypetroleum ether and ethyl acetate mixture showed 10 peaks that represents the presence of ten compounds in investigated plant. The benzoic acid, 5-methoxy-2-[(trimethylsilyl)oxy]-,trimethylsilyl ester (cas) 5-methoxy-salicylic acid-ditms, was found as one of the most predominant constituent (7.95 %) followed by another benzoic acid, 2-[(trimethylsilyl)oxy]-,methyl-ester (cas), methyl-o-,trimethyl-silylsalicylate (7.43 %);propanoic acid, 2-[(trimethylsilyl)oxy], trimethylsilyl ester (3.57 %);butanedioic acid, bis (trimethylsilyl)-,ester(cas) di-tms succinate, (0.63 %);vanilethanediol 3 tms (0.59%);acetic acid, [(trimethylsilyl)oxy]-,trimethylsilyl ester (cas)-glycolic acid-ditms, (0.41 %),and (3-Hydroxy-4-, methoxyphenyl) ethylene glycol tris(trimethylsilyl) ether (0.30%);benzoic acid, 3-methoxy-4-,[(trimethylsilyl)oxy]-, trimethylsilyl ester, (0.25%), and 2-[(trimethylsilyl) oxy]-, methyl ester (cas) methyl-o-trimethyl-, silylsalicylate, (0.19%); and benzaldehyde, 3-methoxy-4-[(trimethylsilyl)-oxy]-, (cas) monotrimethylsilyl vanillin (0.17%).Among ten compounds identified from *Pericampylus glaucus* fractions, only three compounds were documented to have biological and pharmacological activities.The result indicates that *Pericampylus glaucus* leaves contain several bioactive constituents andits ethnopharmacological effects most probably due to the presence of those chemical constituents, the therapeutic activities of which are required to evaluate.

Keywords : *Pericampylus glaucus* (Lam) Merr,GC-MS analysis; fraction of extract; ethanolic extract; phytochemicals; phytochemical analysis, Malaysia

Introduction

The plant *Pericampylus glaucus*(Lam) Merrbelongs to the family of *Menispermaceae*, exists in all region of Malaysia and is claimed to be widely used in folk medicine for the remedy of various diseases[1]. It is commonly originated in humid and subtropical territory. The plant is a sun loving but yet some shadows is also compulsory for their early stage of growth and development. The plant is widely dispersed from Himalayan to

the Southeast Asian countries including India (hill of Sikkim Assam particularly) and Northern Bengal, Malaysia, China, Japan, Bangladesh, Indonesia, Myanmar, Taiwan, Nepal, Philippine, Vietnam. In China at secondary stage of evolutions, the plant have ability for producing glaucus assemblies and even through on the side of roads[1, 2].The plant is climber shrubs and the stems are slender and yellowish in colors while young, and became glabrescent when old. The leaves are spiral having no stipules. The petioles are 2–4 cm long. The blade is papery, yellowish and 2.9 cm × 3.1 cm–5.5 cm × 6.0 cm. The apex is round and the base is flat. The fruits are horseshoe in shaped and diameters are range from 6–7 mm. The seeds are spiny with 3 mm × 5 mm in length[3]. In traditional system of medicines all partsof the plant are used in various Ayurvedic formulations for the curing and remedies of various diseases that affecting the human body. In Malaysia, different parts, like the root and leaves, have been claimed to be used in treating of wide range of diseases, such as the leaves are used against high grade fever and for the treatment of shortness breath and cough. The crushed leaves are superficially useful for alleviating the headache[4]. The roots are reported to be used in traditional system of medicine by the one of the indigenous people of Malaysia 'Orang Asli' to treat headache, asthma, cough, diabetes, obesity and high cholesterol level as oral decoction[5]. In all over the world, various parts of the plant are use in traditional system of medicines to stop-bleeding, arthritis, sore throat, productive cough, colds, headache, abdominal pain, relief of loss of movement tongue, fever, joint pain, muscles pain, diabetes, constipation, snake biting and abdominal distention[6, 7].

The plant *Pericampylus glaucus* have been approved scientifically for having many pharmacological activities including free radical scavenging, anti-diabetic, anti-hyperlipidemic and also has good properties against AIDS, HBV and HCV virus. Various phytochemicals have been originated from *Pericampylus glaucus* that belongs to different chemical groups, such as flavonoid, alkaloids, triterpenoid phenols etc., which are isolated from different natural products and have showed their own remedial effect against various diseases[8].

Several researchers had documented the remedial importance of different extracts of *Pericampylus glaucusin-vitro* and *in-vivo*. The ethanol extract of *Pericampylus glaucus* was approved for having attenuation affects on blood glucose and lipid profile in dose dependent manner at 400, 600 and 800 mg/kg b.wt in STZ induced diabetic rats fed with a high-fat-diet [9]. The alkaloids including Periglaucines A–D and three known alkaloids, norruffscine (5), (-)-8- oxotetrahydropalmatine (6), and (-)-8-oxocanadine (7) isolated from active ethanolic extract of *Pericampylus glaucus* and have been reported to posses significant activity against Hepatitis B, C and HIV-1 virus[10]. The plant extract have also been reported to possess *in-vitro*free radical scavenging effect against standard ascorbic acid [8]. The authors have also evaluated the acute and sub-acute activity of crude ethanolic extract of *Pericampylus glaucus* on Balb/c mice and have documented that the lethal dose are more than 4000 mg/kg (b.wt)[1.] GC-MS take part an important responsibility in the investigation and detections of unidentified components existing in natural products. The main function of GC-MS is to measure the mass numbers of unknown compounds following ionizing the plant compounds and identification of unknown compounds spectrum by comparing with standard spectrum peaks of compound[11].

Basically, the volatile components present in investigated plant sample are separated through Gas chromatography technique with the help of using proper capillary column, which is coated either with intermediate polar, polar and non-polar, chemicals [12]. In the injection portion of the GC apparatus, the Phyto-chemical present in test sample of plant is evaporated and isolated in the column by adsorption procedure with the help of appropriate temperature of the oven (between 60 to 270ºC) which is regulated by software. Based on the melting point of the particular components various components in the sample are eluted from the column. Retention time (RT) is a time at which individual's component is eluted from the GC column and is detected in the mass detector.

Spectrum of unidentified components present in plant sample are matched with the spectrum of the known components are that are stored in NIST library and thus provides retention index, chemical IUPAC names to unknown spectrum [13].Therefore, an attempt was made on the analysis of Phyto-chemical in fraction of ethanolic extract of *Pericampylus glaucus* leaves through GC-followed by Mass spectrometry.

Materials and Methods

Collection and preparation of *Pericampylus glaucus* plant extract

The leaves of *Pericampylus glaucus* was collected from Negeri Sembilan, Malaysia in June 2014 and was authenticated from Forest Institute Malaysia (specimen herbarium number (FRIM/394/490/5/18(118) in September 2014. The leaves were dried and grinded into coarse powder (Sieve 20). The powder was then extracted by continuous hot extraction method using the soxhlet apparatus at a temperature of 78°C using ethanol as a solvent. The ethanolic extract was then concentrated under reduce pressure through rotary evaporator (N- 10000, Eyela, Japan) and got 5% yield of extract. The dried ethanolic extract was preserved in desiccators for further fractionation through column chromatography.

Phytochemical Screening

The presence of phytochemicals in *Pericampylus glaucus* ethanolic extract was previously screened by chemical identification test[8] before GC-MS analysis.

Fractionation of *Pericampylus glaucus* ethanolic extract

The crude ethanolic extract of *Pericampylus glaucus* was fractionated through column chromatography by the procedure that was used by Natarajan et., al 2014 and Ezema, BE., 2011 [14, 15] with some modifications in procedure. An amount (20gram) of crude ethanolic extract was taken and was subjected to the processes of fractionations. The process of fractionation was carried out through chromatography over a column of stationary silica gel. The column was loaded up to the level of ¾ of the total column with slurry of silica gel that was admixed with non-polar n-hexane solvent[16]. The crude ethanolic plant extract was combined with equivalent amount of 20gram of silica gel and dried completely. The powder mixture was later gradually discharge into the column, which was previously packed with 3/4 of slurry of silica gel contained n-hexane solvent[17]. The column was effectively eluted through the use solvents system based on polarity preliminary from n-hexane and then petroleum ether and ethyl acetate in different concentration ratio like n-hexane (100%) and petroleum ether and ethyl acetate mixture (70:30) v/v[18, 19]. The fraction, which was collected from petroleum ether and ethyl acetate mixture (70:30), was further underwent for GC-MS analysis.

Identification of phytochemicals in petroleum ether and ethyl acetate fractions

The GC-MS analysis of petroleum ether and ethyl acetate fraction of plant extract, collected through column chromatography, was carried out on a GC CLARUS 550 PerkinElmer system that was composed of a gas chromatograph connected to a mass spectrometer (GC-MS) apparatus providing the following specifications:[20]

The non-polar Elite-1 column which comprised dimethyl-poly-siloxane (100%) was fused to capillary column (Restek silica) (30×0.25 mm ID×1EM df, working in Electron-Ionization system with ionization energy mode at 70 (e.V) for the quantitative analysis and presence of compounds present in active fraction. The neutral gas helium was employed as carrier gas at a continuous flow rate of 1ml/min at 173 kpa pressure and an injection volume of 0.5 EI was employed with split ratio of 10:1injector temperature 250°C; ion-source temperature 280°C.The oven temperature was fixed from 110°C (isothermal for 2 min), with an increase of temperature 10°C/minutes, up-to 200°C, then increase temperature 5°C/min to temperature 280°C and ending with a 9 min isothermal at 280°C respectively[21].

The mass spectrums were taken at70 e.V; with scan duration of 0.5 sec and the fragments were from 40 to 550 Daltons respectively and were searched on computer NIST MS data library for comparing the spectrum achieved through GC–MS phytocomponents[22].

The identification of individuals plant compounds existing in petroleum ether and ethyl acetate collected fractionof *Pericampylus glaucus*(Lam) Merrwas achieved with the help of direct comparison of retention time and mass spectra with the spectra of known compounds using the record library combined with the NIST) library[23]. The name, molecular formula, molecular weight and area under peak (percentage by peak area) of the components of the test materials (petroleum ether and ethyl acetate fraction of *Pericampylus glaucus)*were determined[23.]

Results and Discussion

The purpose of the present research work was to identify the presence of phytochemicals in petroleum ether and ethyl acetate fraction, collected from the active ethanolic extract of *Pericampylus glaucus* leaves from Malaysia through GC-MS analysis. Before going for GC-MS analysis, we have screened the ethanolic extract for the presence of phytochemicals by chemical identification method which resulted the existence of saponins, reducing sugar, flavonoid, sterol, alkaloids, terpenoids, tannins, and phenols [8]. The GC-MS chromatogram obtained from petroleumether and ethyl acetate fraction collected from ethanolic extract of *Pericampylus glaucus* leaves are presented in (Figure 1). In GCMS analysis the collected petroleum ether and ethyl acetate fraction showed ten peaks that indicates the presence of ten phytoconstituents in petroleum ether and ethyl acetate fraction of *Pericampylus glaucus*.

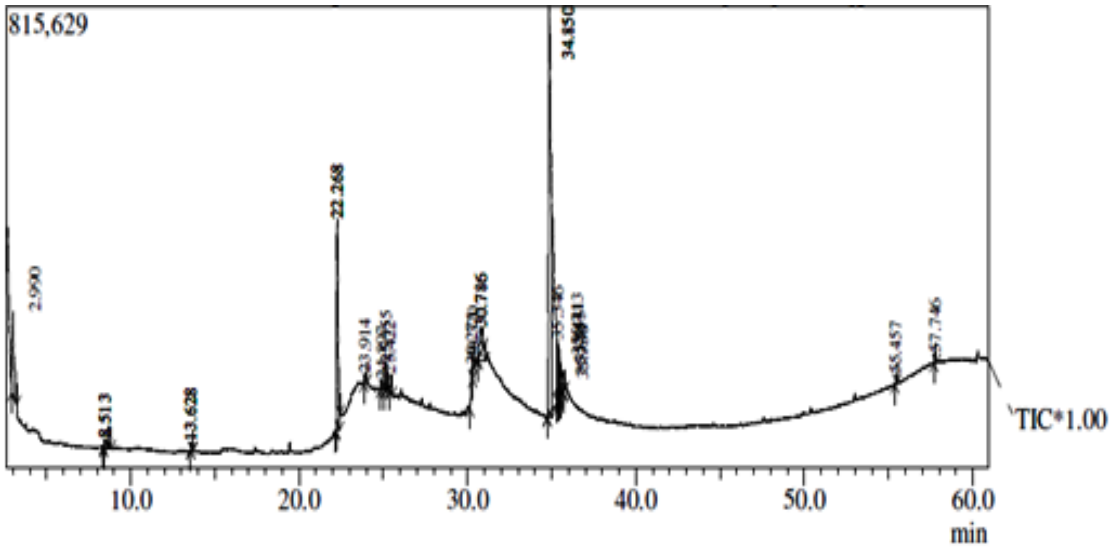

Figure 1.GCMS chromatogram of petroleum ether and ethyl acetate fractions from crude ethanolic extract of *Pericampylus glaucus*.

Table1.List of compounds identified inpetroleum ether and ethyl acetate fractions from crude ethanolic extract of *Pericampylus glaucus*

S.No	List of compounds identified	m/z	RT	AREA	S.I	M.wt	%Area
1	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	73	2.990	1015462	99	234	3.57
2	Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester (cas) glycolic acid-ditms	73	8.513	115772	97	220	0.41
3	Butanedioic acid, bis(trimethylsilyl) ester (cas) di-tms succinate	147	8.513	178105	99	262	0.63
4	Benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate	209	13.628	54140	90	224	0.90
5	Benzaldehyde, 3-methoxy-4-[(trimethylsilyl)oxy]- (cas) monotrimethylsilyl vanillin	194	13.628	48497	99	224	0.17
6	Benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate	209	22.268	2110418	98	308	7.43
7	Benzoic acid, 5-methoxy-2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (cas) 5-methoxysalicylic acid-ditms	297	22.268	2258651	99	312	7.95
8	(3-hydroxy-4-methoxyphenyl)ethylene glycol tris(trimethylsilyl) ether	297	23.914	85336	99	400	0.30
9	Benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester	297	24.897	71312	98	312	0.25
10	Vanilethanediol 3tms	73	25.155	168080	99	400	400

Table2.List of the active compounds identified in sample fraction of *Pericampylus glaucus*

	Name of Compounds Identified	Activity reported
1	Propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	Antioxidant, Anti-proliferative[24]
2	Benzoic acid, 3-methoxy-4-[(trimethylsilyl)-oxy]-, trimethyl-silyl ester	Antifungal, anti-bacterial [25]
3	Vanilethanediol 3tms	Anti-cancer, oral microbial diseases[26]

The mass spectrum range of the components was compared with the compounds record in the NIST libraryand ten phytocomponents were characterized and recognized. The names of the identified compounds, molecular weight (MW), along with their retention time (RT) based on capillary column fuse silica, molecular formula and their concentration (Peak %) are tabulated in below (Table 1). Among the detected and identified constituents in thepetroleum ether and ethyl acetate fraction of plant sample of *Pericampylus glaucus*, three compounds were noted registered in research for having biological and pharmacological activities in the treatment and medications of various diseases affecting human body. The active constituents, collected from fraction of petroleum ether and ethyl acetate contributes to the medicinal activities of *Pericampylus glaucus*,are shown in below (Table 2).

These identified compounds mainly comprised of esters, fatty acid, aldehyde, hydrocarbons and alcohols. The identified compounds, recognized in petroleum ether and ethyl acetate fraction sample of *Pericampylus glaucus*, the benzoic acid, 5-methoxy-2-[(trimethylsilyl)oxy]-,trimethylsilyl ester (cas) 5-methoxy-salicylic acid-ditms was found as one of the most prevailing constituent (7.95 %) followed by another benzoic acid, 2-[(trimethylsilyl)oxy]-,methyl-ester (cas), methyl-o-, trimethyl-silylsalicylate, (7.43 %);propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester (3.57 %);butanedioic acid, bis(trimethylsilyl),-ester(cas) di-tms succinate, (0.63 %), andvanilethanediol 3tms (0.59%); Acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl ester (cas)-glycolic acid-ditms, (0.41 %), and (3-Hydroxy-4-, methoxyphenyl) ethylene glycol tris(trimethylsilyl) ether (0.30%);benzoic acid, 3-methoxy-4-,[(trimethylsilyl)oxy]-, trimethylsilyl ester, (0.25%), and 2-[(trimethylsilyl) oxy]-, methyl ester (cas) methyl-o-trimethyl-, silylsalicylate, (0.19%); and benzaldehyde, 3-methoxy-4-[(trimethylsilyl)-oxy]-, (cas) monotrimethylsilyl vanillin (0.17%). In the same way, the retention time for benzoic acid, 5-methoxy-2-[(trimethylsilyl)- oxy]-, trimethyl-silyl ester (cas) 5-methoxy-salicylic acid-, ditms, was high (22.268min), followed by benzoic acid, 2-[(trimethylsilyl)-oxy]-, methyl ester (cas) methyl o-trimethyl-, silylsalicylate, (22.268 min), propanoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl-, ester, (2.990 min), butanedioic acid, bis(trimethylsilyl)-, ester (cas) di-tms succinate, (8.513 min), vanilethanediol 3tms, (25.155 min), acetic acid, [(trimethylsilyl)oxy]-, trimethylsilyl-, ester (cas) glycolic-acid-ditms, (8.513 min), (3-Hydroxy-4-, methoxyphenyl)-, ethylene glycol tris(trimethylsilyl) ether, (23.914 min),benzoic acid, 3-methoxy-4-[(trimethylsilyl)oxy]-, trimethylsilyl ester (22.268 min), benzoic acid, 2-[(trimethylsilyl)oxy]-, methyl ester (cas) methyl o-trimethylsilylsalicylate (24.897 min), and benzaldehyde,3-methoxy-4-[(trimethylsilyl)-, oxy]- (cas) mono-trimethylsilyl vanillin (13.628 min).

Conclusions

The results from our present investigation demonstrated the presence of 10 phytochemicals from the petroleium and ethyl acetate fractionsof ethanolic extract of leaves of *Pericampylus glaucus*. The presence of those phytochemicals may be responsible to exhibit different biological activities in the ethnopharmacological use of the plant for the treatment of various diseases. Further, isolation and biological evaluation of those identified compounds are warranted for the discovery of drugs as well as to establish the traditional use of the plants.

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