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PHYTOCHEMICAL SCREENING, TLC PROFILE AND ^{1}H NMR ANALYSIS OF *Passiflora foetida* EXTRACTS

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Abstract: *Passiflora foetida*, also known as red fruits passion flower is widely found in Malaysia. Previous studies on this plant reported its therapeutic properties such as anticancer, antidiabetic, anti-hyperpigmentation, antioxidant, analgesic, anti-inflammatory, anti-acetylcholinesterase, and antimicrobial activities. In this study, different parts of the plant (aerial, stem, leaf, and fruit) were used for the phytochemical screening including alkaloid, flavonoid, terpenoid, and saponin tests. The aerial, leaf, and stem parts show positive results in all tests. However, there are slight differences in color intensity of each test. With the exception of terpenoids, the fruit shows positive results in alkaloid, flavonoid, and saponin tests. Then, a thin-layer chromatography (TLC) profile was carried out using various solvent systems including chloroform/methanol (9:1) and ethyl acetate/hexane (1:9). The TLC profile reveals that the chloroform/methanol (9:1) solvent system provides better separation of compounds compared to the ethyl acetate/hexane (1:9) solvent system. Characteristic spots were observed on the TLC plate, possibly indicating the presence of flavonoids, steroids, and terpenoids. Moreover, the extract of the aerial part of *P. foetida* was analyzed using ^{1}H-NMR which revealed signals indicating the presence of phenolic and flavonoid compounds in the extract. This study serves as a preliminary investigation, providing valuable information on the metabolite profile of various parts of *P. foetida* that might be useful for future studies.

Keywords: *Passiflora foetida,* phytochemical screening, TLC profile, metabolite profile, ^{1}H-NMR analysis.

Introduction

Passiflora foetida is the member of Passifloraceae family and it is known as red fruit passion flower. In Malaysia, it is also called as "pokok lang bulu" or "timun dendang". It can be found easily in the riverbed and dry forest floor (Dasuki Sulain, 2017). Generally, the fruits of *P. foetida* are yellowish orange in colour, turn reddish-orange when ripe and it is juice with seeds that taste sour-sweet. *P. foetida* has been used widely in the treatments of various ailments and is expected to have more contributions. Traditionally, *P. foetida* has been used for tumour treatment. The extract of the fruits had been verified to have anticancer activities (Dasuki Sulain, 2017). The Passifloraceae family has high variety of bioactive phytochemical compounds especially flavonoids and glycosides (Ozarowski & Thiem, 2013). Since *P. foetida* is a member of Passifloraceae, it also contains flavonoids which are good for the immune system. The effectiveness of *P. foetida* in traditional treatment are including to treat hysteria and insomnia (Nwosu, 1999), diarrhea, fever, skin diseases especially inflammation (Chopra *et al.,* 1986).

Phytochemicals are also commonly known as secondary metabolites produced by plants that are non-essential nutrients (Thakur *et al.,* 2020). For the human benefit, phytochemicals play a very important role in preventing the body from diseases such as heart disease, inflammation, high blood pressure, and etc. It also acts as the substrate for biochemical reactions and cofactors and inhibitors of enzymatic reactions.

Organic compounds such as carbohydrates, flavonoids, alkaloids, and phenolic compounds are examples of phytochemicals (Revathy & Sunilkumar, 2019). Phytochemical screening is essential for identifying possible secondary metabolites in herbal plants and determining the composition of these plants.

In this study, a preliminary assessment of the metabolite profile of different parts of *P. foetida* was conducted through phytochemical screening tests, thin layer chromatography (TLC), and proton nuclear magnetic resonance (^{1}H NMR) analyses. The findings from this study might be beneficial for future research related to the applications of this plant.

Materials and Methods

Sample Preparation

P. foetida plant (aerial, leaf, stem, and fruit parts) (Figure 1) were harvested within the coastal area nearby Universiti Malaysia Terengganu. The collected *P. foetida* was rinsed by using tap water to remove the dust particles. The cleaned plants were placed on a paper in a ventilated room and air-dried for an hour at room temperature to remove excess surface water after the rinsing process.

Sample Extraction

Different parts of the *P. foetida* plant, including the aerial parts, leaves, stems, and fruits, were freeze-dried and ground into a powdered form. Approximately, 1 g of the sample was extracted in 20 mL of methanol using the sonication method for 30 minutes at room temperature. The mixture was then filtered and concentrated using a rotary vacuum evaporator. The extracts were stored at 4ºC in the refrigerator until they were used for TLC and ^{1}H-NMR analyses.

(A) Aerial part (B) Leaf (C) Stem (D) Fruit

Figure 1: The different parts [Aerial part (A), Leaf (B), Stem (C), and Fruit (D)] of *Passiflora foetida* plant

Phytochemical Screening

Mayer's Test

About 5 mL of 10% ammonia chloroform solution was added to 2 g of pounded sample. The extract was then filtered into test tube and 1 mL of sulfuric acid (H_2SO_4) was added into the test tube. The mixture was shaken for 15 seconds and left until two phases formed. The upper layer was removed into new test tube and 1 mL of Mayer's reagent (potassium mercuric iodide solution) was added into the test tube. White precipitate indicates the present of alkaloid in the sample.

Alkaline Reagent Test

About 15 g of samples was immersed into methanol, warmed for 30 mins and filtered to gain the extract. About 5 mL of 20% sodium hydroxide was added into 1 mL of methanol extract and shaken. The yellow precipitate indicated the presence of flavonoids.

Salkowski Test

About 2 mL of chloroform and 3 mL of concentrated H_2SO_4 were slowly added into 5 mL of methanol extract. The formation of reddish brown indicated the presence of terpenoids.

Test for Saponin

Approximate 2 g of sample was heated in 20 mL of distilled water. Then the extract was filtrated and the extract was transferred into a test tube. About 5 mL of distilled water was added into the test tube and shaken. Few drops of olive oils were added into the test tube and shaken again. The formation of emulsion indicated the presence of saponin.

Thin Layer Chromatography Profile

Crude methanol extracts from different parts (aerial, leaf, stem, and fruit) of the plant were used for TLC analysis. Each sample of the plant was spotted on the solvent line of TLC plate by using a capillary tube. The plate was placed in a beaker with an appropriate solvent system. For visual detection, the TLC plate was exposed to UV light with a short wavelength of 254 nm and a long wavelength of 365 nm for visual detection. Then, the concentrated H_2SO_4 acid (50%, v/v) was sprayed to the TLC plate and heated on hot plate to observe the colourless compounds.

^{1}H-NMR Analysis

The aerial part extract was chosen for further ^{1}H-NMR analysis. About 20 mg of extract was added with 800 µL of deuterated methanl (CD_3OD) and 200 µL of deuterium oxide (D_2O) (pH 6.0) containing 0.1% trimethylsilylpropanoic acid (TSP) salt. The mixture was vortexed for 5 minutes and centrifuged at 13000 rpm for 10 minutes. Then, the supernatant was transferred to NMR tube for ^{1}H-NMR analysis. ^{1}H-NMR analysis was carried out using 400 MHz Bruker NMR machine. The parameters used in this experiment were as followed: Pulse width (PW) 21.0 **µs** (90°) and relaxation delay (RD) 2.0 seconds, to obtain the acquisition time of 30 minutes (256 scans). TSP was used as an internal standard. Spectral pre-processing that included baseline correction, phasing, and alignment was performed using online MNOVA software version 6.0. Metabolite identification was conducted using Chenomx Profiler software version 7.62 and further confirmed by comparing the spectral data with available databases and published literature.

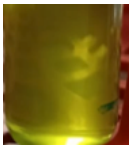
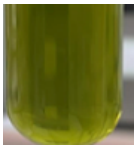
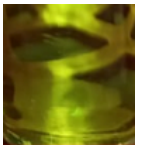
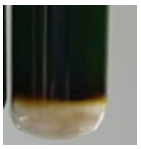
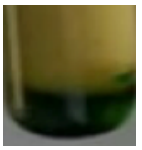
Results and Discussion

Phytochemical screening

Phytochemical tests were performed to indicate the presence of various secondary metabolites in *P. foetida* (Table 1). According to the phytochemical test results, the aerial, leaf, and stem parts contained flavonoid, alkaloid, terpenoid, and saponin. Meanwhile, fruit contains alkaloid, flavonoid, and saponin. All parts contained alkaloids, but the aerial part and fruit exhibited the least intensity in the test, suggesting they contained a very small amount of alkaloid. Similarly, although all parts contain saponins, the stem part showed the weakest intensity indicating it contained a very small or trace amount of saponin.

In the previous studies, the preliminary phytochemical screening of ethanol extract of *P. foetida* showed the presence of phenols, carbohydrate, proteins, phytosterols, phenolic compounds, steroids, glycosides, gums, tannins, flavonoids (pachypodol, ermanin), cyanogenic compounds, and alkaloids such as Harman alkaloids and β-carbolines. (Sathish *et al.,* 2011; Asadujjaman *et al.,* 2014; Olaoluwa *et al.,* 2019; Revathy & Sunilkumar, 2019). The raw fruits of *P. foetida* contained the essential amino acids, unsaturated fatty acids, minerals, and phenolic compounds (Song *et al.,* 2018). The phenolic compound is the main constituent that has antioxidant activity as it inhibits oxidative damage, maintains the stability of cell membrane, also, anti-inflammatory (Cordova *et al.,* 2013; Fidelis *et al.,* 2019). The major flavonoids reported in this plant are O-glycoside or C-glycoside type flavonoids (Chiavaroli *et al.,* 2020). Different phytochemicals contribute to various bioactivities. For instance, plants containing flavonoids can exhibit analgesic and anti-inflammatory activities (Sasikala *et al.,* 2011).

Table 1: Phytochemical screening results of *Passiflora foetida*

Tests	Observation	Different Parts of *P. foetida*			
		Aerial	**Stem**	**Leaf**	**Fruit**
Alkaloid	Presence of white precipitates	 (+)	 (++)	 (++)	 (+)
Flavonoid	Yellow solution formation	 (++)	 (++)	 (+)	 (++)
Terpenoid	Dark brown or reddish-brown precipitate formation	 (+)	 (++)	 (+)	 (-)
Saponin	Formation of emulsion	 (++)	 (+)	 (++)	 (++)

Note: (-): Negative, (+): Positive with weak intensity, (++): Positive with medium intensity.

Thin Layer Chromatography Profile

TLC test was performed to monitor the presence of the compounds in the extracts and support the results of the qualitative phytochemical tests. For the visualization of spots, TLC plate was placed at short wavelength (254 nm) and long wavelength (365 nm) under UV lamp. To visualize other specific compounds, concentrated H_2SO_4 was sprayed on TLC plate and heated on hot plate at temperature between 90°C to 100°C. Figure 2 and Figure 3 shows the results of TLC profile of the aerial part and different parts of *P. foetida*, respectively.

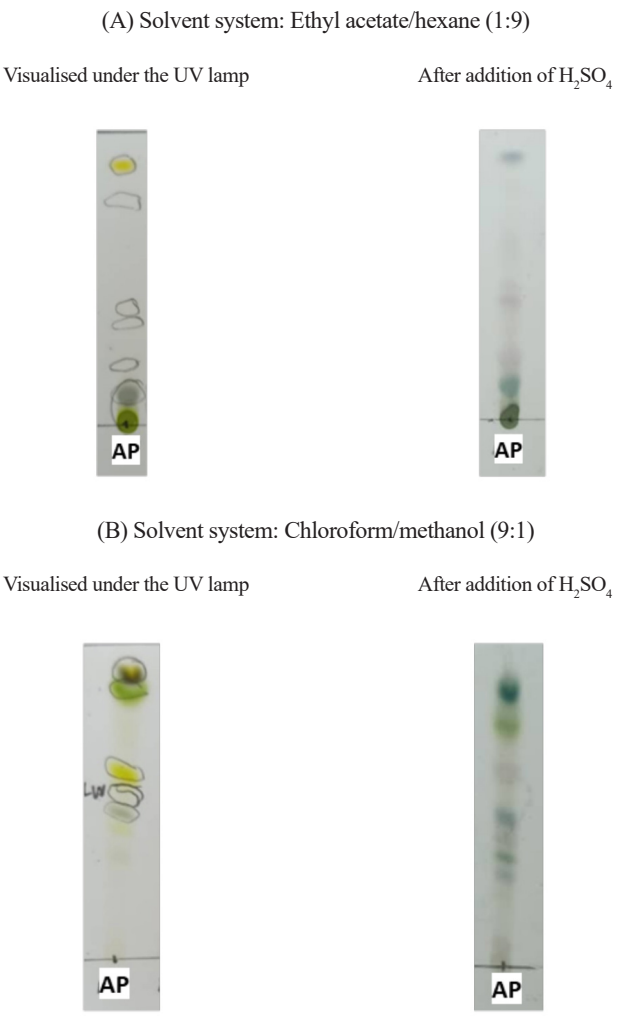


Figure 2: Thin layer chromatography (TLC) profile of the aerial part extract of *P. foetida* developed in solvent systems ethyl acetate/hexane (1:9) (A) and chloroform/methanol (9:1) (B).
Note: AP: Aerial part, LW: Long wavelength (366 nm)

The TLC profile of the aerial part extract showed that chloroform; methanol (9:1) [Figure 2(B)] gave the better solvent system as compared to ethyl acetate; hexane (1:9) [Figure 2(A)]. From the chloroform; methanol (9:1) solvent system, the yellow spot that is observed under the UV lamp (short wavelength 254 nm) possibly was flavonoid compound while the green spot was chlorophyll. Furthermore, in the chloroform: methanol (9:1) solvent system, purple-blue spots were observed on the TLC plate after spraying concentrated H_2SO_4 and followed by applying heat. The blue purple spots commonly are steroids and terpenoids (Krzakowa & Grzywacz, 2010; Napiroon *et al.,* 2017).

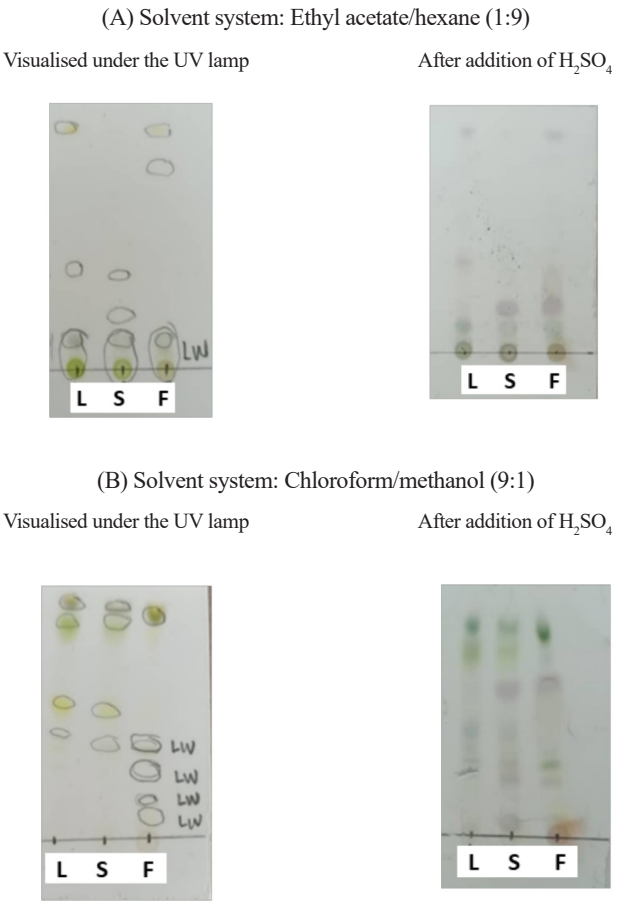


Figure 3: Thin-Layer Chromatography (TLC) profile of leaf, stem, and fruit of *P. foetida* developed in solvent systems ethylacetate/hexane (1:9) (A) and chloroform/methanol (9:1) (B).
Note: L: Leaf, S: Stem; F: Fruit; LW: Long wavelength (366 nm)

Similar to the TLC profile of the aerial part, the chloroform/methanol (9:1) solvent system showed better results compared to the ethyl acetate/hexane (1:9) solvent system for the separation compounds from different parts of the samples. Figure 3 showed an orange spot found in the baseline of the TLC plate of the fruit extract possibly indicating the presence of sugar (Al-Kayali *et al*., 2021). Overall, TLC analysis revealed differences between the spots of leaf, stem, and fruit extracts. Through TLC profiling, the suitability of the solvent system can be assessed and further applied for the isolation of compounds using chromatographic techniques. The Retention factor (Rf) values and the number of spots of the different parts of the plant are listed in Table 2.

Table 2: Retention factor (Rf) values and number of spots of the different parts of *P. foetida*

No. of Spot	Ethyl Acetate/Hexane (1:9)				Chloroform/Methanol (9:1)			
	Aerial Part	Leaf	Stem	Fruit	Aerial part	Leaf	Stem	Fruit
1	0.09	0.12	0.11	0.11	0.07	0.32	0.05	0.0
2	0.23	0.37	0.20	0.22	0.28	0.35	0.24	0.29
3	0.35	0.89	-	0.88	0.35	0.44	0.32	0.32
4	0.41	-	-	-	0.4	0.74	0.44	0.65
5	0.76	-	-	-	0.47	0.84	0.61	0.89
6	0.88	-	-	-	0.63	-	0.77	-
7	-	-	-	-	0.77	-	0.89	-
8	-	-	-	-	0.89	-	-	-

^{1}H-NMR Analysis

The ^{1}H-NMR spectrum (Figure 4) shows the signals of metabolites presented in the plant at the region **δH** 0.5 - 10.5 ppm. The observable signals at **δH** 3.0 - 5.0 ppm, there might be the signals of protons attached to alkene group (-C = C-), hydroxyl group (-C-OH) and methoxy group (-OCH_3) (Astuti *et al.,* 2017). The chemical shifts in between **δH** 6.0 - 8.5 ppm indicated that the proton attached to aromatic carbon compounds. The aromatic region might belong to phenolics and flavonoids compounds. Previous study by Nguyen *et al.* (2015) found that this plant contains flavonoid structure compounds such as apigenin, luteolin, chrysoeriol, tricin, quercetin-4'-methyl ether, vitexin, vitexin-2"-O-xyloside, orientin, apigenin 7-O-β-D-glucopyranoside, and luteolin 7-O-β-D-glucopyranoside. Moreover, phenolic compounds such as syringaresinol, berchemol, threo-guaiacylglycerol, p-hydroxybenzaldehyde, 3,4,5-trimethoxyphenyl-O-β-D-glucopyranoside, and trans-p-coumaric acid also found in previous studies including new phenylethanoid that named as passifoside (Van Linh *et al.,* 2022). The presence of these compounds with various substitutions indicated by the signals between **δH** 2.0 - 8.0 ppm available in the spectrum. Different substitutions on the ring will give different signals that possibly indicate the structure of the flavonoid and phenolic compounds. Both groups of compounds can be further determined using ^{13}C-NMR, 2D NMR, and Mass Spectroscopy analyses.

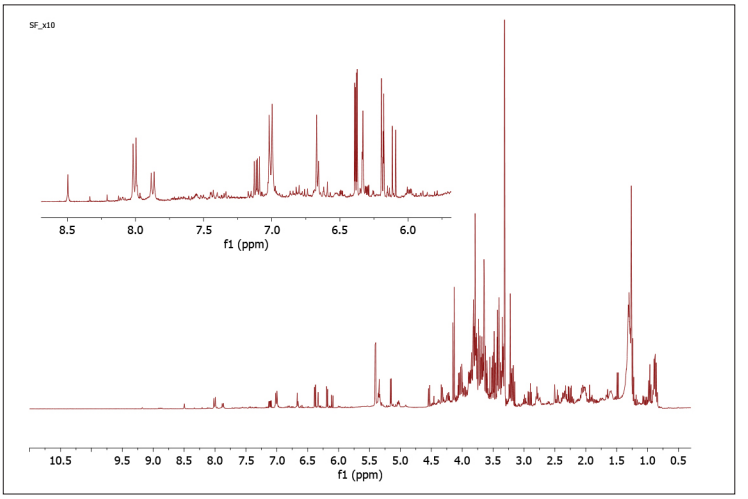


Figure 4: ^{1}H-NMR spectrum of aerial part extract of *P. foetida*

The region **δH** 3.0 - 4.0 ppm showed the presence of signals that are the characteristics of hydroxyl functional group (-OH) of the carbohydrates. The signal at **δH** 5.40 ppm, 5.20 ppm, and 4.55 ppm further confirmed the presence of sucrose, α-glucose, and β-glucose, respectively (Shin & Cho, 2008; Cao *et al.,* 2015). In the aliphatic region (**δH** 0.8 - 2.0 ppm), the signals that possibly attributed to organic acids and fatty acids are found (Zhang *et al.,* 2016). The organic acids found in the sample are formic acid (**δH** 8.45 ppm) and oxoglutaric acid (**δH** 2.99 ppm). Meanwhile, fatty acids can be identified through the presence of signals at **δH** 1.28 - 1.32 and **δH** 0.89 ppm, referring to aliphatic chain and terminal CH_3 of fatty acids, respectively. Amino acids such as glutamic acid (**δH** 2.32 ppm), alanine (**δH** 1.46 ppm), valine (**δH** 1.04 ppm) and isoleucine (**δH** 0.99 ppm) were also identified. For comprehensive metabolite identification and detailed analysis of the different parts of *P. foetida*, further investigation utilizing metabolomics techniques could be conducted.

Conclusion

In conclusion, phytochemical screening is an easy and efficient method for preliminary determination of metabolites presence in natural products. In this study, the aerial part, leaf, and stem of *P. foetida* showed positive results for alkaloids, flavonoids, terpenoids, and saponins, while the fruit contained alkaloids, flavonoids, and saponins. Subsequently, TLC profile of the extracts from different plant parts using the chloroform/methanol (9:1) solvent system exhibited better compound separation compared to the ethyl acetate/hexane (1:9) solvent system. Several potential compounds were observed on the TLC plate, with the yellow spots possibly indicating flavonoid compounds, the green spot potentially representing chlorophyll and the blue-purple spots likely corresponding to steroids and terpenoids. Furthermore, ^{1}H-NMR spectroscopy was employed to analyze the aerial part extract of *P. foetida* revealing the presence of various compounds within the extract.

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References

Al-Kayali, R., Jalab, J., Kitaz, A., & Abdelwahed, W. (2021). Evaluation of antibacterial activity of some medicinal plants by bioautography. *Universal Journal of Pharmace utical Research 2021*, *6*(4), 42-49. https://doi.org/10.22270/ujpr.v6i4.639

Asadujjaman, M., Mishuk, A. U. lla., Hossain, M. A. Sla., & Karmakar, U. K. Uma. (2014). Medicinal potential of Passiflora foetida L. plant extracts: Biological and pharmacological activities. *Journal of Integrative Medicine*, *12*(2), 121–126. https://doi.org/10.1016/S2095-4964(14)60017-0

Astuti, M. D., Winarti, T. S., & Mustikasari, K. (2017). Isolasi dan identifikasi senyawa terpenoid dari ekstrak n-heksana daun kelopak tambahan tumbuhan permot (*Passiflora foetida*). *Jurnal Sains dan Terapan Kimia*, *11*(2).

Cao, R., Nonaka, A., Komura, F., & Matsui, T. (2015). Application of diffusion ordered-1H-nuclear magnetic resonance spectroscopy to quantify sucrose in beverages. *Food Chemistry*, *171*, 8-12.

Chiavaroli, A., Di Simone, S. C., Sinan, K. I., Ciferri, M. C., Angeles Flores, G., Zengin, G., ... & Orlando, G. (2020). Pharmacological properties and chemical profiles of *Passiflora foetida L*. extracts: Novel insights for pharmaceuticals and nutraceuticals. *Processes*, *8*(9), 1034. https://doi.org/10.3390/pr8091034

Chopra, R. N., Nayar, S. L., & Chopra, I. C. (1986). *Glossary of Indian medicinal plants (Including the supplement)* (pp. 330). New Delhi: Council Scientific Industrial Research.

Cordova, F. M., Zibadi, S., & Watson, R. R. (2013). Antioxidant and anti-inflammatory actions of passion fruit peel extract in modifying osteoarthritis, hypertension, and asthma. In *Bioactive food as interventions for arthritis and related inflammatory diseases* (1st ed.). Elsevier Inc. https://doi.org/10.1016/B978-0-12-397156-2.00255-6

Dasuki Sulain, M. (2017). Antiproliferative, antioxidative and compounds identification from methanolic extract of passiflora foetida and its fractions. *Journal of Analytical & Pharmaceutical Research*, *6*(1), 1–10. https://doi.org/10.15406/japlr.2017.06.00166

Fidelis, M., De Moura, C., Kabbas, T., Pap, N., Mattila, P., Mäkinen, S., Putnik, P., Kovačević, D. B., Tian, Y., Yang, B., & Granato, D. (2019). Fruit seeds as sources of bioactive compounds: Sustainable production of high value-added ingredients from by-products within circular economy. *Molecules*, *24*(21), 1–54. https://doi.org/10.3390/molecules2 4213854

Krzakowa, M., & Grzywacz, E. (2010). Phenolic compounds pattern in sweet clover (*Melilotus officinalis*) vs white clover (*M. alba*) revealed by 2D TLC (two-dimentional thin-layer chromatography) and its taxonomic significance. *Herba polonica*, *56*(3).

Napiroon, T., Sookchaloem, D., & Vajrodaya, S. (2017). Thin layer chromatography screening and profiling of Terrestrial aroids (*Araceae*) lipophilic extracts from Saiyok forest, Thailand. *Journal Tropical Forest Science*, *1*(1), 1-10.

Nwosu, M. (1999). Herbs for mental disorders. *Fitoterapia*, *70*(1), 58-63. https://doi.org/10.1016/s0367-326x(98)00024-0

Olaoluwa, O. O., Iganboh, P. D., & Taiwo, O. M. (2019). Chemical constituents and biological activities of *Passiflora foetida* (*Linnaeus*) stem and fruit essential oils. *The Pharmaceutical and Chemical Journal*, *6*(6), 21–28.

Ozarowski, M., & Thiem, B. (2013). Progress in micropropagation of Passiflora spp. to produce medicinal plants: A mini-review. *Revista Brasileira de Farmacognosia*, *23*(6), 937-947. https://doi.org/10.1590/S0102-695X2013000600011

Revathy, S., & Sunilkumar, T. (2019). Phytochemical and nutritional studies on the fruit pulp extract of *Passiflora foetida Linn*. *Journal of Pharmacognosy and Phytochemistry*, *8*(4), 732–734.

Sasikala, V., Saravanan, S., & Parimelazhagan, T. (2011). Analgesic and anti–inflammatory activities of *Passiflora foetida L. Asian Pacific Journal of Tropical Medicine*, *4*(8), 600-603.https://doi.org/10.1016/S1995-7645(11)60155-7

Sathish, R., Sahu, A., & Natarajan, K. (2011). Antiulcer and antioxidant activity of ethanolic extract of *Passiflora foetida L. Indian Journal of Pharmacology*, *43*(3), 336. https://doi.org/10.4103/0253-7613.81501

Shin, S. J., & Cho, N. S. (2008). Conversion factors for carbohydrate analysis by hydrolysis and ^{1}H-NMR spectroscopy. *Cellulose*, *15*(2), 255-260.

Song, Y., Wei, X. Q., Li, M. Y., Duan, X. W., Sun, Y. M., Yang, R. L., ... & Wang, H. (2018). Nutritional composition and antioxidant properties of the fruits of a Chinese wild *Passiflora foetida*. *Molecules*, *23*(2), 459. https://doi.org/10.3390/molecules23020459

Thakur, M., Singh, K., & Khedkar, R. (2020). Phytochemicals. In *functional and preservative properties of phytochemicals* (Issue I). Elsevier Inc. https://doi.org/10.1016/b978-0-12-818593-3.00011-7

Van Linh, N., Trung Tuong, N., Xuan Phong, P., Trang, D. T., Nhiem, N. X., Hoai An, D., & Huu Tai, B. (2022). New phenylethanoid and other compounds from *Passiflora foetida L.*, with their nitric oxide inhibitory activities. *Natural Product Communications*, *17*(11), 1934578X221141163.

Zhang, C. Y., Luo, J. G., Liu, R. H., Lin, R., Yang, M. H., & Kong, L. Y. (2016). ^{1}H NMR spectroscopy-guided isolation of new sucrose esters from Physalis alkekengi var. franchetii and their antibacterial activity. *Fitoterapia*, *114*, 138-143.