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Antioxidant, antiproliferative and anti-inflammatory activities of squirting cucumber (*Ecballium elaterium* L)

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Abstract: Plants constitute a source of natural phytochemical components which are widely known for their potential biological activities. This work concerned a study of the antioxidant, anticancer and anti-inflammatory activities of squirting cucumber (*Ecballium elaterium* L.) parts (flowers, fruits, leaves and stems) using different solvent extracts (cyclohexane, dichloromethane, ethyl acetate, methanol and water). The different squirting cucumber parts had an appreciable anti-tumour activity in the less polar extracts contrarily to the antioxidant activity. Squirting cucumber flowers had an appreciable anti-inflammatory activity in the polar extracts with the highest NO inhibition in the ethyl acetate and methanol extracts. However, the anti-inflammatory activity of squirting cucumber stems had an affinity for the apolar extracts with the highest nitric oxide inhibition in the cyclohexane and the dichloromethane extracts. Squirting cucumber leaves and fruits had an important anti-inflammatory activity in both polar (particularly ethyl acetate for leaves and water for fruits) and apolar (cyclohexane for these two organs) extracts. Squirting cucumber exhibited a potent antioxidant, antiproliferative and anti-inflammatory activities owing to its bioactive chemical components and may be a good plant based product for pharmaceutical industry.

Keywords: squirting cucumber (*Ecballium elaterium* L.), antioxidant activity, antiproliferative activity, anti-inflammatory activity, plant organs, solvent extracts.

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INTRODUCTION

Plants are known to produce variety of phytochemicals, making them rich sources of different types of medicines. These phytochemicals usually extracted from plants used as medicines, food additives, dyes, insecticides, cosmetics, perfumes and fine chemicals (Abu-Hijleh *et al.,* 2018). In some countries, 80% of the population is depending on medicinal plants to maintain their health and cure their diseases (Aidi Wannes *et al.,* 2016a).

The plant *Ecballium elaterium*, known as squirting cucumber, is a weed belongs to the Cucurbitacea family. The plant is perennial, fleshy, rough hairy with stems 30-100 cm long. It has hairy vine, palmately lobed, bristly leaves. The flowers are greenish-yellow and the fruits are large juicy berry. *E. elaterium* was traditionally used for the treatment of fever, cancer, liver disorders, jaundice, constipation, hypertension, dropsy, rheumatic diseases and fungicidal (Souilah *et al.,* 2020). It was experimentally approved for the treatment of various diseases including sinusitis (Sargin *et al.,* 2013), rhinosinusitis (Mazokopakis *et al.,* 2009), antimicrobial (Abbassi *et al.,* 2014), anti-inflammatory (Bourebaba *et al.,* 2020) and anticancer activities (Touihri *et al.,* 2015). This plant is considered rich in proteins, lipids, cucurbitacins (B, D, E, I and L,) and cucurbitacin derivatives such as glycosylcucurbitacins and triterpenoid glycosides (Greige-Gerges *et al.,* 2007). Several recent studies have revealed that the extracts of *E. elaterium* contained a wide range of active ingredients such as phenolic compounds, flavonoids, alkaloids, sterols, amino acids, vitamins, tocopherols and fatty acids justifying their usage in food systems (Touihri *et al.,* 2015). Due to the variety of these bioactive compounds contained in plant materials and their differing solubility properties in different solvents, the optimal solvent for extraction depends on the particular plant materials and the compounds that are to be isolated (Salih *et al.*, 2021). In fact, the objective of extraction process is to maximize the amount of bioactive compounds and to obtain the highest biological activity of these extracts (Omeroglu *et al.,* 2019). Therefore, the aim of this study was to investigate the effect of five solvents (cyclohexane, dichloromethane, ethyl acetate, methanol and water) on the antioxidant, anticancer and anti-inflammatory activities of the different squirting cucumber parts (flowers, fruits, leaves and stems). To our knowledge, it is the first time to investigate the antiproliferative activity of different squirting cucumber parts against human lung carcinoma A549, human colon adenocarcinoma DLD-1 and healthy human skin fibroblast WS-1 cell lines using different solvent extracts as well as the anti-inflammatory potential of these different solvent extracts by the inhibition of nitric oxide production in lipopolysaccharide-stimulated RAW 264.7 macrophage cells..

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MATERIALS AND METHODS

Plant materials

Ecballium elaterium were harvested in the region of Manouba, northwest of Tunisia (36°48' 26" N, 10°06' 03" W), during the flowering period. The plant was taxonomically identified at the Technopark of Borj-Cedria by the botanist Pr Samoui. A. Whole plant (leaf, stem, fruit and flower) were lyophilised in the laboratory and then powdered. The respective plant powders were used for extraction.

Preparation of plant extract

In this study, cyclohexane, dichloromethane, ethyl acetate and methanol were used separately for preparation of the various extracts of leaves, stem, flowers and fruit from *E. elaterium*. 10g of powdered samples were separately packed in a Soxhlet apparatus and extracted with 100 mL of each solvent.

For preparation of aqueous extract, 10 g of each powdered samples were added in 100mL water for 3 h using a shaker apparatus. The respective filtrates were freeze-dried in a lyophilizer. The residues were dissolved in DMSO until further analysis.

Cell lines and culture conditions

Cancer cell lines A-549 (human pulmonary carcinoma CCL-185), DLD-1 (adenocarcinoma colorectal CCL-221), WS-1 (Human Skin Fibroblast CRL-1502) and RAW 264.7 (mouse macrophages TIB-71) were obtained from the American Type Culture Collection (ATCC, Manassas, USA.

Phytochemical characterization

The contents of total phenolics (TPC) and flavonoids (TFC) total tannin content (TTC) and condensed tannin content (CTC) were measured using a colorimetric assay used by Aidi Wannes *et al.,* (2016b). Furthermore, plant extracts were screened for the presence of alkaloids, terpenoids, sterols and saponins according to Tona *et al.,* (1998).

In vitro antioxidant activities

The free radical scavenging activity of the *E. elaterium* extracts was measured in terms of their hydrogen donating or radical scavenging ability using DPPH radical (Aidi Wannes *et al.,* 2016b).

The reducing property was determined by assessing the ability of extracts to reduce ferric chloride ($FeCl_3$) solution as described by Mejri *et al.,* (2022). The effective concentration providing 0.5 nm of absorbance (EC_{50}, µg/mL) was calculated from the graph of absorbance at 630 nm against concentration.

The total antioxidant activity was evaluated by the formation of phosphomolybdenum complex as described by Dibacto *et al.*, (2021). Total antioxidant activity is expressed as the milligrams of gallic acid equivalents per gram of dry extract (mg eq GA/g DE).

Ex vivo anticancer activity-cytotoxicity assay

The Alarmar Blue (resazurin) reduction assay was used to evaluate the anticancer or cytotoxic effects of different *E. elaterium* parts on A549 lung cancer cells and colon cancer cells DLD-1. This assay measured the mitochondrial activity as a proof of cell viability (El-Haci and Bekkara, 2011). The cytotoxicity activity of *E. elaterium* extracts which kills 50% of cells (IC_{50} µg/mL) were investigated in all studied cell lines.

In vitro anti-inflammatory activity

The Griess assay was performed to measure NO production (Hamidi *et al.,* 2020). RAW 264.7 macrophages were plated (2×10^5 cells/100 µL) in 24-well microplates (BD Falcon). For 24 h of incubation, RAW 264.7 cells were stimulated with 100 µg/mL lipopolysaccharide (LPS) followed by various concentrations of *E. elatrium* extracts (1.25-160 µg/mL). The treatment with N(G)-nitro-L-arginine methyl ester hydrochloride (L-NAME) was used as a positive control. The final concentration of solvent in the culture medium was maintained at 0.5% (v/v) to avoid solvent toxicity. After 24h of incubation, cell-free supernatants were collected and mixed with an equal volume (1:1) of Griess reagent. After 15 min incubation at room temperature, the absorbance at 540 nm was measured using an automated 96-well Varioskan Ascent plate reader and the nitrite levels were determined by comparison to a standard curve.

STATISTICAL ANALYSIS

The results of chemical compositions, antioxidant, anticancer and anti-inflammatory activities of each solvent were the mean ± S.D of three experiments. The one-way analysis of variance (ANOVA) followed by the Tukey HSD test were employed and the differences between individual means were deemed to be significant at $p<0.05$.

RESULTS

Extraction yield

The extraction yields from squirting cucumber fruits, flowers, leaves and stems using different solvents, are shown in fig. 1. The recovery percentage of extractable compounds were ranged from 0.81 to 16% for leaves, 1.20 to 13.9% for stems, 8.33 to 10.60% for fruits and 0.61 to 9.70% for leaves. The methanol solvent gave the highest extraction yield from *E. elaterium* leaves (16%), stems (13.90%) and fruits (10.60%). However, the highest extraction yield was obtained by water for *E. elaterium* flowers (9.70%).

Phytochemical analysis

The contents of total polyphenols (TPC), total flavonoids (TFC), total tannins (TTC) and condensed tannins (CTC) of different squirting cucumber organs using different extraction solvents are given in table 1. The highest TPC, TFC, TTC and CTC were detected in the methanol extracts of fruits (52.60mg GAE/g, 96.25mg QE/g, 3.87mg GAE/g and 2.38mg CE/g, respectively), leaves (5.85mg GAE/g, 78.73 mg QE/g, 10.54mg GAE/g and 4.89mg CE/g, respectively) and stems (2.51mg GAE/g, 12.55mg QE/g, 7.77mg GAE/g and 4.45mg CE/g, respectively). In flowers, the aqueous extract showed the highest TPC (9.70mg GAE/g) while the dichloromethane contained the highest TFC (16.57mg QE/g). The highest TTC (5.93mg GAE/g) and CTC (4.82mg CE/g) were observed in the cyclohexane extracts of squirting cucumber flowers.

In this study, it is interesting to mention that other secondary metabolites were qualitatively detected in squirting cucumber extracts depending on organ and solvent factors. In fact, the preliminary characterisation showed that alkaloids were present in the dichlormethane extract of flowers as revealed by an intense orange color using dragendorff reagent while in the ethyl acetate extract of leaves and the methanol extract of fruits, the orange color was less intense. Saponins were only present in the methanol extracts of fruits and leaves, as revealed by an intense purple color using komawisky reactif. However, sterols and triterpenoids were mostly found in apolar solvents, especially the dichloromethane extract of fruits, as shown by the intense blue color using Liebermann-Burchard reactif.

In vitro antioxidant activity

The antioxidant activities of squirting cucumber flowers, fruits, leaves and stems using different extraction solvents were evaluated *in vitro* by three techniques, namely, TAC, DPPH and reducing power assays (table 2). Results showed that the solvent type and plant part significantly affected the antioxidant activities of *E. elaterium* extracts. TAC was detected with a maximum in the dichloromethane extracts of flowers (20.97mg GAE/g). For the other parts, the methanol extracts had the highest TAC with 20.97mg GAE/g for leaves, 19.90mg GAE/g for fruits and 11.87mg GAE/g for stems, in ethyl acetate extracts, respectively. As compared to the synthetic standard BHT (IC_{50}=17.34µg/mL), the ethyl acetate, methanol and aqueous extracts of flowers (IC_{50}=223.33, 896.66 and 388µg/mL, respectively), fruits (IC_{50}=313.36, 155 and 366.66µg/mL, respectively) and stems (IC_{50}=196.33, 766.66 and 288.13µg/mL, respectively) were very weakly able to reduce the stable free radical DPPH. For leaf, only the methanol extract had a weak DPPH activity (IC_{50}=506.6µg/mL). For reducing power activity, the synthetic standard ascorbic acid had an EC_{50}=40µg/mL. The ethyl acetate and water extracts of flowers had higher reducing power towards the Fe^{3+}/ferricyanide complex with EC_{50}=6.73 and 13.23µg/mL, respectively. Additionally, an important reducing power activity was detected in the methanol extract of fruits (IC_{50}=1.13µg/mL) and leaves (EC_{50}= 29.33µg/mL).

In vitro anticancer activity

The anticancer or the cytotoxicity effects of squirting cucumber parts were evaluated on human cancer cell lines such as A549 (pulmonary carcinoma), DLD-1 (adenocarcinoma colorectal) and normal fibroblast cells, WS-1 (table 3).

In flowers, the ethyl acetate extracts had a potent cytotoxic activity to both cell lines with against A549 (IC_{50}=4.20µg/mL) and DLD-1 (IC_{50}=17µg/mL). The A549 cells were more sensitive with the dichloromethane extracts (IC_{50} = 3.39µg/mL,) and a lower activity of the methanol extract was observed (IC_{50}=97.13µg/mL). Both cyclohexane and aqueous extracts of flowers were not significantly affected the viability of normal cell lines WS-1 (IC_{50}>200µg/mL).

In fruit, the dichloromethane, cyclohexane and ethyl acetate, extracts had the most potent cytotoxic activity against A549 cells (IC_{50}=3.7, 8.6 and 13µg/mL, respectively) and DLD-1 cells (IC_{50}=12, 6 and 27µg/mL, respectively). Aqueous extracts showed marginal anti-A549 activity (IC_{50} = 145µg/mL). Concerning normal cell lines WS-1, the methanol and aqueous extracts did not shown any toxicity effects (IC_{50} > 200µg/mL).

In leaves, the dichloromethane and cyclohexane extracts exerted the most potent anti-DLD-1 activity with IC_{50} = 3 and 14µg/mL, respectively. For A549 cells, the cyclohexane extract was inactive (IC_{50}>200µg/mL) while the dichloromethane extract had a moderate cytotoxic activity with IC_{50}=96µg/mL. Except for the dichloromethane and cyclohexane extract, all other extracts (ethyl acetate, methanol and aqueous extracts) did not exhibit cytotoxic activity against any of the tested cell lines (IC_{50}>200µg/mL). All solvent extracts of leaves had not toxic effect against non tumoral cell lines (WS-1).

In stems, the dichloromethane extracts were the most strong against DLD-1 (IC_{50}=4µg/mL) and A549 (IC_{50}= 24µg/mL) cells, respectively. The ethyl acetate and aqueous extracts had a moderate anticancer activity with IC_{50} values ranging from 67 to 170µg/mL, but the methanol extract lacked any anti-A549 and anti-DLD-1 activities. The dichloromethane, ethyl acetate and methanol extracts had no toxicity against the normal WS-1 cell lines (IC_{50}>200µg/mL).

These results highlighted for the first time the noteworthy cytotoxic effects of different *E. elaterum* parts and a good

selectivity against different cancer type. For DLD-1 cells, the highest anticancer activity was detected in leaves (dichloromethane extracts), in stems (dichloromethane extracts) and in fruit (cyclohexane extracts) (IC_{50}=3, 4 and 6µg/mL, respectively) as compared to the both positive controls Betulinol (IC_{50}=25µg/mL) and Etoposide (IC_{50}=8µg/mL). For A549 cells, the fruit extracts (dichloromethane) and the flowers extracts (dichloromethane and ethyl acetate) exhibited a pronounced cytotoxicity effects (IC_{50}=3.39, 3.7 and 4.2µg/mL, respectively) as compared to the both standard anticancer drug, Betulinol (IC_{50}=8.2µg/mL) and Etoposide (IC_{50}=4.08µg/mL). These standard drug, Betulinol and Etoposide, showed significant cytoxicity against normal cell lines (IC_{50}=8.2 and 12µg/mL).

In vitro anti-inflammatory activity

As shown in fig. 2, all solvent extracts of different squirting cucumber organs inhibited LPS-induced NO production in a dose-dependent manner. Our results were compared to the positive control L-NAME which had a NO inhibition of 53.94% at the concentration 250µM and 37.40% at the concentration 1mM.

In flowers, the ethyl acetate and methanol extracts had the highest inhibition of NO production (55.08% and 58.02%, respectively) at the concentration 80 µM as compared to the other solvent extracts.

In fruits, the cyclohexane extract significantly inhibited NO production (56.55%) at concentration as low as 5 µM. Moreover, a significant inhibition of NO production was also obtained by the dichloromethane (64.35%) and aqueous (61.35%) extracts at 10 µM and by the ethyl acetate (68.65%) and methanol (76.90%) extracts at 20 µM. A greater inhibition of NO production was obtained at 40 µM for the ethyl acetate extract (95.85%) and at 160 µM for the methanol (97.45%) and cyclohexane (85.55%) extracts. The IC_{50} were very low in the aqueous and cyclohexane extracts having 3 and 4.5 µg/mL, respectively. They were followed by the dichloromethane (IC_{50} = 7.5 µg/mL), the methanol (IC_{50} = 11 µg/mL) and the ethyl acetate (IC_{50} = 15 µg/mL) extracts.

In leaves, a significant inhibition was determined at the low concentration 1.25 µM for ethyl acetate extract (62.80%). The cyclohexane and dichloromethane extracts significantly inhibited NO production (55.75% and 72.25%, respectively) at 2.5 µM. A maximum of NO production (100%) was obtained at 20 µM for the ethyl acetate extract, at 40 µM for the dichloromethane extract, at 80 µM for the cyclohexane and water extracts and at 160 µM for the methanol extract. The ethyl acetate extract had the highest anti-inflammatory activity with IC_{50} = 1 µg/mL, followed by the cyclohexane (IC_{50} = 2 µg/mL), the dichloromethane (IC_{50} = 2.50 µg/mL), the methanol (IC_{50} = 3.75 µg/mL) and the aqueous (IC_{50} = 15 µg/mL) extracts.

In stems, only the cyclohexane and dichloromethane extracts significantly inhibited the NO production (77.05% at 10µM and 54.80 at 80µM, respectively). A maximum of NO production was observed at 20µM for the cyclohexane extract (97.35%) and at 160µM for the dichloromethane extract (89.55%). The IC_{50} of the cyclohexane and dichloromethane extracts were 5.50 and 70µg/mL, respectively.

DISCUSSION

In this study, the antioxidant, anticancer and anti-inflammatory activities of squirting cucumber (*Ecballium elaterium* L.) parts (flowers, fruits, leaves and stems) were investigated using different solvent extracts (cyclohexane, dichloromethane, ethyl acetate, methanol and water). The methanol solvent gave the highest extraction yield from *E. elaterium* leaves (16%), stems (13.90%) and fruits (10.60%). However, the highest extraction yield was obtained by water for *E. elaterium* flowers (9.70%). Our results confirmed the effect of solvent extraction and the plant organ on the extraction yields accordingly to Felhi *et al.,* (2017). These authors also found that the highest extraction yield of *E. elaterium* fruit peels was obtained by methanol (11%) as compared to diethyl ether (1%) and acetone (1%) extracts. This difference could be explained by the higher solubility of extractable bioactive components in methanol than the other solvents as reported by Felhi *et al.,* (2016). So, the variation in the extraction yields could be attributed to the difference in solvent polarities which plays a key role in increasing the solubility of phytochemical compounds Mejri *et al.,* (2022).

Depending on both plant organ and solvent extraction, different results were obtained by Abbassi *et al.,* (2014) who determined TPC, TFC and TTC of the hydromethanol extracts from squirting cucumber leaves (46.84mg GAE/g, 17.38mg CE/g and 3.54mg GAE/g, respectively), fruits (43.61mg GAE/g, 9.91mg CE/g and 4.29mg GAE/g, respectively), flowers (11,56mg GAE/g, 2.52mg CE/g and 3.09mg GAE/g, respectively) and stems (6.74mg GAE/g, 4.42mg CE/g and 2.09mg GAE/g, respectively). These results were similar to those of El-Haci and Bekkara (2011) concerning TPC and TFC of the hydromethanol extracts of leaves (48.22mg GAE/g and 45.43mg CE/g, respectively) and stems (10.71mg GAE/g, 5.45mg CE/g, respectively). Felhi *et al.,* (2016) reported that TPC and TFC of the methanol extract from fruit peels were 107mg GAE/g and 18mg QE/g, respectively. Hamidi *et al.,* (2020) noted that the methanol extract of squirting cucumber leaves had higher TPC (39.97mg GAE/g) than fruits (30.90mg GAE/g) and only leaves had TFC (49.17mg QE/g). Felhi *et al.,* (2016) found that TPC and TFC of Tunisian squirting cucumber fruit juice depended on the collected area, with 106.4mg GAE/g and 6.5mg QE/g, respectively for Cap-Bon region, 86.2mg GAE/g and 3.9mg QE/g, respectively for Sidi Bouzid

region and 78.7mg GAE/g and 0.6mg QE/g, respectively for Kef region. So, several factors could be influenced the content of these components as biotic (species, organ and physiological stage) and abiotic (environmental, handling and solvent extraction) factors.

The antioxidant activities of squirting cucumber flowers, fruits, leaves and stems using different extraction solvents were evaluated *in vitro* by three techniques, namely, TAC, DPPH and reducing power assays. Results showed that the solvent type and plant part significantly affected the antioxidant activities of *E. elaterium* extracts. Our results were higher than those of Hamidi *et al.,* (2020) in the case of the methanol extracts of *E. elaterium* leaves (IC_{50}=1150µg/mL) and fruits (IC_{50}=1180µg/mL). Felhi *et al.,* (2017), also found that DPPH activity of fruit peel extract had an IC_{50} = 1200 µg/mL in methanol, 1500 µg/mL in acetone and 2300 µg/mL in diethyl ether. However, the higher results of DPPH activity were obtained by Bourebaba *et al.,* (2020) in the case of the ethanol extracts of squirting cucumber flowers (IC_{50} = 46.01µg/mL), leaves (IC_{50}=61.78µg/mL) and fruits (IC_{50}=226.08µg/mL). Felhi *et al.,* (2017) reported that the best reducing power activity of fruit peel extract was determined in methanol (EC_{50}=1040µg/mL), followed by acetone (EC_{50}=1520µg/mL). The efficiency of methanol was related to its intermediate polarity, which allows it to solvate low molecular weight organic compounds possessing protonatable functional groups such as CDDH and DH (Arakaki *et al.,* 2016). El-Haci and Bekkara (2011) found that the reducing power of the ethyl acetate extract from *E. elaterium* leaves presented the most ability to reduce the iron (EC_{50}=39.60µg/mL). It can be deduced that the antioxidant activity of the different extracts was strongly dependent on the extraction solvent.The selection of solvent extraction is a delicate step for the assessment of antioxidant activity of extracts as the solvent polarity determines the recovery of natural antioxidants from herbals (Clericuzio *et al.,* 2004). In our study, a good correlation was found between TPC of the different extracts and DPPH assay of leaves (r = 0.908) and stems (r = 0.703). Reducing power assay was significantly correlated with TPC of flowers (r = 0.731), fruits (r = -0.632) and leaves (r = -0.841) while with TTC for stems (r = -0.819). TAC was significantly correlated with TTC and CTC in all different part extracts (r > 0.900). Additionally, the extraction yield of all solvent part extracts was significantly correlated with TPC (r = 0.533) and TFC (r = 0.715). So, these results suggested that the antioxidant activity of squirting cucumber extracts might be exerted by polyphenols, flavonoids and tannins which acted as good electron donors and could terminate the radical chain reaction by converting free radicals to more stable products.

The anticancer or the cytotoxicity effects of squirting cucumber parts were evaluated on human cancer (A549 and DLD-1) and normal fibroblast (WS-1) cell lines. The different squirting cucumber parts had an appreciable anti-tumour activity in the less polar extracts contrarily to the antioxidant activity. In addition, the anticancer activity of different squirting cucumber parts was not significantly correlated with TPC, TFC, TTC and CTC. So, the phytochemical characterization of the different solvent extracts of *E. elaterium* parts showed the presence of other bioactive constituents, namely alkaloids, terpenoids and saponins. *E. elaterium* was found to be a powerful antitumor agent owing to the presence of triterpenoids, especially cucurbitacins (Duangmano *et al.,* 2012). Jafargholizadeh *et al.,* (2016) isolated cucurbitacins D, E and I from chloroform and ethyl acetate fractions of a methanolic extract of squirting cucumber fruits and they assessed their cytotoxic effects on the AGS cell line by MTT assay. These authors mentioned that squirting cucumber fruit may have some cytotoxic effects on gastric cancer cells due to its cucurbitacins with IC_{50}=0.3µg/mL for cucurbitacin D, 0.1µg/ml for cucurbitacin E and 0.5µg/ml for cucurbitacin I. Yıilmaz *et al.,* (2018) reported the potent cytotoxic effects of the fruit juice, chloroform extract from squirting cucumber and Curcubitacin I against breast cancer MCF-7 and MDA-MB-231 cell lines. Molavi *et al.,* (2020) had determined the antiproleferative activities of different solvents extracts (dichloromethane, *n*-hexane and methanol) from *E. elaterium* aerial parts and rhizomes against MCF-7 (human breast adenocarcinoma), Hep G2 (liver adenocarcinoma), SW480 (Colon adenocarcinoma) as cancerous and HFFF2 (human fibroblast cell line) as normal cell lines. They found that only *n*-hexane extract of *E. elaterium* aerial parts showed a moderate anticancer activity against MCF7 cell line with IC_{50} = 264.3µg/mL. Additionally, Touihri *et al.,* (2015) found that the seed oil of *E. elaterium*, isolated by n-hexane Soxhlet extraction at 80°C, had antiproliferative effects on colon cancer HT29 (IC_{50}=4.86µg/mL) and adenosarcoma HT1080 (IC_{50} = 4.16µg/mL) cell lines. The ethyl acetate extract of whole *E. elaterium* was found to inhibit cell proliferation of hepatocarcinoma cell line HepG2 with IC_{50}=19.12µg/mL (El-Sayed *et al.,* 2012). Hamidi *et al.,* (2020) reported that the methanol extracts of both leaves and fruits showed a moderate cytotoxic activity on human breast cancer MDA-MB-468 cell lines (IC_{50}=264 and 50µg/mL, respectively). However, these extracts did not show a significant cytotoxicity on Human poorly differentiated gastric cancer MKN-45 and human dermal fibroblast MCF7 cells. Bohlooli *et al.,* (2012) showed that the freeze-dried extract of *E. elaterium* fruit exerted cytotoxic effects on gastric adenocarcinoma cell line AGS and esophageal squamous cell carcinoma cell line KYSE30 by inducing apoptosis. From these results, it was obvious that *E. elaterium* organ extracts have strongly inhibited the proliferation of the cancer cells with lowest IC_{50} values. High selectivity in cytotoxic response between cancer and normal cell lines enhanced prospect of these organ extracts to contain important compound which could serve as a new anticancer drug.

Table 1: Total phenolic, total flavonoid, total tannin and condensed tannin contents of squirting cucumber (*Ecballium elaterium* L.) parts using different solvents

Plant part	Solvent	TPC (mg GAE/g DE)	TFC (mg QE/g DE)	TTC (mg GAE/g DE)	CTC (mg CE/g DE)
Flowers	Cyclohexane	-	-	-	-
	Diclorometh ane	2.29 ± 0.28^{c}	19.91 ± 0.50^{a}	5.93 ± 0.49^{a}	4.82 ± 0.08^{a}
	Ethyl acetate	5.92 ± 0.35^{b}	16.57 ± 1.56^{b}	2.83 ± 0.05^{d}	1.8 ± 1.11^{d}
	Methanol	2.41 ± 0.25^{c}	7.63 ± 0.69^{c}	3.92 ± 0.57^{b}	4.25 ± 1.21^{b}
	water	9.70 ± 0.35^{a}	4.58 ± 0.14^{d}	3.01 ± 0.11^{c}	2.25 ± 0.37^{c}
Fruits	Cyclohexane	-	-	0.14 ± 0.05^{d}	1.26 ± 0.01^{e}
	Diclorometh ane	3.07 ± 040^{c}	8.46 ± 0.01^{b}	2.11 ± 0.03^{c}	1.87 ± 0.02^{d}
	Ethyl acetate	3.29 ± 0.10^{b}	2.11 ± 0.92^{c}	2.51 ± 0.97^{b}	1.99 ± 0.05^{c}
	Methanol	52.60 ± 1.55^{a}	96.25 ± 4.83^{a}	3.87 ± 0.05^{a}	2.38 ± 0.07^{b}
	Water	2.06 ± 0.01^{d}	-	0.55 ± 0.08^{d}	2.71 ± 0.47^{a}
Leaves	Cyclohexane	3.38 ± 0.50^{d}	4.68 ± 0.18^{e}	5.77 ± 0.55^{c}	2.82 ± 0.58^{c}
	Diclorometh ane	0.62 ± 0.10^{e}	29.89 ± 4.43^{c}	7.80 ± 0.93^{b}	4.87 ± 0.28^{b}
	Ethyl acetate	3.58 ± 0.02^{c}	34.98 ± 3.44^{b}	10.19 ± 0.14^{a}	4.73 ± 0.46^{a}
	Methanol	5.85 ± 0.05^{a}	78.73 ± 3.09^{a}	0.54 ± 0.10^{e}	1.94 ± 0.37^{d}
	water	5.01 ± 0.57^{b}	20.81 ± 0.07^{d}	1.68 ± 0.17^{d}	1.42 ± 0.48^{e}
Stems	Cyclohexane	0.07 ± 0.01^{e}	4.33 ± 3.59^{d}	5.41 ± 0.02^{d}	4.45 ± 1.32^{a}
	Diclorometh ane	1.14 ± 0.32^{d}	3.61 ± 2.53^{c}	6.33 ± 0.95^{c}	3.63 ± 1.22^{c}
	Ethyl acetate	2.38 ± 0.33^{b}	3.61 ± 2.53^{c}	6.57 ± 0.05^{b}	3.09 ± 0.73^{d}
	Methanol	2.51 ± 0.23^{a}	8.54 ± 0.28^{b}	7.77 ± 0.29^{a}	4.11 ± 0.33^{b}
	water	1.49 ± 0.13^{c}	12.55 ± 0.04^{a}	0.50 ± 0.04^{e}	1.13 ± 0.94^{e}

TPC. total phenol content; TFC. total flavonoid content; TTC. total tannins content; CTC. total-condensed tannins. GAE. gallic acid equivalents; QE. quercetin equivalents; CE. catechin equivalents; DE. Dry extract. Values represented in the results were means ± SD (n = 9). Superscript letters (a-e) within the same column indicated significantly differences ($P < 0.05$) according to the Tukey HSD test.

Table 2: *In vitro* antioxidant activities of squirting cucumber (*Ecballium elaterium* L.) parts using different solvent extracts

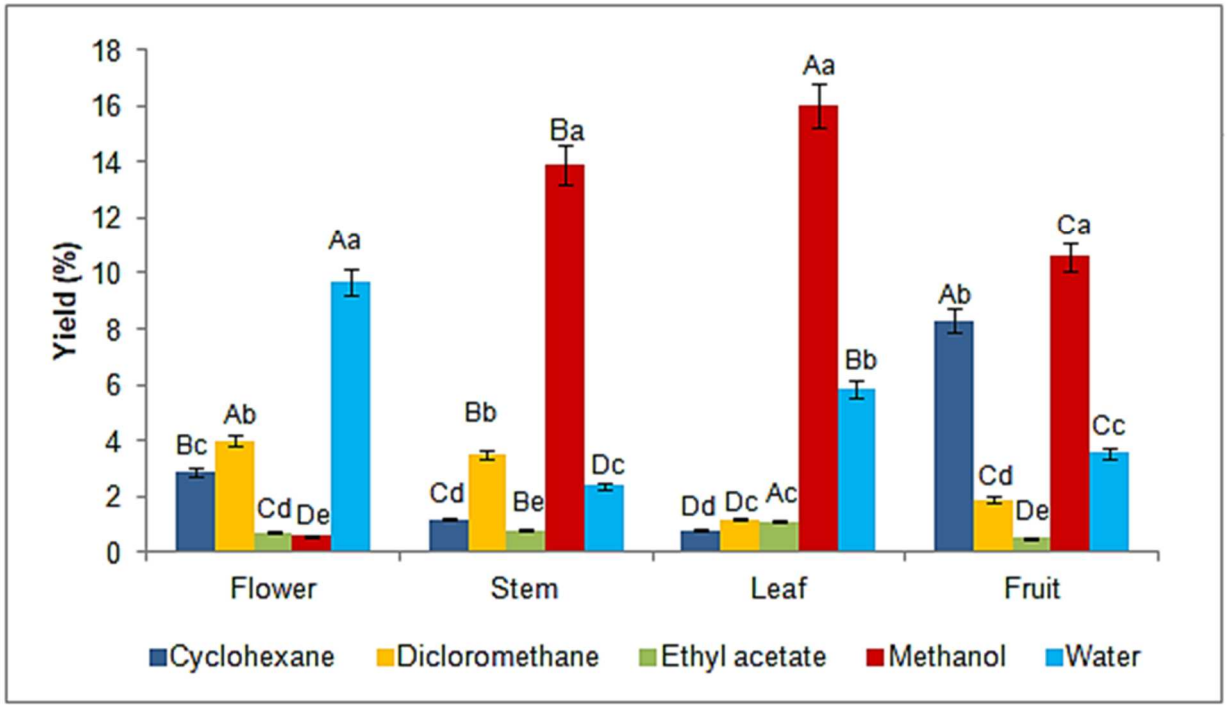
Plant part	Solvent	TAC (mg GAE/g)	DPPH IC_{50} (µg/mL)	Reducing power EC_{50} (µg/mL)
Flowers	Cyclohexane	-	-	-
	Diclorometh ane	10.12 ± 0.04^{a}	-	63.33 ± 1.53^{b}
	Ethyl acetate	1.69 ± 0.37^{d}	223.33 ± 5.77^{c}	6.73 ± 0.27^{d}
	Methanol	5.73 ± 0.42^{c}	896.66 ± 5.77^{a}	128.5 ± 1.25^{a}
	Water	6.00 ± 0.35^{b}	388.00 ± 2.64^{b}	13.23 ± 1.66^{c}
Fruits	Cyclohexane	2.30 ± 0.02^{e}	-	149.33 ± 9.05^{b}
	Diclorometh ane	7.49 ± 0.08^{b}	-	51.66 ± 0.69^{c}
	Ethyl acetate	4.41 ± 0.46^{d}	313.36 ± 12.09^{b}	44.33 ± 1.83^{d}
	Methanol	19.90 ± 1.14^{a}	155 ± 18.02^{c}	11.13 ± 1.00^{e}
	Water	5.51 ± 0.01^{c}	366.66 ± 23.05^{a}	164 ± 4.16^{a}
Leaves	Cyclohexane	14.08 ± 0.17^{b}	-	-
	Diclorometh ane	0.97 ± 0.01	-	535.00 ± 8.61^{a}
	Ethyl acetate	10.82 ± 1.24^{c}	-	34.33 ± 0.19^{b}
	Methanol	20.97 ± 0.29^{a}	506.66 ± 30.55^{a}	29.33 ± 3.15^{d}
	Water	4.35 ± 0.69^{e}	-	32.83 ± 0.48^{c}
Stems	Cyclohexane	9.24 ± 0.43^{b}	-	-
	Diclorometh ane	2.44 ± 0.01^{e}	-	180.33 ± 0.5^{b}
	Ethyl acetate	6.97 ± 0.09^{c}	196.33 ± 5.50^{c}	106.66 ± 0.38^{c}
	Methanol	11.87 ± 0.25^{a}	766.66 ± 11.54^{a}	-
	Water	3.18 ± 0.24^{d}	288.13 ± 2.11^{b}	203.33 ± 3.84^{a}
Positive control				
BHT		-	17.34 ± 0.22	-
Ascorbic acid		-	-	40 ± 0.13

Values represented in the results were means ± SD (n = 9). Superscript letters (a-e) within the same column indicated significantly differences ($P < 0.05$) according to the Tukey HSD test.

Table 3: *In vitro* anti-cancer activity of squirting cucumber (*Ecballium elaterium* L.) parts using different solvent extracts

Plant part	Solvent	A549 IC_{50} (µg/mL)	DLD-1 IC_{50} (µg/mL)	WS-1 IC_{50} (µg/mL)
Flowers	Cyclohexane	>200^{a}	>200^{a}	>200^{a}
	Dicloromethane	3.39 ± 0.68^{c}	>200^{a}	123 ± 09^{b}
	Ethyl acetate	4.20 ± 0.40^{c}	17 ± 2^{b}	117 ± 12^{a}
	Methanol	97.13 ± 3.24^{b}	97 ± 3.68^{b}	>200^{a}
	Water	>200^{a}	>200^{a}	>200^{a}
Fruits	Cyclohexane	8.6 ± 0.70^{b}	6 ± 2^{c}	11.3 ± 0.80^{a}
	Dicloromethane	3.7 ± 0.30c	12 ± 9^{b}	16 ± 2^{a}
	Ethyl acetate	13 ± 1^{b}	27 ± 4^{b}	31 ± 3^{b}
	Methanol	>200^{a}	>200^{a}	>200^{a}
	Water	145 ± 51^{b}	>200^{a}	>200^{a}
Leaves	Cyclohexane	>200^{a}	14 ± 6^{b}	>200^{a}
	Dicloromethane	96 ± 0.22^{b}	3 ± 0.2^{c}	123 ± 4^{a}
	Ethyl acetate	>200^{a}	>200^{a}	>200^{a}
	Methanol	>200^{a}	>200^{a}	>200^{a}
	Water	>200^{a}	>200^{a}	>200^{a}
Stems	Cyclohexane	113.3 ± 75^{c}	41 ± 8^{c}	137 ± 21^{b}
	Dicloromethane	24 ± 3.7^{b}	4 ± 2^{c}	>200^{a}
	Ethyl acetate	142 ± 56^{b}	130 ± 26^{c}	>200^{a}
	Methanol	>200^{a}	>200^{a}	>200^{a}
	Water	170 ± 51^{a}	67 ± 9^{b}	37 ± 42^{c}
Positive control				
Betulinol		8.2 ± 0.7^{b}	25 ± 2^{a}	8.9 ± 0.9^{b}
Etoposide		4.8 ± 0.6^{c}	6.20 ± 1^{b}	8.4 ± 0.8^{a}

The data shown was presented as mean of three replicates (n = 3) ± standard deviation (SD). A549: Human lung carcinoma. DLD-1: Human colorectal adenocarcinoma. WS-1: Human normal skin fibroblasts. Superscript letters (a-c) within the same column for each assay indicated significantly differences ($P < 0.05$) according to the Tukey HSD test.



Values represented in the results were means ± SD (n = 9). Superscript letters (a-e) within the same histogram indicated significantly differences ($P < 0.05$) according to the Tukey HSD test.

Fig. 1: Extraction yields from *Ecballium elaterium* fruits, flowers, leaves and stems using different solvents.

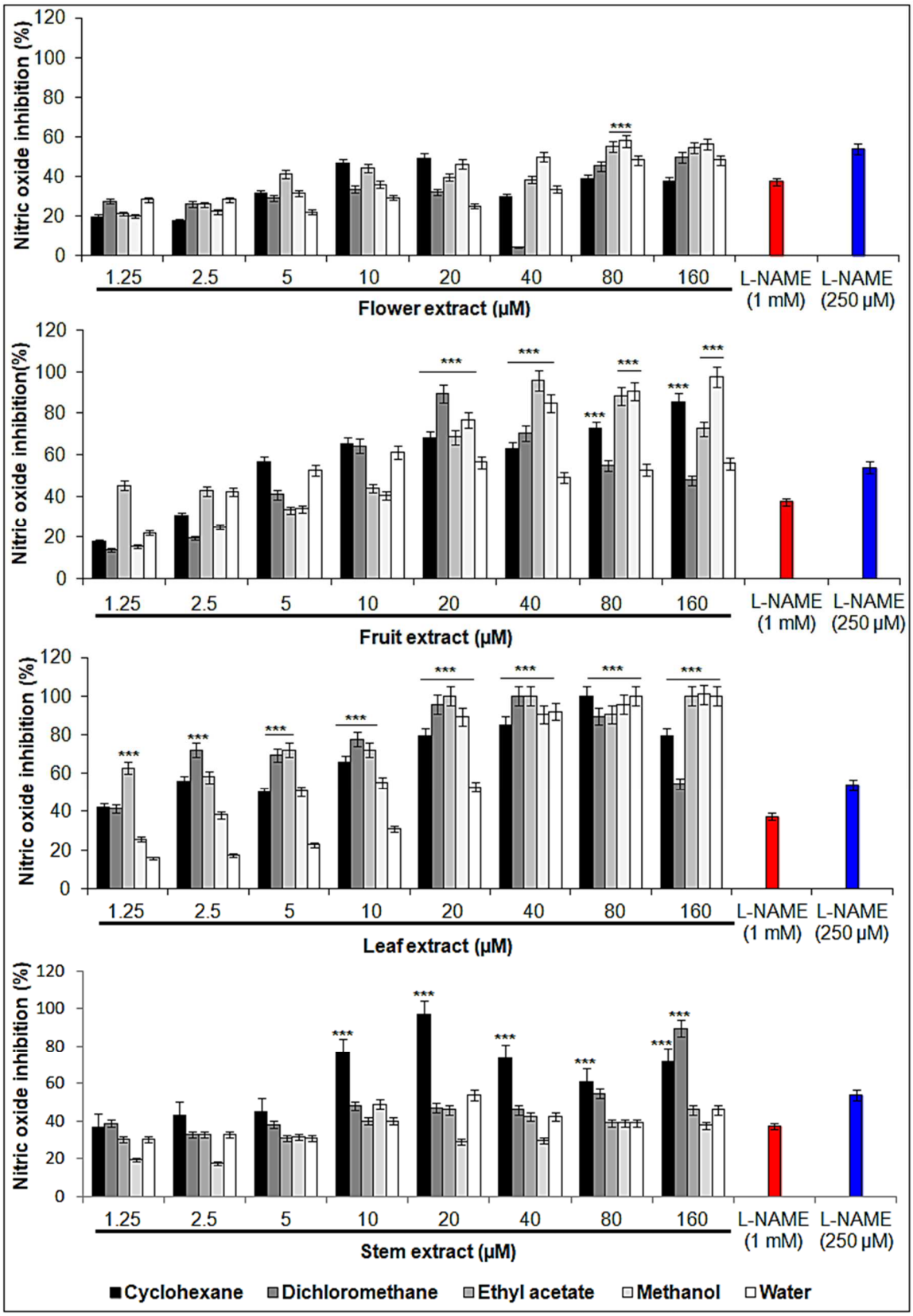


Fig. 2: Effect of cyclohexane, dichloromethane, ethyl acetate, methanol and water extracts from different *Ecballium elaterium* parts at various concentrations on the overproduction of NO by LPS-stimulated RAW 264.7 macrophages. *** $p < 0.001$, *$p < 0.1$.

The nitric oxide (NO) production was used as tools for measurement of the inflammation; reduction of NO production has been characterized as an effective strategy for the discovery of anti-inflammatory agents. Results showed that squirting cucumber flowers had an appreciable anti-inflammatory activity in the polar extracts with the highest NO inhibition in the ethyl acetate and methanol extracts. The anti-inflammatory activity of squirting cucumber flowers was significantly correlated with TFC and reducing power activity explicating that the inhibition of NO production in macrophages could be mainly due to the presence of flavonoids in flowers. However, the anti-inflammatory activity of squirting cucumber stems had an affinity for the apolar extracts with the highest NO inhibition in the cyclohexane and the dichloromethane extracts. The anti-inflammatory activity of squirting cucumber stems was significantly correlated with the anticancer activity (r = -0.554). Similarly to the anticancer activity, the anti-inflammatory activity of squirting cucumber stems could be generally due to the presence of alkaloids, triterpenoids and saponins. Squirting cucumber leaves and fruits had an important anti-inflammatory activity in both polar (particularly ethyl acetate for leaves and water for fruits) and apolar (cyclohexane for these two organs) extracts. The anti-inflammatory activity of squirting cucumber leaves and fruits was significantly correlated with TTC (r = -0.817 and 0.885, respectively), reducing activity (r = -0.508 and -0.832, respectively) and anticancer activity (r = 0.528 and 0.511, respectively). In fact, the inhibition of NO production in macrophages could be not only related to the presence of phenols (especially tannin compounds) but also to the other phytochemical compounds like alkaloids, terpenoids and saponins. Our study is the first report on the *in vitro* anti-inflammatory activity of different solvent extracts from *E. elaterum* parts through the NO production inhibitory in LPS-stimulated RAW 264.7 macrophages. Bourebaba *et al.,* (2020) studied the *in vivo* and *in vitro* anti-inflammatory activity of the ethanol extracts from squirting cucumber flowers, fruits and leaves. They found that all extracts were active by inhibiting heat induced protein denaturation in a non-dose dependent manner, protection against hypotonic hemolysis and reduction of mice hind paw edema, 3 h after carrageenan injection with the dominance of fruit extract at 100mg/kg dose (59.49% of edema reduction) and flower extract at 200mg/kg dose (82.93% of edema reduction). Felhi *et al.,* (2017) also investigated the *in vivo* anti-inflammatory effect of the methanol extracts from squirting cucumber fruits by carrageenan induced rat paw edema assay in mice and the results revealed that a dose of 75mg/kg induced a significant inhibition of 66.4% at 2h. These authors noted that this activity was correlated to the richness of the methanol fruit extract in flavonoids. Bourebaba *et al.,* (2020) detected kaempferol-3-O-rutinoside in the ethanol extracts of squirting cucumber fruits and flowers and this compound had been reported as suppressor of carrageenan-induced mouse paw edema (Wang *et al*., 2014). This effect was also described for rutin (Selloum *et al.,* 2003) which was detected in the methanolic extracts of squirting cucumber leaves, flowers and fruits (Jaradat *et al.,* 2012). Additionally, the same activity was observed for isorhamnetin-3-O-rutinoside (Ahmed *et al.,* 2005) detected in the ethanol extracts of squirting cucumber flowers by Bourebaba *et al.,* (2020). This anti-inflammatory activity cannot be attributed only to flavonoids but to the combination of these compounds with others as alkaloids, triterpenoids and saponins present in the extract. In this way, it was also reported that squirting cucumber extracts had an anti-inflammatory activity induced by cucurbitacins. So, Bourebaba *et al.,* (2020) reported that the ethanol extract of *E. elaterium* fruits contained two cucurbitacins D and P, unlike to flower and leaf extracts having only cucurbitacin D. Greige-Gerges *et al.,* (2007) mentioned the presence of cucurbitacins B, D, E and I in the methanolic extract of *E. elaterium* fruit juice.

CONCLUSION

In summary, these results demonstrated that the solvent used in extraction affected the extract yields and the contents of total phenols, total flavonoids, total tannins, condensed tannins, alkaloids and saponins as well as the antioxidant, anticancer and anti-inflammatory activities of different squirting cucumber parts. Independent of plant part and solvent extract, squirting cucumber exhibited a potent antioxidant, antiproliferative and anti-inflammatory activities justifying its usage in traditional medicine due to its richness in phytochemical constituents and it could be a good candidate for pharmaceutical plant based products. Further studies on bioactive compounds identification and isolation need to be conducted to explore the phytochemistry and mechanisms of action of pharmacological properties of different squirting cucumber parts.

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