Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS OF *CORRIGIOLA TELEPHIIFOLIA* POURR.

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Received: 30 Dec 2012, Revised and Accepted: 28 Mar 2013

ABSTRACT

Background: The use of natural products as anticancer and antioxidant agents has a long history. Several chemotherapy drugs were isolated from medicinal plants. Objective: The present study was aimed to provide information on cytotoxic and antioxidant activity of cyclohexane, dichloromethane and methanol extracts of *Corrigiola telephiifolia*.

Materials and method: The plant roots were dried, powdered and extracted successively by cold maceration with: cyclohexane, dichloromethane and methanol for 48h. All extracts have been assessed for antioxidant activity against DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and cytotoxic activity against murine colon adenocarcinoma CT-26 and melanoma cell lines WM-266.

Results: The dichloromethane extract exhibited potent cytotoxicity with an IC50 of 80 ± 4.56 for CT-26 cells and 70 ± 6.1 µg/ml for WM-266 cells. The extracts exhibited moderate antioxidant activity with IC50 value of 10.53 µg/ml of the dichloromethane extract compared to the IC50 value of 4.71 ± 0.83 µg/ml as shown by the reference antioxidant Trolox.

Conclusion: The potential activity of *Corrigiola telephiifolia* root extract may due to their phytochemical constituents, these species could be considered as potential sources of anticancer compounds. Further studies are necessary for chemical characterization of the active principles and more extensive biological evaluations.

Keywords: *Corrigiola telephiifolia*, cytotoxic effect, DPPH assay, free radical scavenging activity.

INTRODUCTION

Plant based treatments continue to play an essential role in primary health care. The improvement of medicinal plants value can be achieved by searching newer, more effective and less toxic therapeutic molecules, which will add great values to the resources that can be later, integrated into the therapeutic arsenal. Plants based anticancer and antioxidant, have recently received a great attention [1]. Medicinal plants present a potential source of drugs or molecular models for new drugs, in fact some species provided many effective anticancer agents in current use such as vinblastine, irinotecan, topotecan, vincristine, taxanes [2] etc, the imminent strategy of the World Health Organization (WHO) lead scientists to improved herbal remedies and the discovery of a natural chemotherapy drug which guarantees the safety, efficacy and quality as a chemical drug. Plants secondary metabolites proved strong biological activities [3], polyphenolic compounds are believed to have a strong antioxidant [4-7], chemopreventive and suppressive activities against cancer cells by inhibiting many metabolic enzymes involved in the activation of potential carcinogens or arresting the cell cycle [8]. The importance of natural compounds from plants materials is also raising interest among scientists to discover new anticancer molecules. All these make the knowledge of chemical, biological and therapeutic activities of medicinal plants become necessary.

Morocco is one of the Mediterranean countries which has a long history and knowledge of traditional therapy [9]. The varied climate and heterogeneous ecologic condition in Morocco have favored the proliferation of more than 42 000 species of plants; divided into 150 families and 940 genuses [10, 11]. Medicinal plant can grow naturally or be cultivated due to their economic and medicinal values. Among these plants, *Corrigiola telephiifolia* plant is used in traditional medicine of many countries, especially in Morocco, this species is found in the Mediterranean region, woody used in Morocco for medicinal purposes, root decoction is reported to be traditionally prescribe to treat the cancer [12], the plant is used by several traditional healers for the treatment of dermatological diseases and cough; it is an antiasthenic, antispasmodic, diuretic, and aphrodisiac [13], a mixture with honey can be used for the treatment of stomach aches, chills, lung diseases and rheumatic diseases [14], the extracted oil from the roots is used for perfume production and its smoked are used to treat flu and headaches [13].

Very few studies worldwide which aimed at the documentation of this species. In order to justify and validate scientifically its uses in traditional medicine; this study is contributed to determine the free radical scavenging effect of *C. telephiifolia* Pourr. Root extracts and its cytotoxic activity against two different human cancer cell lines by using the MTT assay.

MATERIALS AND METHODS

Plant material

Corrigiola telephiifolia Pourr. was collected based on ethnopharmacological information and traditional uses, from villages around the Ben Slimane region, with the agreement from the authorities and respecting the United Nations Convention of Biodiversity and with assistance of traditional medical practitioner. The plant was identified with a botanist of Scientific Institute (Pr. M. Fennane). A voucher specimen (RAB77766) was deposited in the Herbarium of Scientific Institute, University Mohammed V-Rabat-Morocco.

Preparation of extract

Classical extraction

970 g of *C. telephiifolia* root parts were extracted successively with cyclohexane, dichloromethane and methanol by maceration at room temperature (22°C) over period of 24 h the extraction process was repeated again for a three time, the extracts were then filtered through Whatman paper and the solvents were vacuum-distilled in a rotary evaporator (Rotavap: Buchi). The remaining extracts were finally dried in the oven at 30°C for 2 h to ensure the removal of any residual solvent. Final extracts were a yellow powder in percentage dry weight 0.11, 0.18 and 7.32% of cyclohexane, dichloromethane and methanol extract respectively; the extracts were kept in deep freeze at -20 °C until use.

Aqueous extract

Water extract was prepared by adding 175ml of distilled water to 100g of powdered plant's root and macerated within a period of 48 h. The respective aqueous extract was separated from its residues by gravity filtration and then lyophilized (Free Zone® Dry 4.5, USA). For each study, the lyophilized aqueous extract was carefully prepared under the same condition used throughout the studies (time, temperature and the amount of plant material and water used for extraction under reflux and lyophilization) and each time the quality of extraction was checked by the yield of the lyophilized material [15].

In vitro cytotoxic activity assay

Cell lines and culture medium

Human Melanoma cell lines (WM-266) and murine colon adenocarcinoma (CT-26) obtained from the American Type Culture Collection (ATCC) were used in this study. Cells were grown at 37 °C, maintained in humidified 5% CO_2 and 100% relative humidity atmosphere in Dulbecco's Modified Eagle Media (DMEM) and Roswell Park Memorial Institute medium (RPMI) respectively, supplemented with 10% fetal calf serum, 2mM glutamine,100 µg/mL streptomycin and 100 U/mL penicillin.

Cytotoxicity assay

For the assays, 96-well microplates were seeded with 100 µl medium containing 10 000 cells in suspension with fourfold concentrations of crude extracts ranging from 5 mg/ml to 78.12 µg/ml, dissolved in DMSO, the extracts were incubated for 24h. Doxorubicin (0.58 µg/ml) was used as positive control and the cells in cell culture medium no treated corresponding to 100% of cell viability, all experiments were done 4 times in quadruplicate. Growth of tumoral cells was quantified by the ability of living cells to reduce the yellow dye 3-(4, 5-dimethyl- 2- thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product [16]. 10 µl MTT solution of 0.5 mg/ml was then added to each well and incubated for 4 h. The medium of all the plates was removed and the resulting MTT-formazan product was dissolved by the addition of 100 µl of 0.08 N HCl–isopropanol solution into each well, followed by mixing and measuring the absorbance at 595 nm using a microplate reader.

Antioxidant activity

Free Radical Scavenging Activity

The free radical scavenging activity of the extracts of *C. telephiifolia* root parts was measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH) [17,18]. Briefly, 0.2 mM solution of DPPH in methanol was prepared and 0.5 ml of this solution was added to 2.5 ml of plant extract and was allowed to stand at room temperature for 30 min, and then absorbance was read at 517 nm against blank samples. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation:

% RSA = $[(A_{DPPH} - A_{Extr})/ A_{DPPH}] \times 100$. Where A_{DPPH} is the absorbance value of the DPPH blank sample, and A_{Extr} is the absorbance value of the test solution. A_{Extr} was evaluated as the difference between the absorbance value of the test solution and the absorbance value of its blank.

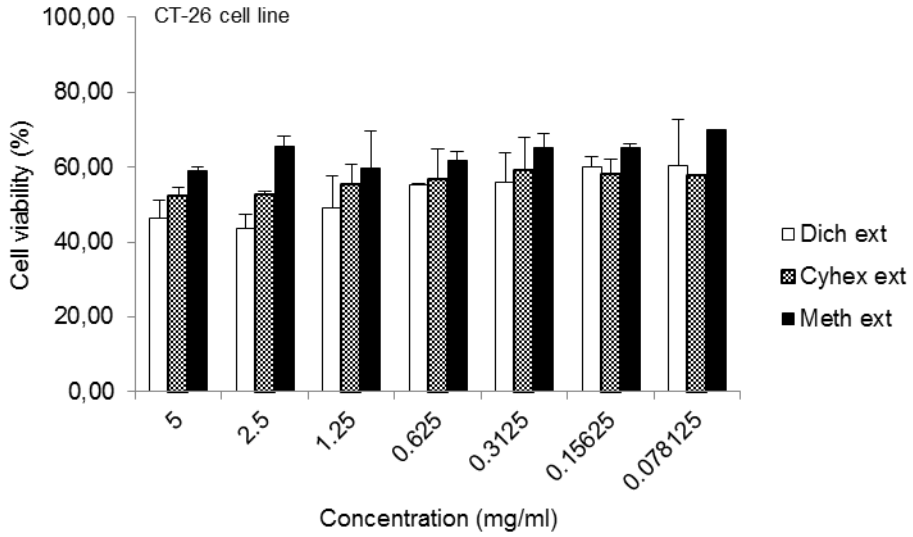
Statistical analysis

The statistical analysis was performed by one-way ANOVA analysis of variance, results were considered to be statistically significant with a 95 % confidence level ($P<0.05$).

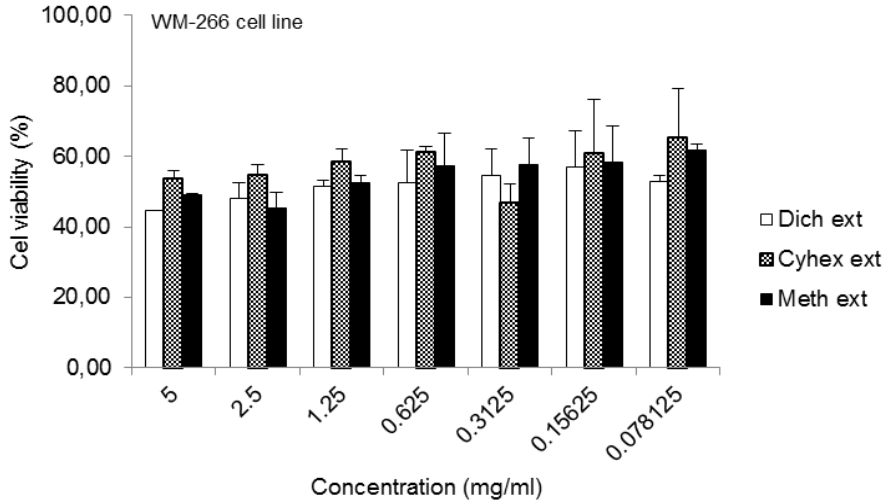
RESULTS AND DISCUSSION

Cytotoxic activity

Cancer known as one of the most malignant diseases worldwide [19] is characterized by uncontrolled growth and local tissue invasion with sometimes distant metastases of abnormal form of body's cells [20]. It has been known that plants have a long history of use in the treatment of cancer [21], and herbal medicines have a vital role in the prevention and treatment of cancer [22], the use of plant derived natural compounds as part of herbal preparations and alternative sources of drugs continues to play major roles in the general wellness of people all over the world [23,24]. The search for anticancer agents that may inhibit cancer development is becoming an important objective for scientists. In this study we have explored the therapeutical and cytotoxic effect of the *C. telephiifolia* a Moroccan species, the choice of the plant is justified by its anticancer and properties in traditional medicine [12]. Various extracts were prepared using different extraction solvents; plant extracts (cyclohexane, dichloromethane and methanol) were subjected to cytotoxicity assays against WM-266 and CT-26 cell lines by MTT assay, this method is important to select plant extracts with potential anticancer properties [25]. The extracts were found to show dose dependent cytotoxicity against WM-266 and CT-26 cell lines between the concentration ranges of 5 mg/ml –78.12 µg/ml. The cyclohexane extract showed a significant cytotoxicity against CT-26 cell lines. The IC50 values were also confirmed that extract showed cytotoxicity against this tested cell line more than that of WM-266 cells (CT-26 IC50 = 70 µg/ml, WM-266 IC50= 120 µg/ml) (Fig. 1).



(a)



(b)

Fig. 1: Percentage cell viability curve of C. *telephiifolia* (CT) extracts against (a) CT-26 and (b) WM-266 cell lines.

Dich ext.: dichloromethane extract, Cyhex ext.: cyclohexane extract and Meth ext.: methanolic extract. Cell viability was plotted via the concentration and all samples were run in quadruplicate (n=4). The percent viable cells were calculated in comparison to untreated cells taken as 100%. Values were expressed as mean ± standard deviation. Bars having different letters indicate significant statistical difference (P<0.05).

Cytotoxic activity of the dichloromethane extract was also determined against CT-26 and WM-266 cancer cells in different concentrations; this extract exerts a pronounced dose-dependent inhibitory effect. The cytotoxicity is clearly identified against both cell lines with IC50 values of 80 ± 4.56 and 70 ± 6.1 µg/ml, respectively. Cytotoxic activity of the methanolic extract was also tested, the extract was found active on CT-26 (IC50 value of 70 ± 6.1 µg/ml) and moderately active on WM-266 (IC50 value of 160 ± 4.56 µg/ml) cell line. Numerous studies have reported medicinal and toxicity profile [26] of the *C. telephiifolia* extracts, it is important to examine the therapeutic effects of this species against cancer cell lines in an effort to identify preliminary candidates for alternative cancer therapeutics. In this work *C. telephiifolia* crude extracts showed varying levels of cytotoxicity on the cancer cells. We adopted the criteria of the American National Cancer Institute to consider a crude extract promising for further purification based on the IC50 values lower than 30 µg/ml in order to discover and develop potential anticancer natural compounds [27,28]. The results obtained in this study indicate that the dichloromethane extract of the plant *C. telephiifolia* was shown to induce significant inhibitory activity against human cancer cell lines tested, the cytotoxic activity could be due to the presence in the dichloromethane extracts of active products that could probably have highly anti-growth effects. It would be an attractive extract to further explore in depth its anticancer properties, it is important to examine the effects of *Corrigiola telephiifolia* species against specific cancer cell lines to identify preliminary candidates for alternative cancer therapeutics.

Free Radical Scavenging Activity

The free radical scavenging activity of *C. telephiifolia* extracts was assessed by DPPH assay. DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods or natural products. The DPPH radical contains an odd electron, which is responsible for the absorbance at 515-517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance [29].

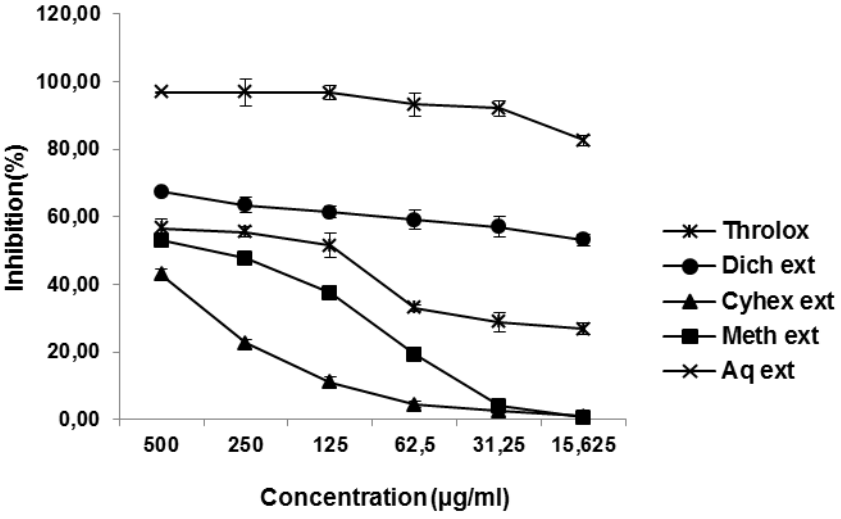


Fig. 2: Free-radical scavenging activity of *C. telephiifolia* extracts measured using the DPPH assay.

Dich ext.: dichloromethane extract, Cyhex ext.: cyclohexane extract, Meth ext.: methanolic extract and Aq ext.: aqueous extract. Values are means ± standard deviation with respect to positive control (Trolox). Bars having different letters indicate significant statistical difference (P<0.05).

As shown in Figure 2, the DPPH scavenging activity in all extracts was concentration-dependent (increasing from15.62 µg/ml to 500 µg/ml) compared with that of Trolox, the extracts tested exhibited moderately DPPH radical-scavenging activities. Overall, the cyclohexane, dichloromethane, methanol and aqueous extracts of *C. telephiifolia* were able to inhibit the formation of DPPH radicals with a percentage inhibition of 56.66 %, 67.50 %, 53.10 % and 43 % respectively at the highest concentration. The DPPH radical scavenging capacity of all the extracts of *C. telephiifolia* was less to this of Trolox (96.80 %, IC50: 4.71 ± 0.83 µg/ml), the IC50 values of all the plant extracts have been furnished in the Table 1.

Table 1: Free radical scavenging activity of *Corrigiola telephiifolia* Pourr. extracts by DPPH reduction

Plant	Family	Part used	Extracts	IC50 (µg/ml)
Corrigiola telephiifolia Pourr.	Caryophyllaceae	Roots	Cyhex ext.	11.34 ± 0.6
			Dich ext.	10.53 ± 0.46
			MeOH ext.	12.06 ± 0.62
			Aq ext.	11.88 ± 0.83

Values are means of three independent analyses ± standard deviation (n = 4)

In recent years, researches on antioxidant activities of medicinal plants have remarkably augmented by virtue of increased interest in their potential high antioxidant capacity and positive health benefits [30-33]. Antioxidants have been reported to prevent oxidative damage by free radical and Reactive Oxygen Species (ROS) [34,35] and may prevent the occurrence of disease including brain disorders, cancer, atherosclerosis, inflammatory disease and variety of other disorders [36-38]. The dichloromethane extract tested show a considerable cytotoxic and antioxydant effects, we could infer that the activities of plant to the synergistic effect of it composition and to the presence in the plant of active products that could probably have highly anti-growth effects. It would be interesting to fractionate the dichloromethane plant extract to identify the active compounds.

CONCLUSION

It can be noted that extracts from *C. telephiifolia* show average cytotoxic and inhibitory values that give an idea of the interesting anticancer activity of such extract; with a high potential to take advantage of and develop anticancer or Pharmaceutic products for the cancer disease.

ACKNOWLEDGEMENTS

The authors wish to thank all the individuals and institutions who made this study possible.

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