RESEARCH ARTICLE

Editorial Process: Submission:02/08/2020 Acceptance:08/06/2020

Evaluation of Cytotoxic Effects of Methanolic Extract of *Pergularia tomentosa L* Growing Wild in KSA

Essam Nabih Ads[1,2]*, Amr S Abouzied[3,4], Mohammad K Alshammari[3]

Abstract

Background: Pergularia tomentosa is a member of the Apocynaceae family found in a wide geographical region including the Gulf region, North Africa and the Middle East. It is known as Fattaka, Ghalqa or Am Lebina in Saudi Arabia, It is used as a remedy for the treatment of skin sores, asthma, and bronchitis. **Objective:** This study aims to investigate the cytotoxic effects of methanolic extract and Latex (milky secretion) extract. **Methods:** The stem of Pergularia tomentosa was cut, air dried and soaked for 72 h with methanol repeatedly three times. The crude latex (milk extract) was collected from healthy stem parts of P. tomentosa L by cutting the petiole of leaves, and left to flow where a thick white liquid (Milky) were secreted, collected in amber glass tube and extracted with methanol. Further, the methanolic extract was fractionated by subsequent extraction with various solvents, viz. n-hexane, ethyl acetate and methanol. The cytotoxic effects of *Pergularia tomentosa L* were evaluated using three cancer cell lines of colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2) and breast carcinoma (MCF-7). The cytotoxic effects of *Pergularia tomentosa L* extracts against HCT-116, HepG2, and MCF-7 were determined by crystal violet staining method. **Results:** The potency of plant extract to decrease the cell viability of human cancer cells was arranged in descending order as follows: Methanol extract (IC50 = 10.2 µg/ml, 13.6 µg/ml and 29.5µg/ml, respectively). > Milky secretion extract (IC50 = 52.6 µg/ml, 58.6 µg/ml and 120 µg/ml, respectively). Methanolic extract was strong cytotoxic activity against HCT-116 and HepG2 (IC50= 10.2, 13.6 µg/ml, respectively) and moderately activity against MCF-7 (IC50= 29.5 µg/ml). The Milky extract exhibited moderate activity against HCT-116 and HepG2 (IC50= 52.6-58.6 µg/ml, respectively) and weak activity against MCF-7 (120.0 µg/ml). **Conclusion:** The methanol extract of *Pergularia tomentosa L* showed higher cytotoxic effect as compared to the Latex (Milky secretion) extract. These extracts can be used as natural antitumor. In Future modern chromatographic separations are needed to get more quantity of metabolites. Further detailed investigation of the isolated metabolites is required to identify the phytoconstituents responsible for antioxidant and cytotoxic effects.

Keywords: *Pergularia tomentosa L*- cytotoxicity- antitumor- anticancer- latex (milky) extract

Asian Pac J Cancer Prev, **22**, Anticancer Activity of Natural Compounds: HOW's on Methods and Reports Suppl, 67-72

Introduction

Pergularia tomentosa is a member of the Apocynaceae family (subfamily: Asclepiadeae). It has a characteristic peculiar odor and it is growing around fifty cm high. The plant is found in a wide geographical region including Gulf region (Saudi Arabia and Oman), Africa (north Sudan, Egypt, Ethiopia, Algeria, Niger and Kenya) and Middle East (Jordan, Iraq, Iran, Pakistan, and Afghanistan). (Babaamer et al., 2013). It is a native species of the North Africa and Middle East (Alsaid et al., 1988; Gohar et al., 2000; Mansour et al., 1988). It is known as Bou Hliba in Tunisia, and Fattaka ,Ghalqa or Am Lebina in Saudi Arabia, the genus of Pergularia is represented in Saudi Arabia by two species, P.tomentosa L and P.daemia (Gohar et al., 2000). Leaves of this plant that contain plant latex (milky secretion) has been used in the treatment of skin infections, such as Tinea capitis. Number of researches has been carried out on P.tomentosa (Green et al., 2011). The antifungal activity of this plant was tested and results obtained shows that the plant has antifungal effect against Aspergillus niger (Shinkafi, S. A., 2014). It is extensively in traditional medicine for the treatment of different diseases, leaves and stems is used for the treatment of bronchitis and tuberculosis, a medication that should be taken with great care and is forbidden for pregnant women. The plant was reported to have molluscidal activity (Hammiche et al., 2006; Hussein et al., 1999). Many reports mentioned this plant that used for the treatment of respiratory disorder and as antirheumatic agent (Hammiche et al., 2006; Arafa et al., 2006) and persistent hypoglyceaemic effects (Shabana et al., 1990; Favel et al.,

*[1]Department of Chemistry, Faculty of Science, University of Hail, Hail, Saudi Arabia. [2]Faculty of Science, Zagazig University, Zagazig, Egypt. [3]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Hail, Hail, Kingdom of Saudi Arabia. [4]National Organization for Drug Control & Research, Cairo, Egypt. *For Correspondence: Essam.Adss@yahoo.com*

1994). Phytochemical analysis of the aerial components of P. tomentosa incontestible the presence of cardenolide glycosides (Green et al., 2011; Van-Buren et al., 1969), and βsitosterolglucoside (Gohar et al., 2000). Previous studies reported the presence of the ghalakinoside, calactin, uzarigenin, and pergularoside within the roots and desglucouzarin, coroglaucigenin, and uzarigenin together with α-sitosterol glycoside in the leaves (Alsaid et al., 1988; Gohar et al., 2000). According to numerous reports, P. tomentosa leaves are a rich source of flavonoids and cardenolides it can be a good source of natural antioxidant, antibacterial, cytotoxic, and allelopathic compounds (Cherif et al., 2016; Hosseini Kahnouj et al. 2017; Lahmar et al., 2017). Despite P. tomentosa has several traditional uses and various biological effects, very little information is available concerning their effects on the arthropod fauna and only few studies refer to the insecticidal activities of this plant (Acheuk and Doumandji-Mitiche 2013; Paul et al., 2011). *Pergularia tomentosa L* produces corrosive white latex that may severely harm the human skin. It has been shown that the crude alkaloids extract of P.tomentosa has a considerable larvicidal, antifeedant and weight loss effects against the fifth instar larvae of the migratory locust Locusta migratoria, suggesting this plant could be used as a natural product for locust larval control (Acheuk and Doumandji-Mitiche, 2013). In Indian traditional medicine, the Pergularia is commonly used to treat wound and related injuries (Jain 1964; Ayyanar et al., 2009). The P.tomentosa possess potent toxicity as well as repellent activity against L.migratoria nymphs. The compound seemed to present anti-insect properties by disturbing neuro-endocrine and growth regulatory processes as evidenced by the exuviations difficulties, the reduction in AChE activity, and the alterations of the brain structure, particularly in the neurosecretory cells. It was also found that the PME extract induced cellular immune reactions by affecting the number and morphology of differential haemocytes. (Miladi et al., 2019). The phytochemical constituents of P.tomentosa plants grown in the district of Hail and other geographical regions. In addition, there are several natural phytocompounds with an antimicrobial activity (Rayyan et al., 2018). In view the medicinal importance of P.tomentosa L, the aim of this study to extract different fraction from the stem of P.tomentosa L for the cytotoxic evaluations.

Materials and Methods

Chemicals

All solvents used in this study were obtained from Sigma Aldrich, Fisher Scientific, Scharlau Spain and VWR BDH Prolabo chemical (analytical grade).

Plant material and extraction

Fresh Stem of *Pergularia tomentosa L* were collected during spring seasons (March-2019) from the Hail region with GPS coordinates (270 03\ 30.4\\ N, 420 09\ 15.9\\ E), (270 58\ 43.8\\ N, 420 15\ 44\\ E), (270 55\ 94.4\\ N, 420 159\ 48.8\\ E), Saudi Arabia. The plant was identified and the specimen deposited in the Herbarium of the Department of Biology, University of Hail. Stem of *Pergularia tomentosa L* (1kg) was air dried and soaked for 72 h with methanol repeatedly (3 times × 2 L). The crude latex (milk extract) was collected from the stems healthy parts of P. tomentosa by striping and cutting the petiole of youngest leaves, and left to flow as a tears where a thick white liquid (Milky) were secreted and collected in amber galas tube . The extract was gently agitated during collection to overcome the tendency of the coagulation-like effect of the materials. After being collected, lattice was immediately brought to the laboratory and stored at + 4°C until use in the experiments. 25ml of latex (milk extract) was extracted with methanol by using separating funnel. The methanol extract in both two extracts was filtered, charcoaled and then solvent was evaporated under reduced pressure by using a rotary evaporator vacuo at 40°C, model W2-100 SENCO, rpm of 100; Shanghai SENCO technology Co, Ltd Japan. The crude extract was fractionated by subsequent extraction with various solvents, viz. n-hexane, chloroform, ethyl acetate, methanol and Acetonitril, finally with formic acid. Column Chromatography (CC) and thin Layer Chromatography (TLC) Techniques used for Separation and purification of the plant metabolite. *Pergularia tomentosa L* extracts were applied on TLC plates (silica gel G-60 aluminium sheet, Merck, Germany), using CAMMAG Linomat 5 application system and analyzed using CAMMAG TLC scanner unit for determination of optimum solvent system that can separate crude extract to its individual metabolites. Developing processes were carried out using variable solvent systems: chloroform: methanol (9: 1, v/v), toluene: ethyl acetate: formic acid (6.5: 5: 1.5, v/v/v) and hexane: ethyl acetate (2: 1, v/v). The compounds were detected in UV range between 254 and 365 nm or in the visible range to detect the characteristics colored spots and Rf value were recorded. Twenty three fractions (F1–F23) were obtained and All identical fractions were merged to obtain three fractions (F7,F14 and F18) with very low quantity. TLC plates were visualized by placing them into iodine vapor (Ads et al., 2017).

Evaluation of Cytotoxic Effects of Pergularia tomentosa L Chemicals

Dimethyl sulfoxide (DMSO) and, crystal violet was purchased from Sigma (St. Louis, Mo., USA), Fetal Bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM); RPMI-1640; HEPES buffer solution, L-glutamine, gentamycin and 0.25% Trypsin-EDTA were purchased from Lonza (Basel, Switzerland).

Mammalian cell lines: HCT-116 cells (ATCC No. CCL-247™ human colon carcinoma cell line), HepG-2 cells (ATCC No. HB-8064™ human Hepatocellular carcinoma cell line) and MCF-7 cells (ATCC No. HTB-22™ human breast cancer cell line) were purchased from American Type Culture Collection, USA.

Cell culture, growth conditions, and treatment

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with double distilled H_2O and filtered through a Whatmann No.1 filter paper.

Cell line Propagation: The cells were propagated

in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50µg/ml gentamycin. All cells were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and were subcultured two times a week to keep the cell viability and exponential cell growth. The plant extracts were dissolved in DMSO and serial two-fold dilutions with growth medium to give final concentrations of extract ranging from 1-500 µg/ml. untreated control culture received only the vehicle (0.1% DMSO).

Cytotoxicity evaluation using viability assay

The cytotoxicity was assessed via a colorimetric crystal violet staining method as described by (Feoktistova et al., 2016). The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100µl of growth medium dispensed into 96-well, flat-bottomed micro titer plates (Falcon, NJ, USA) using a multichannel pipette. Twenty four hours later, fresh medium containing serial two-fold dilutions of the tested extracts (1, 2, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250 and 500 µg/ml) were added to the confluent cell monolayers using a multichannel pipette. Dimethyl sulfoxide (DMSO) was used as solvent for all the tested extracts. The micro titer plates were incubated at 37ºC in a humidified incubator with 5% CO_2 for a period of 24 h. Three wells were used for each concentration of the test sample. Triton X-100 was used as positive control at the same screening conditions. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for at 37°C, for 24 h, the viable cells yield was determined by a colorimetric method. (Mosmann, 1983).

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each one for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated. The optical density was measured with the microplate reader (SunRise, TECAN, Inc, USA) to determine the number of viable cells and the percentage of viability was calculated as [(ODt/ODc)] x100% where ODt is the mean optical density of wells treated with the tested sample and ODc is the mean optical density of untreated cells. The relation between surviving cells and drug concentration is plotted to get the survival curve of each tumor cell line after treatment with the specified compound.

The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose response curve for each conc. using Graphpad Prism software (San Diego, CA. USA). (Mahmoud et al., 2019), (Gomha et al., 2015)

Statistical analysis

Statistical analysis was done by One-way analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT).

Results

Evaluation of cytotoxicity of Pergularia tomentosa L

Cytotoxic activity of methanol extract and Milky secretion extracts of *Pergularia tomentosa L* (in a concentration of 1-500 µg/ml) was investigated in three cancer cell lines of colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2) and breast carcinoma (MCF-7). The control cells showed high proliferation that has been taken as 100%. The two extracts induced

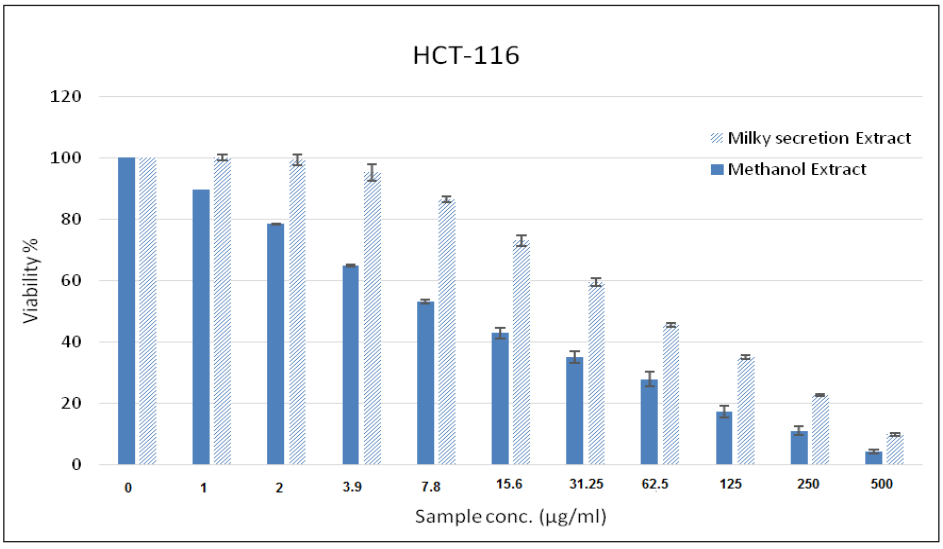

Figure 1. Cytotoxic Activity of Methanol Extract and Milky Secretion Extract of *Pergularia tomentosa L* against HCT-116 Data are Expressed in the Form of mean ± SD. Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT)

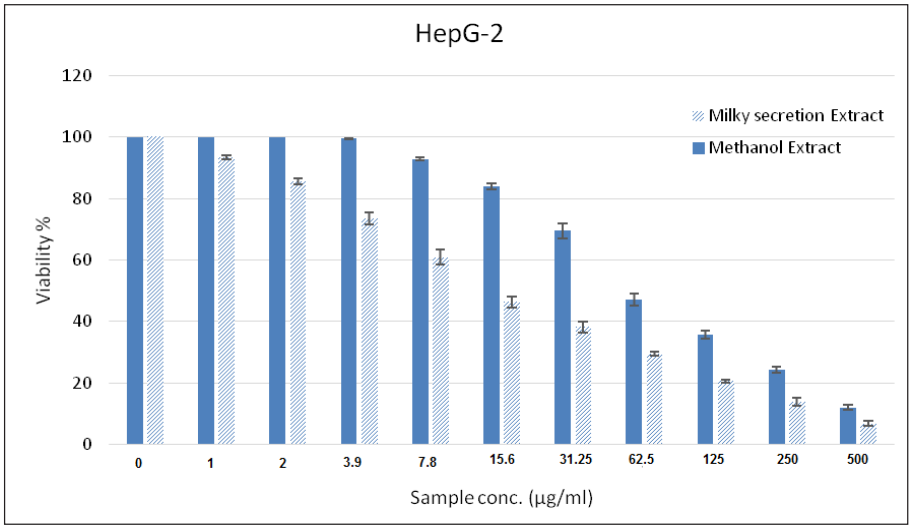

Figure 2. Cytotoxic Activity of Methanol Extract and Milky Secretion Extract of *Pergularia tomentosa L* against HepG-2 Data are Expressed in the Form of mean ± SD. Values not sharing a common superscript differ significantly at p<0.05 (DMRT)

Table 1. Influence of *Pergularia tomentosa L* Extracts on the Cell Viability of Various Human Cancer Cell Lines

Cell lines	Cytotoxic activity (IC_{50}) µg/ml	
	Milky Extract	Methanolic Extract
HCT-116	52.6 ± 1.6	10.2 ± 0.4
HepG-2	58.6 ± 1.3	13.6 ± 0.5
MCF-7	120.0 ± 3.1	29.5 ± 0.9

dose-dependent reduction in the viability of cell-lines (Figures 1, 2 and 3). The IC_{50} of methanol extract in HCT-116, HepG2 and MCH-7 cells was 10.2 µg/ml, 13.65µg/ml and 29.5µg/ml, respectively. While the IC_{50} of Milky secretion extract in HCT-116, HepG2 and MCH-7 cells was 52.6 µg/ml, 58.65µg/ml and 120.0 µg/ml, respectively. The results indicate that the methanol extract showed higher cytotoxic effect as compared to milky secretion extract. Previous studies showed that there were two important sources of antioxidants in *Pergularia tomentosa L*, which are phenolic compounds and hydroxylated flavonoids. Phenolic compounds which is considered as a good source of powerful antioxidants, while flavonoids have the capability to react with DPPH radical by hydrogen atom donation to free radicals while a highly positive correlation between total phenolic content and antioxidant activity was established in case of many plant species (Mallah et al., 2016; Al-Nadaf et al., 2018). The methanolic and aqueous extracts that contained many flavonoids exerted an antioxidant activity against DPPH radicals, peroxyl radicals, hydroxyl radicals, and hydrogen peroxide (Kubinova et al., 2013). Chromatographic fractionation of Pergularia tomentosa isolatede of lupeol acetate (Hassan et al., 2013), fifteen phenolics and flavonoids and four cardenolides were also identified in leaves extracts of P. tomentosa by used LC-MS techniques (Hosseini Kahnouj et al., 2017). Flavonoids and alkaloids (Sak; Everaus 2015; Nwodo et al., 2016) isolated from several plants have shown cytotoxic effects in different

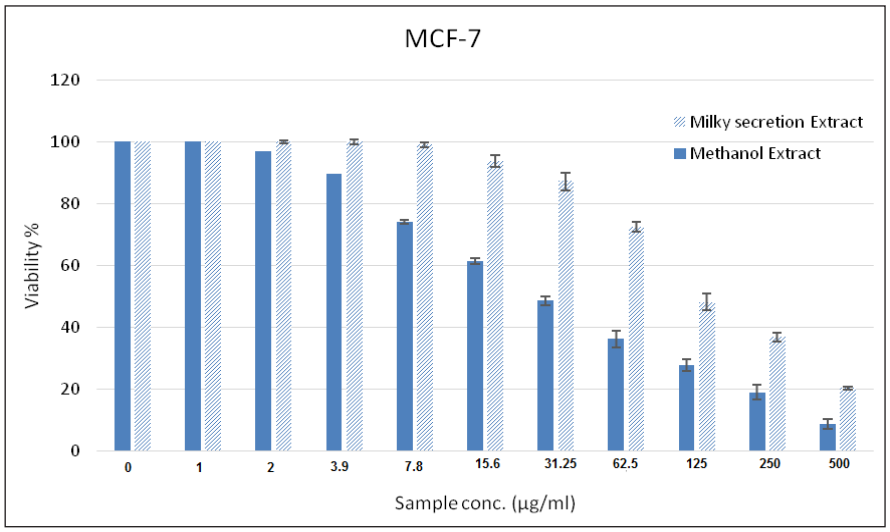

Figure 3.Cytotoxic Activity of Methanol Extract and Milky Secretion Extract of *Pergularia tomentosa L* against MCF-7 Data are Expressed in the Form of mean ± SD. Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

cell lines. These phytochemicals may be responsible for the cytotoxic effects of the *Pergularia tomentosa L*. Other study show that, the cytotoxic effects of roots aqueous extract of Pergularia tomentosa were evaluated in human tumor cell, line cervix carcinoma (Hela), liver carcinoma (HepG 2) and U251 brain cell lines. The activity was achieved with Hepg2 where IC_{50} value was 9.97 µg/ml (Hifnawy et al., 2014), another study has investigated the phytochemical composition and the biological properties of *Pergularia tomentosa L*. grown in the district of Hail. The results of metabolite profiling showed that there are 18 compounds (phenolics and cardenolides) in the extract. Fourteen compounds belonging to the phenolics and flavonoids, and four cardenolides were identified from leaves' aqueous extract of *Pergularia tomentosa L*. (Rayyan et al., 2018). Other reports which investigated the aerial parts of this plant demonstrated that its aerial parts are a rich source of flavonoids (Heneidak et al., 2006).

The cytotoxic effects of *Pergularia tomentosa L* extracts against HCT-116, HepG2, and MCF-7 were determined by crystal violet staining method. The isolated metabolites (F1-F23) of methanolic fraction is very little quantity and not sufficient for cytotoxic tests. So the cytotoxic effects of *Pergularia tomentosa L* extracts against HCT-116, HepG2, and MCF-7 were determined for both methanol and milky secretion extract only. As shown in Table 1, the potency of extracts. As shown in Table 1, the potency of plant extract to decrease the cell viability of human cancer cells was arranged in descending order as follows: methanol extract (IC_{50} = 10.2 µg/ml, 13.6 µg/ml and 29.5µg/ml, respectively. > Milky secretion extract (IC_{50} = 52.6 µg/ml, 58.6 µg/ml and 120 µg/ml). As mentioned by (Atjanasuppat et al., 2009), the cytotoxicity of crude plant extract has classified as follows: active extract (IC_{50}≤ 20µg/ml), moderately active extract (IC_{50}>20–100 µg/ml), weakly active extract (IC_{50}>100–1,000 µg/ml), inactive extract (IC_{50}>1,000 µg/ml). According to these criteria, methanol extract exhibited strongly active cytotoxicity against HCT-116 and HepG2 (IC50= 10.2, 13.6 µg/mL, respectively) and moderately active against MCF-7 (IC_{50}= 29.5 µg/mL). The Milky extract exhibited moderately active against HCT-116 and HepG2 (IC_{50}= 52.6, 58.6 µg/ml, respectively) and weakly active extract against MCF-7 (120.0 µg/ml).

Human cancer cell lines were treated with different concentrations (1-500 µg/ml) of *Pergularia tomentosa L* extracts in 96-well microculture plates for 24 h.

In conclusion, *Pergularia tomentosa L* extracts showed that methanol extract has higher cytotoxic effect than the Latex (Milky secretion) extract. Methanol extract exhibited strong cytotoxic activity against HCT-116 and HepG2 and moderately active against MCF-7., while Latex (milky extract) exhibited moderate activity against HCT-116 and HepG2 and weak activity against MCF-7. Work is in progress for the fractionation of large quantity of extracts from different solvents and the characterization of the isolation of active specific compounds in bioactive fractions. Plans in the future include various modern chromatographic separations to get more quantity of metabolites. Further detailed investigation of the isolated metabolites is required to identify the phytoconstituents responsible for antioxidant and cytotoxic effects. These plans will offer us the opportunity to explore new antioxidant and anticancer agents in *Pergularia tomentosa L* that may have potentials in pharmaceutical and clinical applications.

Acknowledgments

Authors extend their appreciation and thankful to Dr.Abdulrahman S. AlAnazi, Dean of the college of pharmacy, Hail University, KSA and Dr. Kawthar A. Diab, National Research Centre, Dokki, Giza, Egypt. For criticize reading and supporting this research work.

Conflict of interest

The authors declare that there was no conflict of interest.

References

Acheuk F, Doumandji-Mitiche B (2013). Insecticidal activity of alkaloids extract of Pergularia tomentosa (Asclepiadaceae) against fifth instar larvae of Locusta migratoria cinerascens (Fabricius 1781) (Orthoptera: Acrididae). *IJAST*, **3**, 8-13.

Ads E N, Rajendrasozhan S, Hassan SI, Sharawy S, Humaidi. JR (2017). Phytochemical, antimicrobial and cytotoxic evaluation of Ziziphus spinachristi (L.) stem bark. *J Biomed Res*, **28**, 6646-53.

Al-Nadaf AH, Seder NJ, Rayyan WA (2018). Wound healing; antimicrobial and anti-oxidant activity for Jordanian Juglans Regia L. unripe fruits. *J innov pharm biol sci*, **5**, 26-34.

Alsaid M S, Hifnawy MS, McPhail AT, McPhail DR (1988). Ghalakinoside, a cytotoxic cardiac glycoside from Pergularia tomentosa. *Phytochemistry*, **27**, 3245−50.

Arafa I, Alberto P, Balestrieri M, et al (2006). Cardenolide Glycosides from Pergularia tomentosa and their proapoptotic activity in Kaposi's Sarcoma cells. *J Nat Prod*, **69**, 13-9.

Atjanasuppat K, Wongkham W, Meepowpan P, et al (2009). In vitro screening for anthelmintic and antitumour activity of ethnomedicinal plants from Thailand. *J Ethnopharmacol*, **123**, 475–82.

Ayyanar M, Ignacimuthu S (2009). Herbal medicines for wound healing among tribal people in southern India: Ethnobotanical and scientific evidences. *Int J Appl Res Nat Prod*, **2**, 29-42.

Babaamer Z, Sekhri L, Al-Jaber H, Al-Qudah MAbu Zarga M (2013). Extraction and identification of triterpenoids from *Pergularia Tomentosa L. AST,* **5**, 1-5.

Cherif R, Kemassi A, Boual Z et al (2016). Biological activity of aqueous extracts of *Pergularia tomentosa L*. (Asclepiadaceae). *Leb Sci J (LSJ)*, **17**, 25-35.

Favel A, Steinmetz MD, Regh P, et al (1994). In vitro antifungal activity of triterpenoid saponins. *Plantar Medica*, **60**, 50-3.

Feoktistova M, Geserick P, Leverkus M (2016). Crystal violet assay for determining viability of cultured cells. *Cold Spring Harb Protoc*, **4**, doi: 10.1101/pdb.prot087379.

Gohar AM, El-Olemy E, Abdel-Sattar M, El-Said M, Niwa M (2000). Cardenolides and βSitosterol Glucoside from *Pergularia tomentosa L. Nat Prod Sci (NPS)*, **6**, 142-6.

Gomha, SM, Riyadh SM, Mahmmoud EA, Elaasser MM (2015). Synthesis and anticancer activities of Thiazoles, 1,3-Thiazines, and Thiazolidine using Chitosan-Grafted-Poly(vinylpyridine) as basic Catalyst. *Heterocycles*, **91**, 1227-43.

Green P, Veitch N, Stevenson P, Simmonds M (2011).

Cardenolides from Gomphocarpus sinaicus and Pergularia tomentosa (Apocynaceae: Asclepiadoideae) deter the feeding of Spodoptera littoralis. *Arthropod Plant Interact*, **5**, 219-25.
Hammiche V, Maiza K (2006). Traditional medicine in Central Sahara: Pharmacopeia of Tassili N'ajjer. *J Ethnopharmacol,* **105**, 358-67.
Hassan SW, Verma S, Srivastava SK, et al (2013). Activity guided isolation and characterization of antioxidant and antibacterial agents from some local nigerian plants. *Afr J Biotechnol*, **12**, 6315-25.
Heneidak S, Grayer RJ, Kite GC, Simmonds SM (2006). Flavonoid glycosides from Egyptian species of the tribe Asclepiadeae (Apocynaceae, subfamily Asclepiadoideae). *Biochem Syst Ecol*, **34**, 575-84.
Hifnawy MS, El-Shanawany MA, Khalifa MM, Youssef AK, Desoukey SY (2014). Cardiotonic activity of pergularia tomentosa different extracts, fractions &isolated compounds. *J Pharm Biol Sci*, **9**, 54-60.
Hosseini Kahnouj S, Ayyari M, Azarnivand H, Piacente S, Zare Chahouki M. (2017). Pergularia tomentosa, from traditional uses to ecology and phytochemistry. *J Med Plants*, **3**, 108-18.
Hussein H, Al-Rajhy D, El-Shahawi F Hashem S (1999). Molluscicidal activity of Pergularia tomentosa (L.), methomyl and methiocarb, against land snails. International *Int J Pest Manag,* **45**, 211-3.
Jain SK (1964). The role botanist in folklore research. *Folklore*, **5**, 145-50.
Kubinova R, Spackova V, Svajdlenka E, Lucivjanska K (2013). Antioxidant activity of extracts and hplc analysis of flavonoids from capsella bursa-pastoris (l.) medik]. *Ceska Slov Farm*, **62**, 174-6.
Lahmar I, Belghith H, Ben Abdallah F, Belghith K (2017). Nutritional composition and phytochemical, antioxidative, and antifungal activities of *Pergularia tomentosa L. Biomed Res Int*, **2017,** 1-9.
Mahmoud F. Abo-Ashour, Wagdy M. Eldehna, Alessio Nocentini, et al (2019). 3-Hydrazinoisatin-based benzene sulfonamides as novel carbonic anhydrase inhibitors endowed with anticancer activity: Synthesis, in vitro biological evaluation and in silico insights. *Eur J Med Chem,* **10**, 184:111768.
Mallah EM, Rayyan WS, Dayyih WA, et al (2016). Dose-dependent synergistic effect of Pomegranate juice on the bioavailability of sildenafil in rats by using HPLC method. *Am J Pharm*, **35**, 1277-84.
Mansour S, Al-Said A, Hameed U, Hifnawy MS (1988). Pharmacognostical and preliminary phytochemical investigation of the fruit of *Pergularia tomentosa L. Int J Crude Drug Res*, **26**, 1-18.
Miladi M, Abdellaoui K, Hamouda A et al (2019). Physiological, histopathological and cellular immune effects of Pergularia tomentosa extract on Locusta migratoria nymphs. *J Integr Agric,* **18**, 2823-34.
Mosmann T (1983). Rapid colorimetric assay for cellular growth and survival application to proliferation and cytotoxicity assays. *J Immunol Methods*, **65**, 55-63.
Nwodo JN, Ibezim A, Simoben CV, Ntie-Kang F (2016). Exploring cancer therapeutics with natural products from African medicinal plants, Part II: Alkaloids, Terpenoids and Flavonoids. *Anti-Cancer Agents Med Chem*, **16**, 108-127.
Paul WC, Green NC, Veitch PC, Stevenson M, Simmonds SJ (2011). Cardenolides from Gomphocarpus sinaicus and Pergularia tomentosa (Apocynaceae: Asclepiadoideae) deter the feeding of Spodoptera littoralis. *Arthropod Plant Interact*, **5**, 219-25.
Rayyan AW, Alshammari Sami AG, ALSammary Abdulaziz MF, et al (2018). The phytochemical analysis and antimicrobial activity of Pergularia tomentosa in North East Kingdome of Saudi Arabia KSA. Biomed Pharmacol J, **11**, 1763-71.
Sak K, Everaus H (2015). Multi-target cytotoxic actions of flavonoids in blood cancer cells. *Asian Pac J Cancer Prev*, **16**, 4843-7.
Shabana M, Mirhom Y, Genenah A, Aboutabl E, Amer H (1990). Study into wild Egyptian plants of potential medicinal activity. Ninth communication: hypoglycaemic activity of some selected plants in normal fasting and alloxanised rats. *Arch Exp Veterinarmed*, **44**, 389
Shinkafi S (2014). Phytochemical analysis and chromatographic studies of *Pergularia tomentosa L*. and Mitracarpus scaber Zucc. *Br Microbiol Res J,* **4**, 550-9.
Van Buren J, Robinson W (1969). Formation of complexes between protein and tannic acid. *J gric Food Chem,* **17**, 772-7.