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Cite as: AIP Conference Proceedings **2454**, 030024 (2022); https://doi.org/10.1063/5.0078295
Published Online: 09 June 2022

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Biological Activities and LC-MS/MS Profiling of Methanolic Extract of *Opuntia monacantha* Haw. (Cactaceae)

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Abstract. *Opuntia monacantha* Haw. (Cactaceae) is a cochineal prickly pear with various medicinal uses. Its cladodes are consumed widely by local community in Mexico due its nutritional value and therapeutics effects. However, the cactus only known as an ornamental plant in Malaysia. To date, limited numbers of scientific studies have been conducted to explore its medicinal properties. Hence, the present study was carried out to evaluate the cytotoxicity and antioxidant of the cladodes of *O. monacantha* crude methanolic extract (MEOM), as well as phytoconstituents profiling. The cladodes of *O. monacantha* was extracted using maceration method with methanol in ratio (sample:methanol, 1:20L for 72hrs), followed by concentrated by means of rotary evaporator to obtain the crude methanol extract of the cladodes. Afterwards, the MEOM was tested for its toxicity using brine shrimp lethality test (BSLT), antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH), total phenolics content (TPC) and total flavonoids content (TFC). Finally, chemical compounds were identified using UHPLC-Q-TOF/MS. According to Meyer and Clarkson toxicity criterion, the BSLT assay showed the extract possessed significant low toxicity LC_{50} of 1367.91 ppm. As for the antioxidant activity, the results exhibited that the extract could be categorized as having moderate antioxidant activity with IC_{50} value of 3.764 mg/mL DPPH activity, contain the TPC at 4.222±0.339 mg GAE/g extract, while no flavonoids were detected. According to the library of UHPLC-MS spectra, it has been identified the extract contain 22 potential active compounds from the cladodes of MEOM. The strongest peak identified as isoliquiritin, a flavonoid glycoside compound which possibly contribute to the moderate antioxidant activity. The findings are in agreement to the traditional uses of cladodes among natives in Mexico which were consumed safely and used for its various therapeutics values. Besides, the plant can be explored further as it has a potential to be developed into alternative-natural products in treating various discomforts and ailments with no side effects.

INTRODUCTION

Medicinal plants are being widespread belief on the part of the general public and known to be used in the treatment of several ailments, scientifically due to their high level of interest and effectiveness with a minimum and/or no adverse side effects [4, 14], and less expensive. However, some of synthetic drugs currently used for a several treatment of ailments has disadvantages or adverse effects, which interfere the organs function that causing gastrointestinal (GI) toxicity, risk of heart failure, respiratory depression, renal adverse drug reactions and others [29, 36]. Thus, it is demands recently based on their ethno-medicinal uses and purposely for research from its variety parts of plants are being globally increased. Therefore, many of medicinal plants in various species, and genus are sources of natural products constitute valuable phytochemicals that could have beneficial therapeutics, significantly contains of antioxidant properties, and valuable bioactive compounds but, some plants probably exerts toxicity and/or carcinogenic effects [3, 4]. Thus, continuously and recommended study are necessity to acknowledge and proven an efficacy of medicinal plant such as *Opuntia* sp.

Opuntia sp. is one of the most diverse and widely distributed genus in America [12], but the highest richness of wild species are found in Mexico, as at least 126 species with different degrees of domestication have been observed [24]. It is have been used for centuries as food resources and in traditional folk medicine for their nutritional properties and their benefit in chronic diseases, particularly diabetes, obesity, cardiovascular diseases and cancer [31]. The genus *Opuntia* is a potential of medicinal plant and commonly known as dropping prickly pear, belongs to family of

International Conference on Bioengineering and Technology (IConBET2021)
AIP Conf. Proc. 2454, 030024-1–030024-7; https://doi.org/10.1063/5.0078295
Published by AIP Publishing. 978-0-7354-4193-4/$30.00

Cactaceae. It is widely grown and distributed in arid/tropical and semi-arid/sub-tropical regions throughout the world [1, 8, 37], and principally tropical savannah climate with dry summers and wet winters. This type of cactus is native to South America, but also grows in Mexico, Australia, Asia, India, South Africa and Spain [18], and could be cultivated under restricted growth conditions that are not preferable for the growth of other fruits and vegetables [37, 38].

In Malaysia, commonly about 2000 medicinal plant species are being reported to possess health beneficial [16]. Based on nutritional studies, these medicinal plants contain diverse nutritive values and contains of potential bioactive compounds related to several activities such as inflammation disorders-related gout [16], or age-related ailments [15]. However, the cactus is a member of the succulent plant family Cactaceae are often used as ornamental plants, but many are also cultivated as crop plants [6]. Unfortunately, information on the chemical composition, nutritional and its therapeutic potential properties of this genus plant are scanty.

Meanwhile, the preliminary analysis was done by several studies indicate that the plant's cladodes content low of alkaloids [47], while the others study, revealed phytochemical constituents of alkaloids, flavonoids, tannins and saponins [37], present in the crude methanolic extract, and have significant antioxidant properties [4, 20]. These active phytochemical constituents have been found and studied from this plant are exhibits interesting several pharmacological activities such as anti-diabetic, anti-microbial and neuroprotective properties [26, 49, 50], anti-glycated activity [39], anti-inflammatory action and anti-ulcer [37], and anti-diuretic [28]. In addition, based on ethno-medicinal uses, cactus's fruits and cladodes from *Opuntia* genus have been widely used as food, in folk medicine and nutritional properties [27, 45]. Furthermore, antioxidants as important agents for the nutritional and protective benefit of *Opuntia*-enriched diets in chronic diseases, in such inflammation and oxidative stress play a major function [31].

Subsequently, antioxidants and radical scavengers are nutraceuticals compounds exerting a protective effect from oxidative damage caused by free radicals, could assist in the treatment of several ailments [25, 47]. In this regards, medicinal and/or edible plants, encompassing valuable mechanistic properties [30], and/or antioxidant complements, may contribute to the reduction of oxidative damages that acts as a body's defense system [52]. In addition, such that to take antioxidant supplements regularly may reduce the risk of cardiovascular disease [51], achieve vasodilation and decrease of blood pressure [11, 17].

In order to evaluate biological activities from the crude methanolic extract of *Opuntia monancatha* (MEOM), it is required a several screening toxicity tests prior to claims a potential an alternative natural product-related to ailments sources [22]. Brine shrimp lethality test (BSLT) is one the recommended screening test and it is a species of aquatic crustaceans known as *Artemia salina* that belongs to the *Artemidea* family [4, 53]. This test method was introduced by Trapley [4], and Clarkson developed the method for screening the active compounds by utilizing larvae of *A. salina* [35], and is an easy, fast and affordable cost technique to screening toxicity of a plenty of medicinal plants' extract, heavy metals, pesticides, food additives and drugs [4], with higher sensitivity for detection of cytotoxicity compounds and use smaller test of samples [54], of crude extract. On the other hand, to observe and screen whether the extract (MEOM) has produced toxicity on larvae of *A. salina* by using method of BSLT. Moreover, indicator of toxicity level of sea water, value of LC_{50} is counted through Probit Analysis, and the potency of crude extract is determined by comparing the LC_{50} which is less or equal to 1,000 ppm [4, 19], or the lethal concentration of plant's extract resulting in 50% mortality of the brine shrimp (LC_{50}) [46].

Therefore, this study was conducted to evaluate the toxicity of crude methanolic extract of *O. monacanthta* (MEOM) cladodes as well as its antioxidant properties (DPPH assays, TPC and TFC) followed by using LC-MS. Finding from this study will contribute scientific data to the field of ethno-pharmacological studies and has a potential to be developed as an alternative natural product to treat various ailments.

MATERIALS AND METHODS

Plant Materials and extraction

The cladodes of *O. monacantha* plant were collected from the coastal area of Tok Bali, Kelantan, Malaysia and certified by Dr. Syamsul Khamis, a botanist at Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. A voucher specimen, SK 2881/15, has been deposited at the Herbarium of the Laboratory of Natural Products, IBS, UPM, Malaysia. The procedure for preparation of MEOM was carried out as described in details [14], but with slight modifications.

Determination of Total phenolic content (TPC) and Total flavonoid content (TFC)

The microplate TPC method was based on the 96-well microplate Folin-Ciocalteu method described in details [55]. Total flavonoid content was determined following the method described in details [56].

Assessment of Antioxidant activity and Toxicity Test

The microplate antioxidant assay methodology was based on the 96-well plate assay described in details [55]. Meanwhile, the method of toxicity test is obtained from the Bioassay Techniques for Drug Development book [7]. Each sample was treated in four replicates. The data obtained was recorded and the value of LC_{50} calculated (Lethal Concentration 50%) using Probit analysis. Level of toxicity of extract was determined using Meyer (LC_{50} < 1000: toxic, LC_{50} > 1000: non-toxic [35], and Clarkson toxicity criterion (LC_{50} 0-100: highly toxic, LC_{50} 100-500 medium toxic, LC_{50} 500-1000: low toxic and LC_{50} >1000: non-toxic [58].

UHPLC-Q-TOF/MS Acquisition Analysis

UHPLC-Q-TOF/MS system was composed of Agilent 1290 UHPLC instrument (Agilent Technologies, Waldbronn, Germany) and Agilent 6520 Q-TOF mass spectrometer (Agilent Corporation, Santa Clara, CA, USA). The analysis method and mass detection of synthetic compounds of MEOM was done by LCMS Unit, Intergrative Pharmacogenomics Institute (iPROMISE), UiTM, Puncak Alam, Selangor, Malaysia.

RESULTS AND DISCUSSION

The antioxidant activity was assessed using DPPH radical scavenging assay, while its, Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) were assessed using Folin-Ciocalteu and aluminum chloride reagents, respectively. Data is presented in Table 1 and in Fig. 1. Results of toxicity test of the crude methanolic extract of *O. monacantha* (MEOM) against *Artemia salina* larvae after 24- and 48-hr exposure are presented (Table 2) and in Fig. 2.

TABLE 1. Total phenolic content, total flavonoid content and DPPH IC_{50} for MEOM

Sample	Total phenolic content (mg GAE/g extract)	Total flavonoid content (mg CE/g extract)	DPPH IC (µg/mL)
MEOM	4.222 ± 0.339	na	3763.868 ± 2340.667 (> 500 µg/mL)
Ascorbic acid	nd	nd	2.834 ± 0.178

GAE - Gallic acid equivalent; CE - catechin equivalent; nd - not determined; na - not detected

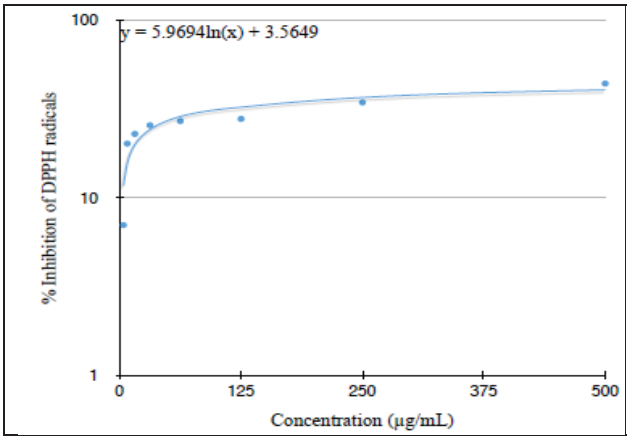

FIGURE 1. DPPH radical scavenging activity of MEOM

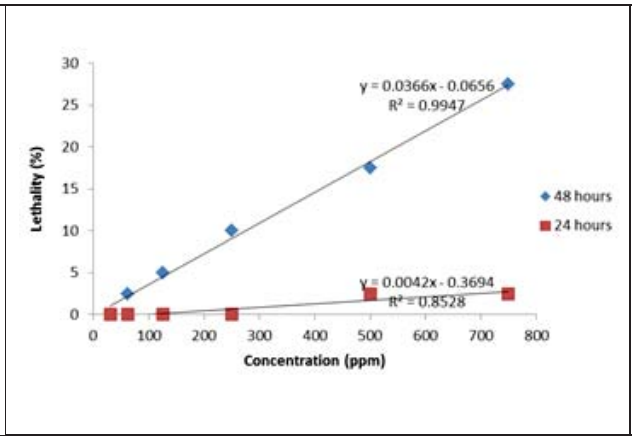

FIGURE 2. Graph of the lethality of the brine shrimps against the concentration of MEOM after 24- and 48-hr exposure

TABLE 2. Effects of the MEOM on the brine shrimp *A. salina* after 24- and 48-hr exposure

Extraction Method	Conc (ppm)	Shrimp Mortality (%)		Acute LC_{50} (after 24hr)	Chronic LC_{50} (after 48hr)
		24hr	48hr		
Maceration	31.25	0	0	11992.71 ppm	1367.91ppm
	62.5	0	2.5		
	125	0	5.0		
	250	0	10.0		
	500	2.5	17.5		
	750	5	27.5		

Furthermore, the extract was also analyzed, its constituents and its chromatogram shown in Fig. 3, while identified compounds using ESI positive mode are presented (Table 3).

Statistical Analysis for the response

LC_{50} values were determined using software Mini tab (ver.16) by Probit Analysis. To determine the difference between LC_{50} of different extract, t-student was used. For significant differences $P<0.05$ was applied.

In an attempt to contribute towards finding new an important alternative-related to natural products, with low or, possibly no adverse side effects, the present study was conducted to determine biological activities of crude methanolic extract of the plant cladodes of MEOM. The phytochemical constituents that were analyzed are in agreement to a report by Bari et al., [37].

The positive results for all tests were indicated by the formation of blue colouration for TPC test, the presence of yellow solution for TFC test, and reduction of purple colour for DPPH. Qualitatively, the slight changes of colour produced by the MEOM in each test indicated low content of phenolics and flavonoids which resulted to a weak antioxidant activity of the crude extract that was macerated in methanol for 72 hours at room temperature. The results showed that the extract contain very low amount of phenolics content which is 4.222±0.339 mg GAE/g extract, whereas no flavonoids are detected. However, Bari et al., [37], reported a moderate antioxidant activities of *O. monancantha* methanol extract when maceration for 6 hours at room temperature. Several studies [12, 21, 33], were also reported that methanol is suitable solvent to extract phenolic and flavonoids, due to its polarity.

Furthermore, the antioxidant of MEOM was determined by DPPH method. It is simple, efficient, quick, practically and relatively inexpensive [2, 13]. Based on the result, it was found the MEOM to be higher than the maximum concentration tested shown in Fig. 1. The calculation from the trendlines equation of the plotted graphs from three replicates of data resulted to the IC_{50} value of 3.764±2.341 mg/mL, which is considered as having a very moderate antioxidant activity as compared to that of ascorbic acid which is 2.834±0.178 µg/mL. Sudjaroen et al., [59] reported that, variation in free radical scavenging ability is occurs due to plant's nature and amount of secondary constituents, and chemical composition of extract [37]. Thus, the antioxidant potency of MEOM was determined by measuring their ability to scavenge DPPH radicals [47]. The crude extract was also able to inhibit the DPPH radicals in dose dependent manner and their IC_{50} value was used to exhibit its effectiveness.

In addition, the results of the toxicity test using the Brine shrimp lethality test (BSLT) method showed that the MEOM (LC_{50} value, 1367.91 ppm) was not toxic because it displayed LC_{50} value above 1000 ppm [13, 35]. Due to its simplicity, low costs and high sensitivity [40], and the method utilizes small amount of test material [57], it has been used to many researchers [40], for identifying of potential therapeutic phytochemicals that can be useful in the treatment of various diseases [4]. Although, most plants are important sources of antioxidants, their toxicity effects have been reported by several studies, which could be due to its compounds and reactions [41].

According to results of UHPLC-Q-TOF/MS chromatogram, showed 22 proposed compounds have been identified (Fig. 3 & Table 3). The compounds are classified as; 3 alkoloids, 5 polyphenols, 3 flavanones, 8 flavanoids, 2 nitrogen-containing compounds, 1 isoflavanoid & 1 flavanoid glycoside. Using LC-MS/MS as a tool for identification, due to it sensitivity, specificity and selectivity of liquid chromatography tandem mass spectrometry, it is considered as an essential tool for the characterization and identification of low molecular compounds such as fatty acids, sterols, cholastane derivatives, nucleosides and others [32]. Based on the chromatogram, the strongest peak is identified as isoliquiritin, a flavonoid glycoside compound that has been reported to exhibit several pharmacological activities including antioxidant, anti-inflammatory, and anti-depression activities [48]. Moreover, this compound has a cytoprotective effect on corticosterone-induced neurotoxicity in PC12 cells, which may be related to its antioxidant action, inhibition of (Ca^{2+}) overload, and inhibition of the mitochondrial apoptotic pathway and others. Meanwhile,

other compound such as Cinnamic acid is an organic acid occurring naturally in plants, has low toxicity and also broad spectrum of antioxidant and biological activities [23]. In addition, quercetin derivatives are such as protocatechuic acid (type of phenolic acid) are quantified and major metabolite antioxidant molecules. Compound 4-O-caffeoylquinic acid which also known as cryptochlorogenic acid, is a cinnamic acid derivative and possesses antioxidant properties [42]. The identified compound such as naringin and naringenin are strong antioxidants [34]. Compound naringin were reported for anti-obesity, hyperlipidemia, hypertension, cardiac function, hyperglycemia and diabetes, hepatic function, inflammation, oxidative stress and free radical damage [44]. A group of polyphenol such as carnosic acid and carnosol potently inhibit human 5-lipoxygenase and suppress pro-inflammatory responses of stimulated human polymorphonuclear leukocytes [10].

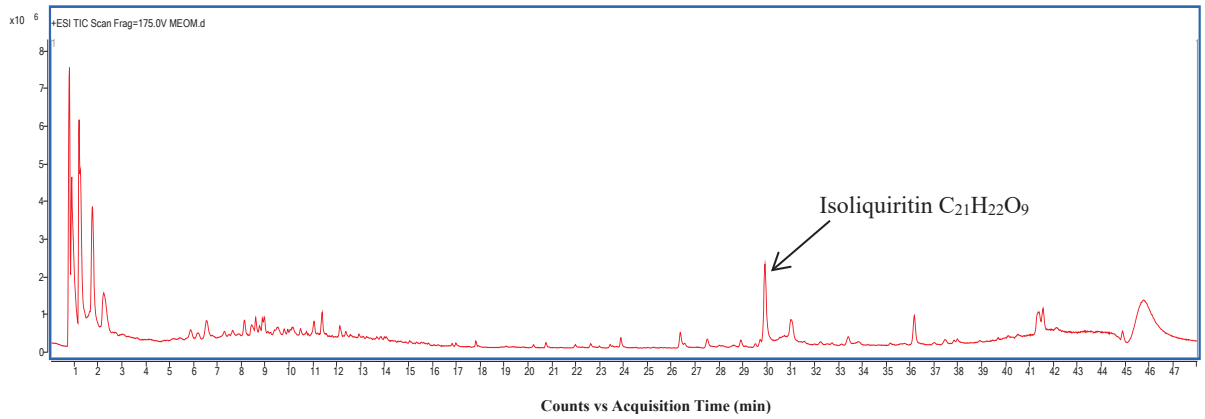

FIGURE 3. LC-MS/MS chromatogram of MEOM by UHPLC-Q-TOF/MS

TABLE 3. Qualitative characterization of isolated compounds in MEOM by LC-MS/MS using ESI positive ion mode

No	Proposed Compounds	Molecular Formula	Retention Time (min)	Molecular weight	Observed (*m/z*) $[M+H]^+$)	Height (Peak)
1	Olivil 4”-o-glucopyranoside	$C_{26}H_{34}O_{12}$	0.791	150.03	151.04	303067
2	Protocatechuic acid	$C_7H_6O_4$	0.791	152.03	153.03	44749
3	Apigenin	$C_{15}H_{10}O_5$	0.798	267.94	268.95	26422
4	Carnosic acid	$C_{20}H_{28}O_4$	0.899	322.07	323.08	12323
5	4-O-caffeoylquinic acid	$C_{16}H_{18}O_9$	0.901	352.08	353.09	23526
6	4’-O-Methylepigallocatechin	$C_{16}H_{16}O_7$	0.966	320.07	321.08	29496
7	(-)-Epicatechin	$C_{15}H_{14}O_6$	0.967	290.06	291.07	11534
8	3’-Hydroxymelanettin	$C_{16}H_{12}O_6$	1.204	300.08	301.09	6268
9	Pyrogallol	$C_6H_6O_3$	1.615	126.04	127.04	42686
10	p-Coumaroyl tyrosine	$C_{18}H_{17}NO_5$	1.652	327.12	328.13	141863
11	Cinnamic acid	$C_9H_8O_2$	1.768	148.05	149.06	23685
12	Dihydrosinapic acid	$C_{11}H_{14}O_5$	1.943	226.09	227.10	13857
13	(-)-Epigallocatechin	$C_{15}H_{14}O_7$	7.274	306.07	307.08	15654
14	Matairesinol	$C_{20}H_{22}O_6$	8.495	372.10	373.11	38037
15	Dihydroferulic acid 4-O-glucuronide	$C_{16}H_{20}O_{10}$	8.968	372.10	373.11	21033
16	Esculetin	$C_9H_6O_4$	8.969	178.06	179.07	43220
17	Delphinidin 3-O-sambubioside	$C_{26}H_{29}O_{16}$	12.123	597.23	598.24	21681
18	1,3,5-Trimethoxybenzene	$C_9H_{12}O_3$	12.932	168.08	169.09	5667
19	Naringenin 7-O-glucoside	$C_{21}H_{22}O_{10}$	13.108	434.16	435.16	8366
20	Isoliquiritin	$C_{21}H_{22}O_9$	29.928	148.02	149.02	754267
21	Protocatechuic acid 4-O-glucoside	$C_{13}H_{16}O_9$	29.93	316.11	317.12	153808
22	Ceanothic acid	$C_{30}H_{46}O_5$	31.045	482.31	483.32	174633

In this study, MEOM had a significant biological activity exerted on the TPC and TFC, and antioxidant potency on ability to scavenge DPPH radicals. The crude extract also significantly, showed low toxicity on the BSLT. The moderate to low biological activities could be contributed by its various classes of compounds and synergistic and/or antagonist reactions.

CONCLUSION

As conclusion, even though the MEOM only showed moderate to low activities, it possess wide range of compounds which could be isolated and analyzed further for its potential. These possibly bioactive compounds have been reported and exhibited various pharmacological effects which beneficial to human health which in future, can be developed into a new alternative-natural product to treat various ailments, with no/minimum severe side-effects.

ACKNOWLEDGMENT

We are grateful for financial supports from the research grant; FRGS R/FRGS/A07.00/00290A/001/2014/000175, and facilities provided by Faculty of Agro-based Industry, Universiti Malaysia Kelantan and Universiti Putra Malaysia.

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