IRSTI 31.23.39; 34.39.49 https://doi.org/10.26577/ijbch.2022.v15.i2.08

Sami A. Al-Robai[1*], Abdelazim A. Ahmed[1,2], Aimun A.E. Ahmed[1,3], Sami A. Zabin[1], Haidar A. Mohamed[1,4], Abdullah A. A. Alghamdi[1]

[1]Albaha University, Al-Baha, Saudi Arabia
[2]University of Khartoum, Sudan
[3]Omdurman Islamic University, Khartoum, Sudan
[4]Medicinal and Aromatic Plants Research Institute, National Center for research, Sudan
*e-mail: dr.alrobai@gmail.com

*(Received 22 August 2022; received in revised form 29 August 2022; accepted 2 September 2022)*

# Phenols, antioxidant and anticancer properties of three medicinal plants: *in vitro* and *in silico* evaluation

**Abstract.** Recently, antioxidant and anticancer properties of phenolic compounds have been an area of growing interest. This work aimed at assessing the phenolic content and selected bioactivity of three medicinal plants. Standard spectrophotometric, chromatographic and biological methods were adopted. *In silico* screening for the phenolic acids as cytoxic agents for cancer was performed. *Euphorbia granulata* showed significantly-high amount of polyphenols. The highest level of flavonoid and tannin contents was detected in *Galinsoga parviflora*. Ten phenolic acids were identified and quantified via GC-MS in all extracts. p-Hydroxybenzoic was the most dominant acid in *Tagetes minuta* while gallic was the predominant acid in *E. granulata*. Studied extracts showed higher reactive oxygen and nitrogen species scavenging activities and exhibited lower hydrogen peroxide inhibition value. *T. minuta* exhibited strong activity on A2780 cell line. The cytotoxicity of *G. parviflora* was highly significant against all cancer cells. *E. granulata* proved to be most active towards MCF7 and A2780 cell lines. *In silico* data revealed that caffeic acid had a high cytotoxicity. Studied crude extracts showed promising antioxidant and anticancer activities which could be due to the different phenolic contents in the studied plants.

**Key words:** phenols, antioxidant, anticancer, medicinal plants.

## Introduction

Nowadays, medicinal plants and their derived bioactive components have gained much attention in non-clinical and clinical researches [1].The health benefit and biological activity of natural phenols from plants have been reported by several works [2,3]. In Saudi Arabia, medicinal plants have been used in traditional medicine since ancient times and local people still use them to cure many diseases [4]. According to Alqahtani eta al. [5] and Abdel-Sattar et al. [6] more than half of the flowering plants in the Kingdom are used in folk medicine or have healing properties. Aati et al. [7] have reported that there are 471 out of 2253 plant species in the flora of Saudi Arabia are traditionally used for medicinal purposes. Al Baha province, southwestern of Saudi Arabia is characterized by a variety of habitats (mountains, escarpments valleys, coastal plain, sand dunes). This geographic diversity has provided an ideal atmosphere for the growth of various medicinal herbs in this region.

In recent years, *T. minuta* is gaining importance in scientific research because of its richness in chemical components that have bioactive and therapeutic properties towards plant, animal and human pathogens [8]. In previous works, many flavonoid compounds (quercetagetin, quercetagetin-7-arabinosyl-galactoside, quercetagetin-3-arabinosyl-galactoside, quercetagetin-7- glucoside, patuletin, patuletin-7-glucoside and isorhamnetin) were detected in leaves extract of *T. minuta*. Quercetagetin-7-arabinosyl-galactoside, which is the dominant compound in the leaves extract, had antimicrobial activity towards some pathogenic microbes [9]. Moreover, the essential oil obtained from leaves showed high antifungal activity towards *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* [10]. The potentiality of *T. minuta* was reported as a good source of antioxidant [11-13] and anticancer [14-16] agents.

*E. granulata* has many ethnobotanicals uses such as controlling human immunodeficiency virus (HIV-1) [17], expelling intestinal worms and having diuretic activity [18] as well as antimicrobial properties [19]. Ghauri et al. [20] have recently demonstrated the analgesic and pyretic activities of the aqueous methanolic extract of *E. granulate* in animal model.

Crude extracts and the pure isolated compounds from *G. parviflora* have been reported as potent cytotoxic, antimicrobial, and antioxidant agents [21]. The chemical constituents of *G. parviflora* are useful for treating snakebites, wounds, eczemas, lichens, flu and colds [22]. The cytotoxic activity and protective property of different extracts from *G. parviflora* on human fibroblast model have been reported by Bazylko, et al. [23].

The targeted plants *T. minuta, E. granulata*, and *G. parviflora* are growing in different places in Al Baha region, KSA and are commonly used by the local inhabitants in folk medicine. The available studies of antioxidant and anticancer properties as well as phenolic constituents of these plants are poorly described. This work aimed at evaluating the phenolic contents, antioxidant and anticancer properties of the methanolic extracts obtained from these plants in order to give scientific evidence for their uses in traditional medicine.

## Materials and methods

*Plant materials and extraction.* Three wild medicinal plants*, T. minuta* L. (Asteraceae), *G. parviflora* Cav. (Asteraceae) and *E. granulata* Forssk. (Euphorbiaceae), were collected from different localities of Al Baha region. Each plant material (both aerial and root parts) was washed by distilled water, dried under shade for 22 days, then gently ground to coarse powder using an electric mill. Plant powdered materials were defatted with petroleum ether (40 – 60 °C) for 24 h, then filtered and dried. Afterwards, each plant sample (10g) was macerated in 100 mL methanol and kept at room temperature for 72 h with shaking using an orbital shaker (150 rpm). The mixture was filtered and the obtained extract was dried using a rotary evaporator (Buchi, USA). The dried extracts were stored at 4 °C prior to analysis.

*Total polyphenol content.* The total polyphenol content in plant extract samples was evaluated spectrophotometrically according to Cliffe et al. [24] method with slight modifications. Plant aliquot of the extract solution (0.1 mL; 0.5 mg /mL) was mixed well with Folin-Ciocalteu reagent (0.5 mL) and distilled water (5 mL) and the mixture was left at 25 °C for 10 min. Then, sodium carbonate solution (1.5 mL; 25%) was added to the reaction mixture and incubated at 40 °C for 30 min. The absorbance was read at 765 nm on a spectrophotometer (PD-303UV, Apel, Saitama, Japan) against a blank contained 0.1 mL of methanol instead of 0.1 mL of plant sample in the reaction mixture. Gallic acid (0.1 – 0.5 mg/mL) was used as a reference standard and the results were expressed as milligram gallic acid equivalents per gram dry weight (mg GAE/g DW).

*Total flavonoid content.* For estimating the amount of total flavonoid in each extract, Dewanto et al. [25] assay was followed with some modifications. A mixture of plant's extract sample (0.5 mL; 0.5 mg/mL), distilled water (1 mL) and sodium nitrite solution (0.2 mL; 5%) was prepared and left to stand for 10 min at 25 °C. To the prepared mixture, 0.4 mL of aluminum chloride (10% w/v in methanol) was added and the mixture was incubated at 25 °C for 5 min. Sodium hydroxide solution (1 mL; 1M) was added, and the volume of the reaction mixture was completed to 6 mL with distilled water. The optical density was recorded at 510 nm against a blank solution (composed of 0.5 mL methanol instead of plant sample in the reaction mixture). Quercetin (0.01 – 0.05 mg/mL) was used as a reference standard and the results of the total flavonoid content were expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

*Total tannin content.* Total tannin content in each plant extract was quantified according to the method described by Bhat et al. [26] with some modifications. One mL (0.5 mg/mL) of each extract or tannic acid solution (0.1 – 0.5 mg/mL) was mixed with 3 mL of vanillin/methanol solution (4%) then concentrated hydrochloric acid (1.5 mL) was added to the mixture. After left for 30 min at room temperature, the absorbance was measured at 500 nm against methanol as a blank. The amount of tannin content was expressed as mg equivalent of tannic acid per gram dry weight of the plant sample (mg TAE/g DW).

*In vitro antioxidant assays.* The antioxidant activity of methanolic extracts from T. minuta, E. granulata and G. parviflora was determined using four different complementary assay methods as described below. Ascorbic acid as natural antioxidant and butylated hydroxytoluene (BHT) as synthetic antioxidant were used as reference standards.

*DPPH radical scavenging activity.* The radical scavenging effect of the plant extract samples against 2,2-diphenyl-1-picrylhydrazl (DPPH) free radicals was performed following Koh et al. [27] assaying method. Every plant extract (1mL) with a concentration of (0.5 mg/mL) or standard was added to 2.7 mL

of (6 x 10-5 mol/L) methanolic solution of DPPH and mixed well. After incubation at room temperature in the dark for 60 min, the absorbance was read at 517 nm. DPPH solution without plant sample or standard was used as a control and methanol as a blank. The radical scavenging activity was estimated using the % of DPPH inhibition as follow:

$$\%\ \text{DPPH radical scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

where, $A_c$ = Control absorbance and $A_s$ = Sample absorbance.

Experiment was repeated thrice and results are presented as mean with standard deviation.

*ABTS radical scavenging assay.* The radical scavenging activity of the extracts together with the standard solutions were measured by ABTS (2,2'-azinobis (3- ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay [28]. Ten mL of potassium persulfate (2.4 mM) was mixed with 10 mL of ABTS solution (7 mM) then the mixture was left to react in the dark for 12 h at room temperature. This ABTS solution was diluted with methanol to obtain an absorbance of 0.700 at 734 nm prior to assay. Plant samples or standard solutions (1 mL., 0.5 mg/mL) were added to 2.5 mL of the diluted ABTS then the reaction mixture was allowed to react for 7 min at room temperature in the dark before recording the absorbance at 734 nm. The ABTS scavenging activity of the targeted plant extracts was expressed as % of ABTS+ similar to DPPH scavenging method as follow:

$$\%\ \text{ABTS radical scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

where, $A_c$ = Control absorbance and $A_s$ = Sample absorbance.

Experiment was repeated thrice and results are presented as mean with standard deviation.

*Nitric oxide radical scavenging activity (NO).* The assay method reported by Ebrahimzadeh et al. [29] was adopted for measuring the percentage of nitric oxide radical scavenging activity in each suspension. A solution containing a mixture of 0.5 mL of plant extract or positive standard (0.5 mg/mL), 2 mL of sodium nitroprusside (10 mM) and 0.5 mL of phosphate buffer saline (50 mM, pH 7.4) was prepared then left for 2.5 h at 25 °C. Griess reagent was prepared by mixing 1.0 mL of sulfonic acid (33%) in glacial acetic acid (20%). The above prepared mixture (0.5 mL) was mixed with the Griess reagent (0.5 mL) and 1.0 mL of naphthylene diamine dichloride (0.1% w/v). After incubation at room temperature for 30 min, the absorbance was recorded at 540 nm.

$$\%\ \text{nitric oxide radical scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

where, $A_c$ = Control absorbance and $A_s$ = Sample absorbance.

Experiment was repeated three times and results are presented as mean with standard deviation.

*Hydrogen peroxide scavenging activity.* Ruch et al. [30] method was adopted for measuring the capacity of each extract to scavenge the hydrogen peroxide radical in the reaction medium. A mixed solution of 40 mM H2O2 and 50 mM phosphate buffer saline (pH 7.4) was prepared and stored in a dark brown bottle. One mL of each sample (0.5 mg/mL) was added to 0.6 mL of the above mixed solution. After 15 min at room temperature, the absorbance was recorded at 230 nm. A blank was prepared by mixing plant samples with the buffer and without hydrogen peroxide.

$$\%\ \text{H2O2 radical scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

Experiment was repeated three times and results are presented as mean with standard deviation.

*Determination of individual phenolic acids (GC-MS analysis).* Five mL of methanol was added to 0.5 g of each plant extract sample, then vortex for 1 min. Extraction was done by sonication in ultrasonic bath for 30 min. Extracts were concentrated to 1 mL under a gentle stream of high purity nitrogen (N2). For derivatization, 20 µL concentrated sample was transferred to 2 mL vial and dried under a gentle stream of high purity nitrogen then 30 µL of N, O-Bis (trimethylsilyl) trifluoroacetamide (BSTFA) was added. After incubation at 70 °C for 3 h, samples were redried under gentle stream of N2 to remove excess BSTFA then redissolved in 300 µL of hexane. Derivatized samples were injected in gas chromatography mass spectrometry (GC-MS) on an Agilent (Palo Alto, CA) 6890N gas chromatograph equipped with an Agilent HP-5MS column (30 m×0.25mm ×0.25µm film thickness) and 5973N mass selective detector. Samples were analyzed by selected-ion-monitoring (SIM) mode using electron impact and negative chemical ionization mass spectrometry. Samples were scanned for individual phenolic acids and quantized against calibration curves made from 10 individual phenolic standards.

*Determination of cytotoxicity (Cell culture).* Different cell lines were brought from the ATCC, USA: MCF-7 (human breast adenocarcinoma), A2780 (human ovary adenocarcinoma), HT-29 (human colon adenocarcinoma); and MRC-5 (Normal human foetal lung fibroblast). Cells were sub-cultured at 37°C, 5% CO2, 95% air and 100% relative humidity. RPMI-1640 medium [10% fetal bovine serum (FBS), L glutamine and 1% antibiotic-antimycotic] was used for all cells except MRC-5 cell, which was maintained in Eagle's minimum essential medium (EMEM, 10% FBS).

*Cytotoxicity assay.* The cytotoxicity of the prepared plant samples was evaluated by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay [31]. The tested cell lines (100 µL) were placed in 69-well (3×103 cells/well) plates then incubated at 37 °C overnight. Final concentrations of each extract were: 0, 0.05, 0.5, 5, 25, 50 µg/mL in media (DMSO, 0.1%). Doxorubicin (0.01 – 10 µg/mL) was used in this experiment as a reference drug. Each concentration was tested in triplicates and the experiment was repeated twice. The plates were incubated for 72 h then MTT solution (10 µL; 5 mg/mL) was added to each well. Optical density of the purple formazan A570 produced by treated cells is compared with the amount of formazan produced by untreated control cells, the strength of the drug in causing growth inhibition can be determined, thus compound concentration causing 50% inhibition (IC50) compared to control cell growth (100%) were determined. GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA was used for analysis.

*In silico molecular docking screening (Protein source as suggested targets).* The crystallographic structure of the five selected protein targets were retrieved from protein data bank as PDB format; Recombinant human placental annexin v complexed with K-201 as a calcium channel activity inhibitor [PDB code: 1HAK], with a resolution of 3.00 Å [32]. Estrogen receptor alpha ligand binding domain in complex with Lsz102 [PDB code: 6B0F], with a resolution of 2.86 Å [33]. Structure, Thermodynamics, and Kinetics of Plinabulin Binding to two Tubulin Isotypes [PDB code: 6S8L], with a resolution of 1.80 Å [34]. Crystal structure of a double-stranded DNA containing the major adduct of the anticancer drug Cisplatin [PDB code: 1AIO], with a resolution of 2.60 Å [35] and Human topoisomerase II alpha bound to DNA [PDB code: 4FM9], with a resolution: Doxorubicin of 2.90 Å [36]. They were downloaded as PDB structure.

*Ligand sources.* The selected ligands (standard drugs and the detected individual phenolic acids) were sketched using built in services in the zinc database in SDF format. The two standards Doxorubicin and Tamoxifen were collected from ZINC database with ID ZINC3918087 [37] in SDF format. Then converted to PDBQT format with three-dimensional structure using Open babel.

*Binding pocket identification and preparation.* AutoDock Tools 1.5.6 [38] were used to prepare and convert the protein to PDBQT format. All five selected protein targets were checked for missing atoms, chain breaks and water molecules. Unnecessary residues were also removed from the protein structures, only the active binding site was kept, hydrogens were added, partial charges were calculated.

*Ligands preparation.* The ligands were converted to their corresponding three-dimension structures using chemical file format converter openbabel 2.4.1 [39] (by adding "--gen3d" argument). Auto Dock Tools 1.5.6 was used to prepare and convert the molecules to PDBQT format, rotatable bonds were detected, polar hydrogens were added, non-polar hydrogens were deleted and their charges were merged with carbon atoms, hydrogen bond donors and hydrogen bond acceptors were defined for each ligand, partial charges were calculated.

*Geometry optimization and pre-docking procedure.* In order to prepare the selected compounds for docking, hydrogens and Gasteiger charges were added and all the hetero-atoms and water molecules were removed from protein structure. The protonated protein initially optimized in order to remove all the bad steric clashes using AMBER 99 force field [40], while MMFF94s force field parameters [41,42] were performed for small molecules. Ligands were optimized by minimizing energy to minimum RMS Gradient of 0.010 kcal/mol using Chem3D.

*Molecular docking approach.* AutoDock Vina 1.1.2 [43] was used to perform molecular docking simulations of the standard ligands and tested compounds that previously prepared in PDBQT format were carried out against the three-dimensional structure of all listed five proteins and binding energy was recorded as described here:

(1) The co-crystallized protein with its inhibitor compound was identified; therefore, the binding site was identified with its residues. (2) The inhibitor compound is then removed, optimized the active site pocket and re-docked again into the active site pocket to gain the same results. (3) A search space (grid box) that covers the entire binding site for each target was

set; other Vina configurations were left as default, such as following:

1HAK: (16.5992, 18.4811, 14.6966) centered (22.7988, 16.0331, -11.475); 6B0F: (15.3105, 18.2803, 20.7096) centered (-38.8628, 5.27725, -28.2858); 6S8L: (17.8539, 20.5174, 23.1907) centered (17.2629, -4.42538, 26.6303); 1DDP: (10.4729, 10.3155, 10.1) centered (-0.495224, -3.74989, 0.609371); 4FM9: (77.1944, 111.975, 70.9791) centered (38.0454, 46.9917, 16.8529). (4) Ligand interactions were computed for the X-ray co-crystallized protein to reveal the different types of interactions as a validation for the coming docking procedure. (5) A cluster of 10 conformers of the ligand were retained with highest and best score by default.

*Ligand efficacy estimation.* A set of standard ligands and the detected phenolic compounds were explored in AutoDock Tool to calculate different parameters and saved in CSV format and then evaluated regarding their efficiencies in order to see the drug candidate properties. The ΔG values for our ligands were calculated by AutoDock 1.1.4. However, the ligand efficiency (LE) value was introduced to normalize the free binding energy values [44]. Ligand efficiency indices (ΔG/NHA) were calculated based on the number of heavy atoms for our ligands using equation to calculate the LE from *in vitro* data: LEv= -p(EC50)/NHA.

*Statistical analysis.* Initially the AutoDock Tool was used to calculate the binding free energy (ΔG) for our ligands clusters *in silico*, while the GraphPad prism software was used to manipulate and compare the data using One-way ANOVA with Dunnett's PostHoc Test and Tukey's Multiple Comparison Test. Results were considered statistically significant at p-value ≤ 0.05.

## Results and discussion

*Total polyphenol, flavonoid and tannin contents.* In this investigation, the amounts of total phenol, flavonoid and tannin contents were varied among the studied plants (Fig.1). The quantity of the flavonoid compounds was significantly ($p < 0.05$) higher in *G. parviflora* extract than the other two plant extracts. The highest tannin content was observed in *G. parviflora* extract (6.07 ± 0.33 mg/g) and the least (1.98 ± 0.24 mg/g) was in the extract of E. granulate. The amount of polyphenols (33.88 ± 0.72 mg/g) in the crude methanolic extract of *E. granulata* was significantly ($p < 0.05$) higher than that in *T. minuta* (19.71± 0.51 mg/g) and *G. parviflora* (19.62 ± 1.15 mg/g). No significant differences between *T. minuta* and *G. parviflora* in their polyphenol contents were observed.

On comparing the observed results reported by Rezaei et al. [45] the polyphenol (34.17 ± 0.6 mg g−1) and flavonoid (14.86 ± 0.4 mg g−1) concentrations in *T. minuta* were higher than our obtained values. Moreover, the total phenolic content in *T. minuta* (67 ± 7 mg g−1) reported by Ranilla et al. [46] was higher than the results of this study for the same plant. The presence of flavonoids and tannins as secondary metabolites in *E. granulata* has been reported by Ali et al. [15] The richness of the genus Euphorbia in phenolic [47] and tannin [48] compounds has been revealed in previous studies. The amount of flavonoids in *G. parviflora* for this study was inconsistence with the result reported by Bazylko et al. [23] for ethanol extract (685.4 ± 69.6 mg g−1) and water extract (41.4 ± 3.5 mg g−1). The results reported by Studzińska-Sroka et al. [49] for total polyphenol (98.30 ±0.14 mg g−1) and flavonoid (6.15 ± 0.41 mg g−1) contents in *G. parviflora* were disagreed with this result. Moreover, flavonoids and other natural polyphenols have been reported in *G. parviflora* by several researchers [21,23,50].

*Individual phenolic acids.* As shown from Table 1, the GC-MS analysis revealed the presence of ten individual phenolic acids in all extracts. The quantity of vanillic acid and 3,4 dihydroxybenzoic acid was very low in all plant extracts. The highest level of the total individual phenolic acids (397µg/g) was detected in *T. minuta* extract followed by *E. granulata* (300 µg/g) and *G. parviflora* (130 µg/g). p-Hydroxybenzoic acid (70 µg/g) was the dominant phenolic acid in *T. minuta* extract. p-Coumaric acid was the second dominant phenolic acid in *T. minuta* extract. The amount of sinapic acid observed in *T. minuta* extracts (42 µg/g) was twice that of *G. parviflora* (21 µg/g). *T. minuta* and *E. granulata* extracts were contained equal amount (25µg/g) of trans-cinnamic acid. *E. granulata* was rich in both gallic acids and ferulic acids as compared with that of *G. parviflora* and *T. minuta*. The concentration of gallic and caffeic acids in *E. granulata* represented 45% of the total content of the individual phenolic acids detected in this plant. Ferulic acid (4-hydroxy-3-methoxycinnamic acid) was the second predominant phenolic acid (62 µg/g) in the extract of *E. granulata*. The amount gallic and caffeic acids present in *E. granulata* was nearly equal to one-third of the total individual phenolic acid content. *E. granulata* and *G. parviflora* contained same amounts (8 µg/g) of 3,4-dihydroxybenzoic acid and syringic acids. The studied plant extracts showed variation in their vanillic acid content.

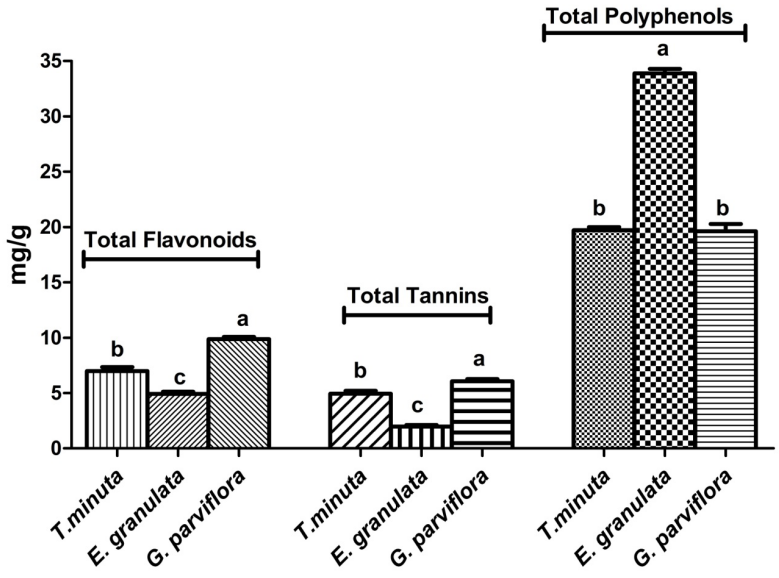


**Figure 1 –** Total polyphenol, flavonoid and tannin contents in the studied plants.
Note: Different letters in the same test are significantly different (Tukey's Multiple Comparison Test, $p \leq 0.05$)

However, phenolic extracts which contained hydroxybenzoic acid has the ability to protect Caco-2 cells against pro-oxidant induced toxicity [51]. The study carried out by Farhoosh et al. [52] has revealed the anti-DPPH radical activity of the derived compounds from p-hydroxybenzoic acid. Ester p-hydroxybenzoic acid has been widely used as an ingredient in cosmetic, food and drug products [53]. p-Coumaric acid has various pharmacological effects such as antioxidant [54,55] anticancer [56] neuroprotective [57]. Recently, the potentiality of this acid to control the inflammatory diseases through different molecular mechanisms has been stated by Ferreira et al. [58] Sinapic acid has various pharmacological and therapeutic applications such as antioxidant, anticancer and anti-inflammatory [59]. However, most of the phenolic compounds in plant tissues are derived from t-cinnamic acid [60]. Gallic acid which was the dominant phenolic acid in *E. granulata* extract possesses various industrial and biological properties [61]. It has a high antioxidant property as well as protective activity in healthy people against apoptosis [62]. The potent antioxidant activity of the gallic acid is attributed to its tendency to donate hydrogen [63]. Gallic acid has been reported as an effective compound against various diseases such as alzheimer, Parkinson [64] and cancer [65]. Ferulic acid plays an essential role for treatment of several chronic disorders such as cancer disease and has cytoprotective potential to inhibit the free radicals in living cells [66]. Ferulic acid which is found in many plants [67] has the ability to scavenging both the reactive oxygen species and nitrogen radicals [68] and has an inhibition effect on the cytotoxic enzymes [69]. Caffeic acid which was found at different concentrations in the studied plants has been reported as antioxidant ingredient [55]. The therapeutic potential of vanillic acid has been reviewed by Brimson et al. [70] who indicated that vanillic acid is a probable anti-inflammatory and anticancer candidate and also inhibit nitric oxide (NO) production. In general, the health benefits of phenolic acids and their uses in food and medicine have been recently confirmed by Yang et al. [71].

*Antioxidant activity.* The potential of the methanolic extracts of the investigated plants to scavenge the free radicals in the reaction medium was carried out using four different *in vitro* methods. Ascorbic acid and butylated hydroxytoluene (BHT) were used as reference compounds. Results, summarized in figures 2 and 3, showed that the values of antioxidant activities of the studied extracts exhibited good antioxidant activities although none of the crude extracts in this investigation had a higher activity than the used controls in each assay. It was observed that the DPPH, ABTS and NO radical scavenging activities of all crude extracts were higher than 80% and slightly less than the activity of the tested controls. The three tested plant extracts showed potent antioxidant activity when using DPPH% and ABTS% methods (Fig. 2). The data of DPPH assay indicated

that there were significant differences ($p \leq 0.05$) between the scavenging activity of the plant extracts and that of the controls. The anti-radical activities of the plant samples were significantly different when compared with ascorbic acid and BTH as controls. The tested extracts of the three plants showed statistically significant difference in their ability to scavenge the free nitric oxide radicals in the reaction medium (Fig. 3). They displayed good ability to scavenge the nitric oxide radicals in the prepared solutions. The antioxidant potential (NO) of *E. granulata* extract (82.77 ± 0.30%) was nearly equal that of BHT (83.14 ± 0.28%) and lower than ascorbic acid (87.76 ± 1.10%) as controls. The potentiality of the three crude extracts to inhibit or quench the hydrogen peroxide radicals in the prepared suspensions was very weak (1.73 ± 0.27 – 6.62 ± 0.44%) as compared with the control (16.27 ± 0.60%). *E. granulata* extract exhibited more DPPH (87.70 ± 0.75%) and ABTS (87.65 ± 0.49%) inhibition as compared with the *T. minuta* (85.41 ± 1.55%) and *G. parviflora* (83.22 ± 0.34%) extracts.

**Table 1** – Individual phenolic acids (μg/g) in the studied herb plants as analysed by GC-MS

| Compound | *T. minuta* | *E. granulate* | *G. parviflora* |
|---|---|---|---|
| t-Cinnamic acid | 25 | 25 | 9 |
| p-Hydroxybenzoic acid | 70 | 43 | 15 |
| Vanillic acid | 20 | 8 | 10 |
| 3,4-Dihydroxybenzoic acid | 19 | 8 | 8 |
| Syringic acid | 35 | 8 | 8 |
| p-Coumaric acid | 57 | 24 | 13 |
| Gallic acid | 44 | 73 | 12 |
| Ferulic acid | 40 | 62 | 14 |
| Caffeic acid | 45 | 25 | 20 |
| Sinapic acid | 42 | 24 | 21 |
| Total | 397 | 300 | 130 |

In previous studies, antioxidant activity was revealed in extracts from some species belong to the genus Euphorbia such as *E. hirta*, *E. retusa* and *E. gaditana* [72]. Mostafa et al. [73] had reported that at concentration of 150 mg/ml, the ethyl acetate fraction from *G. parviflora* demonstrated strong antioxidant activity. The ability of extracts from *G. parviflora* to scavenging DPPH free radicals was reported by Bazylko et al. [74]. A previous study showed that both aqueous and ethanol extracts from *G. parviflora* have the ability to scavenge the generated reactive oxygen species (superoxide anion and hydrogen peroxide) in cell-free systems [23]. The antioxidant property of essential oils obtained from *T. minuta* was reported by Osée Muyima et al. [11] and de Oliveira et al. [13]. The studies carried out by Shirazi et al. [16] and Igwaran et al. [12] revealed that *T. minuta* could be a useful source of antioxidant agents. Thiophene and flavonoid compounds which were isolated from the methanolic extract of *T. minuta* had showed potent antioxidant activity when compared with propyl gallate as a standard [75]. The obtained results revealed that the investigated plants were rich in polyphenols, flavonoids and tannins molecules, which had antiradical activity. The presences of phenolic, flavonoid and tannin compounds in the tested plants could be linked with the antioxidant properties of these extracts. This is consistent with the several recent studies, which suggested that the antioxidant properties could be referred to phenolic compounds present in plants [72,76]. Among the reported secondary metabolites in plants, phenolic components had been recognized as excellent substances for reducing the oxidative damage caused by the free radical in living cells [77]. However, the direct relationship between the phenolic and flavonoid contents in different medicinal plants and antioxidant activity has been revealed by El-Zayat et al. [78]. The potentiality of the medicinal plants as a source of therapeutic agents is often related to their antioxidant capacity, that depend on to the amount of the phenol and flavonoid components present in these plants [79].

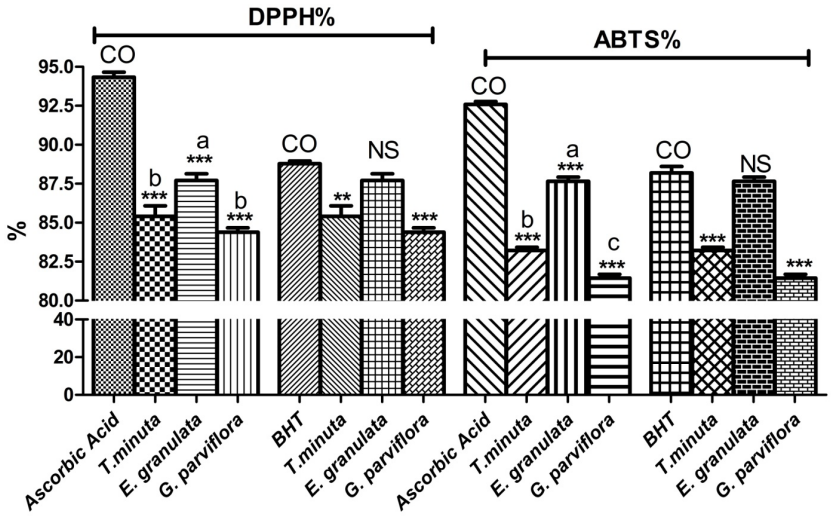


**Figure 2 –** Antioxidant activity of plant extracts evaluated by DPPH and ABTS assays.
Note: The results are presented as mean with standard deviation (±); n = 3, sample values were compared with the control value using one way ANOVA+ Post Hoc test, where p ≤ 0.05 (*), p ≤ 0.01 (**), p ≤ 0.001 (***), Not significant (NS), Control (CO). Different letters in the same lot are significantly different (Tukey's Multiple Comparison Test, p ≤ 0.05)

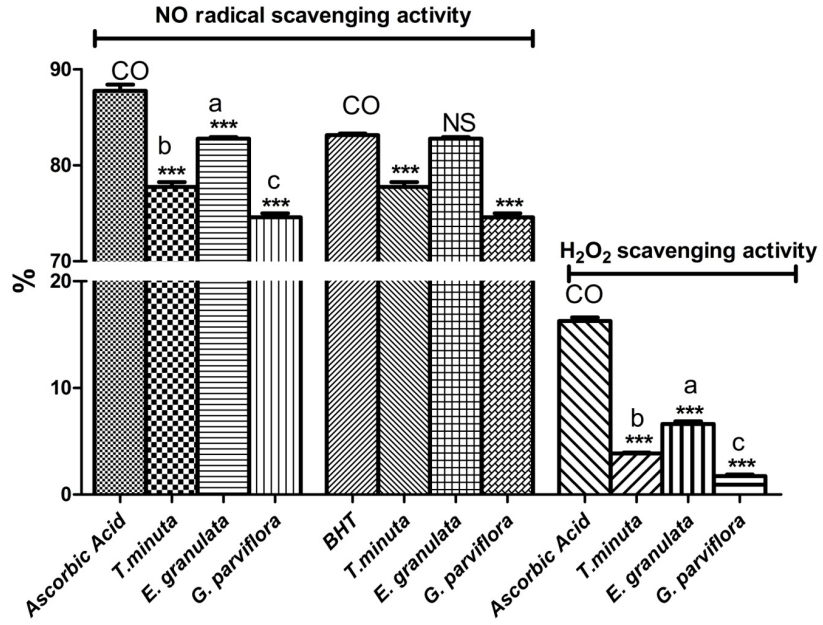


**Figure 3 –** Antioxidant activity of plant extracts evaluated by nitric oxide (NO) and hydrogen peroxide assays.
Note: The results are presented as mean with standard deviation (±); n = 3. sample values were compared with the control value using one way ANOVA+ Post Hoc test, where $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), Not significant (NS), Control (CO). Different letters in the same lot are significantly different (Tukey's Multiple Comparison Test, $p \leq 0.05$)

*Anticancer activity.* The cytotoxic activity results of the tested extracts on MCF7 (human breast adenocarcinoma), A2780 (human ovary adenocarcinoma), HT29 (human colon adenocarcinoma) and MRC5 (normal human fetal lung fibroblast) cell lines are summarized in Table 2. A clear variation was observed among the three plant extracts regarding their toxicity against the tested cell lines. The recorded $IC_{50}$ values of *G. parviflora* extract for the three tested cell lines were significantly ($p \leq 0.001$) high when compared with $IC_{50}$ value of the control. The $IC_{50}$ values of the three extracts were significantly different ($p \leq 0.05$) for A2780 and MCF7 cell lines. No significant difference was observed between *T. minuta* and *G. parviflora* in their inhibition effect towards HT29 cell line. Extracts of *E. granulata* and *G. parviflora* showed same cytotoxic activity on MCF7 cell lines and A2780 cell line, respectively. Both MCF7 and HT29 cell lines were weakly responded to extracts of *T. minuta* and *E. granulata* as compared with the normal cell. The highest anticancer activity (8.92 ± 2.32 μg mL−1) was presented by *T. minuta* extract on A2780 cell line. The lowest cytotoxic activity was demonstrated for *T. minuta* extract (35.01 ± 3.57 μg mL−1) against MCF7 cell line and E. granulate extract (41.89 ± 0.07 μg mL−1) towards HT29 cell line.

According to the Us National Cancer Institute (NCI) a crude extract is considered to be active if it has an $IC_{50}$ value of < 20 μg/mL [80]. Based on this criterion, the $IC_{50}$ values of *T. minuta* and *G. parviflora* extracts on A2780 cell line as well as *E. granulata* extract on MCF7 cell line were within the set limit of NCI guidelines for crude extracts at 50% inhibition. In this study the anticancer activity of *T. minuta* crude extract on MCF7 cell line (35.01 ± 3.57μg mL−1) was high when compared with the result (54.7 ± 6.2 μg mL−1) reported by Ali et al. [15]; and its low as compared with the activity (4.68 μmolL-1) of the pure isolated tagetone (A) compound from *T. minuta* flowers [81]. The activity of *T. minuta* extracts on other cancer cells such as Lewis lung carcinoma has been reported by Ickesh et al. [14]. In addition, the reproductive activity of the essential oils from *T. minuta* against human tumor cell lines (liver hepatocellular carcinoma (HepG2) and nasopharyngeal cell lines has been presented by Shirazi et al. [16]. The anticancer result of *E. granulata* extract on MCF7 cell (16.23 ± 4.50 μg mL−1) obtained in this study was inconsistent with the result reported by Ghauri et al. [20] who stated that the aqueous methanolic extract of *E. granulata* had no anti-proliferative activity towards the breast cancer cell line (MCF7). The weak cytotoxic activity of *G. parviflora* extract on MCF7 cell lines which was reported by Mostafa et al. [73] disagreed with the result obtained in this study. On the other hand, Pan et al. [82] have found that chloroform and ethyl acetate extracts from G. parvoflora have good cytotoxic activity on HL60 (human promyelocytic leukemia cell).

**Table 2** – Inhibition concentration of plant extracts and doxorubicin as a reference drug against three cancer cell lines and one normal fibroblast (MTT 72 h, IC50 ± SD μg mL−1)

| Sample | MRC5 (Control) | A2780 | HT29 | MCF7 |
|---|---|---|---|---|
| *T. minuta* | 37.10 ± 1.61b | 8.92 ± 2.32***, a | 21.22 ± 0.57***, a | 35.01 ± 3.57NS, c |
| *E. granulate* | 40.22 ± 9.48b | 22.80 ± 1.55**, c | 41.89 ± 0.07 NS, b | 16.23 ± 4.50***, a |
| *G. parviflora* | 57.40 ± 1.96a | 16.53 ± 0.92***, b | 22.39 ± 1.73***, a | 27.83 ± 0.13***, b |
| Doxorubicin | 0.74 ± 0.06 | 0.02 ± 0.01 | 0.38 ± 0.03 | 0.01 ± 00 |

Results are presented as mean with standard deviation (±); n = 4. $IC_{50}$ values were compared with the $IC_{50}$ value of MRC5 normal cells in the same row using one way ANOVA+ Dunnett's Multiple Comparison Test, where $p \leq 0.01$ (**), $p \leq 0.001$ (***), Not significant (NS). Means followed by different letters in the same column are significantly different according to Tukey's means test at $p \leq 0.05$.

*In silico molecular docking results.* The *in silico* molecular docking screening results showed that caffeic acid had a least (ΔGbind, kcal/mol) values against the five different selected target receptors, particularly α-estrogen receptor (6b0f) − 6.24 ± 0.08 kcal/mol] and two tubulin isotypes (6s8l) [− 6.17 ± 0.07 kcal/mol] compared to the relevant standard ligands tamoxifen [− 8.40 ± 0.46 kcal/mol], cisplatin-

Zn/plinabulin [− 5.57 ± 0.92 kcal/mol] (Table 3). In addition to, caffeic acid exhibited a ratio of 72 – 95% when it was correlated with both *in vitro* activity against the tested cell-lines and *in silico* virtual efficiency against the five different selected target receptors as estimated from the numbers of heavy atoms (Table 4). On other hand, similar activity to the standards tamoxifen and cisplatin/ plinabulin on estrogen receptors (ERα and/or ERβ) and two tubulin isotypes was observed by caffeic acid (Table 3 and 4).

Lesser the binding energy, is an indication of high activity and better is the binding of the ligand and receptor [83,84]. Referring to this hypothesis the *in silico* molecular docking results proved that caffeic acid had a least (ΔGbind, kcal/mol) value among the ten screened compounds against the different selected targets. This indicated that this compound possessed a high affinity and thus expected to have a greatest cytotoxic activity.

In addition to, caffeic acid exhibited a ratio of (72 – 95%) regarding the correlated *in vitro* activity and *in silico* efficiency; these findings confirmed the predetermined cytotoxic activity of caffeic acid and can be taken as evidence to confirm the cytotoxic activity against the selected cancer cell-lines. This finding was in line with the recent trend that scientists usually combining both *in silico* and *in vitro* approaches to identify and validate the potential biological activity of lead compound(s) at pre-clinical testing level [85-87].

Kuntz et al. [88] mentioned that molecules that achieve a given potency with fewer heavy atoms are more efficient. Accordingly, caffeic acid which possess 14-NHA can be consider as an efficient cytotoxic compound. Strong correlation between the *in vitro* activity and *in silico* virtual efficiency prediction of caffeic acid as cytotoxic agent was observed. Caffeic acid showed closely related values; 0.32, 0.33 with MCF7 and HT29 cell lines *in vitro* and 0.44, 0.44 with 6b0f and 6s8l as target receptors *in silico*. This indicates that docking calculations can be used to determine which compound had the best drug candidate tendency or feature(s) to serve as a future new drug as mentioned by Defant et al. [89]. The obtained results suggested that caffeic acid may act either as estrogen receptors blocker [90] or as tubulin polymerization inhibitor that disrupt the microtubules and induce cell cycle arrest in G 2-M phase and formation of abnormal mitotic spindles [91] or may act by both mechanisms. further confirmatory tests were required to proof or rule out these hypotheses.

**Table 3** – *In silico* molecular docking: affinity for the different standards and tested ligands

| Ligand/ Target | 1hak : Annexin V (C25 H32 N2 O2 S) | 6b0f: α-Estrogen Receptor (C25 H17 F3 O4 S) | 6s8l: Plinabulin + Two Tubulin Isotypes | 1AIO: DNA Containing Cisplatin | 4FM9: α-topoisomerase II |
|---|---|---|---|---|---|
| Standard (Control) | − 6.87 ± 0.07C | −8.40 ± 0.46C | −5.57 ± 0.92C | −2.54±0.09C | − 6.21 ± 0.15C |
| Vanillic acid | − 4.51 ± 0.11*** | −5.42 ± 0.15*** | −5.45 ± 0.045 NS | −5.19 ± 0.07*** | −5.89 ± 0.09* |
| 3,4 Dihydroxy Benzoic acid | −4.58±0.085*** | −5.53±0.18*** | −5.62± 0.09 NS | −5.36 ± 0.07*** | −5.93± 0.06* |
| Gallic acid | −4.93 ± 0.091*** | −5.52 ± 0.16*** | −5.68 ± 0.11 NS | −5.72 ± 0.05*** | −6.51 ± 0.17* |
| p-Coumaric acid | −5.06 ± 0.12*** | −6.11 ± 0.09*** | −5.98 ± 0.04 NS | −4.82 ± 0.11*** | −6.14 ± 0.15* |
| Caffeic acid | −5.10 ± 0.15*** | −6.24 ± 0.08*** | −6.17 ± 0.07 NS | −5.36 ± 0.05*** | −6.21 ± 0.15* |
| Ferulic acid | −5.33 ± 0.09*** | −6.24 ± 0.11*** | −6.07 ± 0.06 NS | −5.20 ± 0.04*** | −6.16 ± 0.13* |
| Sinnapic acid | −5.33 ± 0.12*** | −5.93 ± 0.17*** | −5.79 ± 0.05 NS | −5.33 ± 0.06*** | −6.04 ± 0.08* |
| Syringic acid | −4.54±0.08*** | −5.51±0.07*** | −5.44 ± 0.08 NS | −5.59 ± 0.05*** | −6.23 ± 0.06* |
| p-Hydroxybenzoic acid | −4.46 ± 0.09*** | −5.44 ±0.15*** | −5.34 ± 0.09 NS | −4.80 ± 0.08*** | −5.86 ± 0.09* |
| t-Cinamic acid | −4.70 ± 0.16*** | −5.90 ± 0.11*** | −5.79 ± 0.03 NS | −4.68 ± 0.07*** | −5.63 ± 0.15* |

Standard ligands: K-201, Tamoxifen, Cisplatin-Zn and Doxorubicin. Where $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***), Not significant (NS) and control (C)

**Table 4** – Comparison of calculated LE based on NHA, *in vitro* and *in silico* for the most promising tested compound; caffeic acid from the *T. minuta* plant

| Targets | STD Ligands | Cell line | *In vitro* activity | | *In silico* efficiency | | Ratio |
|---|---|---|---|---|---|---|---|
| | | | $IC_{50}$ | LEv | ΔGbind, kcal/mol | LEs | |
| 1hak : Annexin V (C25 H32 N2 O2 S) | Cisplatin/ K-201 | HT29 | 21.22 ± 0.57 | 0.33 | −5.10 ± 0.15 | −0.36 | 91.7% |
| 6b0f: α-Estrogen Receptor (C25 H17 F3 O4 S) | Tamoxifen/ Lsz102 | MCF7 | 35.01 ± 3.57 | 0.32 | −6.24 ± 0.08 | −0.44 | 72.7% |
| 6s8l: Two Tubulin Isotypes | Cisplatin/ Plinabulin | HT29 | 21.22 ± 0.57 | 0.33 | −6.17± 0.07 | −0.44 | 75% |
| 1AIO: DNA Containing Cisplatin | Cisplatin/ Plinabulin | A2780/ MRC5 | 8.92 ± 2.32<br>37.10 ± 1.61 | 0.36<br>0.32 | −5.36 ± 0.05 | −0.38 | 94.7%<br>84.2% |
| 4FM9: α-topoisomerase II | Doxorubicin | MCF7 | 35.01 ± 3.57 | 0.32 | −6.21 ± 0.15 | −0.44 | 72.7% |

LEv = p ($IC_{50}$)/NHA, LEs = ΔGbind /NHA

## Conclusion

The results obtained showed that the amount of polyphenol content in the studied extracts was in the following order *E. granulata* > *T. minuta* > *G. parviflora*. Both flavonoids and tannins were higher in *G. parviflora* compared to the other two plant extracts. Moreover, ten different phenolic acids were quantified and identified in the studied plants. p-Hydroxybenzoic acid was the most dominant of all phenolic acids in *T. minuta* extract while gallic acid was the major acid in *E. granulata* extract. Vanillic acid and 3,4-dihydroxybenzoic acid were found in a few amounts in the investigated plants. Most of the extracts showed high DPPH, ABTS and NO radical scavenging activity as compared with the control. All extracts displayed various cytotoxic activities towards the tested cell lines. *T. minuta* extract exhibited highest cytotoxicity against A2780 cell line and was the lowest against MCF7 cell line. *In silico* screening revealed that phenolic acids have a noticeable cytotoxic activity and caffeic acid was the most promising compound that possessed high affinity and ligand efficiency against the selected virtual targets. The cytotoxic activity of caffeic acid as estrogen receptor blockers or tubulin polymerization inhibitor required more *in vitro* and *in vivo* confirmatory studies. Further research is required to complete the pharmacological and toxicological profile of the investigated components in the studied plants.

## Acknowledgments

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia for funding this research work through the project number: MOE-BU-7-2020.

## Funding

This study was funded by the Deputyship for Research & Innovation, Ministry of Education, Saudi Arabia under Grant number MOE-BU-7-2020.

## References

1. Süntar I. (2020) Importance of ethnopharmacological studies in drug discovery: role of medicinal plants. *Phytochem. Rev.,* vol. 19, no. 5, pp. 1199-1209. https://doi.org/10.1007/s11101-019-09629-9

2. Yılmaz S., Ergün S. (2018) Trans-cinnamic acid application for rainbow trout (*Oncorhynchus mykiss*): I. Effects on haematological, serum biochemical, non-specific immune and head kidney gene expression responses. *Fish Shellfish Immunol.,* vol. 78, pp. 140-157. https://doi.org/10.1016/j.fsi.2018.04.034

3. von Danwitz A., Schulz C. (2020) Effects of dietary rapeseed glucosinolates, sinapic acid and phytic acid on feed intake, growth performance and fish health in turbot (*Psetta maxima* L.). *Aquaculture*, vol. 516, pp. 734624. https://doi.org/10.1016/j.aquaculture.2019.734624

4. Sher H., Aldosari A. (2012) Overview on the ecological and geographical appraisal of important medicinal and aromatic plants: An endangered component in the flora of Saudi Arabia. *Sci. Res. Essays*, vol. 7, no. 16, pp. 1639-1646. https://doi.org/10.5897/SRE12.183

5. Alqahtani S.N., Alkholy S.O., Ferreira M.P. (2014) Antidiabetic and anticancer potential of native medicinal plants from Saudi Arabia. In: Watson R., Preedy V., Zibadi S. Polyphenols in human health and disease. Academic Press. Cambridge, Massachusetts, United States, pp. 119-132. https://doi.org/10.1016/B978-0-12-398456-2.00011-6

6. Abdel-Sattar E., Abou-Hussein D., Petereit F. (2015) Chemical constituents from the leaves of *Euphorbia ammak* growing in Saudi Arabia. *Pharmacogn. Res.*, vol. 7, no. 1, pp. 14-17. https://doi.org/10.4103/0974-8490.147136

7. Aati H., El-Gamal A., Shaheen H., Kayser O. (2019) Traditional use of ethnomedicinal native plants in the Kingdom of Saudi Arabia. *J. ethnobiol. Ethnomed.*, vol. 15, no. 1, pp. 1-9. https://doi.org/10.1186/s13002-018-0263-2

8. Walia S., Mukhia S., Bhatt V., Kumar R., Kumar R. (2020) Variability in chemical composition and antimicrobial activity of *Tagetes minuta* L. essential oil collected from different locations of Himalaya. *Ind. Crops Prod.*, vol. 150, 112449. https://doi.org/10.1016/j.indcrop.2020.112449

9. Tereschuk M.L., Riera M.V., Castro G.R., Abdala L.R. (1997) Antimicrobial activity of flavonoids from leaves of *Tagetes minuta*. *J. Ethnopharmacol.*, vol. 56, no. 3, pp. 227-232. https://doi.org/10.1016/S0378-8741(97)00038-X

10. Supradip S., Suresh W., Kundu A., Kumar B., Decksha J. (2012) Antifungal acetylinic thiophenes from *Tagetes minuta*: potential biopesticide. *J. Appl. Bot. Food Qual.*, vol. 85, no. 2, pp. 207-211.

11. Osée Muyima N.Y., Nziweni S., Mabinya L.V. (2004) Antimicrobial and antioxidative activities of *Tagetes minuta*, *Lippia javanica* and *Foeniculum vulgare* essential oils from the Eastern Cape Province of South Africa. *J. Essent. Oil-Bear. Plants*, vol. 7, no. 1. pp. 68-78. https://doi.org/10.1080/0972-060X.2004.10643368

12. Igwaran A., Iweriebor B.C., Okoh S.O., Nwodo U.U., Obi L.C., Okoh A.I. (2017) Chemical constituents, antibacterial and antioxidant properties of the essential oil flower of *Tagetes minuta* grown in Cala community Eastern Cape, South Africa. *BMC Complement Altern. Med.*, vol. 17, no.1, pp. 1-10. https://doi.org/10.1186/s12906-017-1861-6

13. de Oliveira D.H., Abib P.B., Giacomini R.X., Lenardão E.J., Schiedeck G., Wilhelm E.A., Luchese C., Savegnago L., Jacob R.G. (2019) Antioxidant and antifungal activities of the flowers' essential oil of *Tagetes minuta*,(Z)-tagetone and thiotagetone. *J. Essent. Oil Res.*, vol. 31, no. 2, pp. 160-169. https://doi.org/10.1080/10412905.2018.1519465

14. Ickesh G.R., Fong H.H.S., Schiff Jr P.L., Perdue Jr R.E., Farnsworth N.R. (1973) Antitumor activity and preliminary phytochemical examination of *Tagetes minuta* (Compositae). *J. Pharm. Sci.*, vol. 62, no. 2, pp. 1009-1011. https://doi.org/10.1002/jps.2600620635

15. Ali N.A.A., Sharopov F.S., Al-Kaf A.G., Hill G.M., Arnold N., Al-Sokari S.S., Setzer W.N., Wessjohann L. (2014) Composition of essential oil from *Tagetes minuta* and its cytotoxic, antioxidant and antimicrobial activities. *Nat. Prod. Commun.*, vol. 9, no. 2, pp. 265 – 268. https://doi.org/10.1177/1934578X1400900233

16. Shirazi M.T., Gholami H., Kavoosi G., Rowshan V., Tafsiry A. (2014) Chemical composition, antioxidant, antimicrobial and cytotoxic activities of *Tagetes minuta* and *Ocimum basilicum* essential oils. *Food Sci. Nutr.*, vol. 2, no. 2, pp. 146-155. https://doi.org/10.1002/fsn3.85

17. Hussein G., Miyashiro H., Nakamura N., Hattori M., Kawahata T., Otake T., Kakiuchi N., Shimotohno K. (1999) Inhibitory effects of Sudanese plant extracts on HIV-1 replication and HIV-1 protease. *Phytother. Res.*, vol. 13, no. 1, pp. 31-36. https://doi.org/10.1002/(SICI)1099-1573(199902)13:1<31::AID-PTR381>3.0.CO,2-C

18. Saleem H., Ahmad I., Gill M.S.A. (2015) Phytochemical screening and diuretic activity of *Euphorbia granulata*. *Bangladesh J. Pharmacol.*, vol. 10, no. 3, pp. 584-587. https://doi.org/10.3329/bjp.v10i3.22844

19. Awaad A.S., Alothman M.R., Zain Y.M., Zain G.M., Alqasoumi S.I., Hassan, D.A. (2017) Comparative nutritional value and antimicrobial activities between three Euphorbia species growing in Saudi Arabia. *Saudi Pharm. J.*, vol. *25*, no. 8, pp. 1226-1230. https://doi.org/10.1016/j.jsps.2017.09.007

20. Ghauri M.A., Iqbal L., Raza A.m Hayat U., Atif N., Javeed A. (2021) *In vivo* anti-inflammatory, antipyretic, analgesic activity and *in vitro* antiproliferative activity of aqueous methanolic extract of *Euphorbia granulata* Forssk. *Future J. Pharm. Sci.*, vol. 7, no. 1, pp. 1-10. https://doi.org/10.1186/s43094-021-00184-9

21. Ali S., Zameer S., Yaqoob M. (2017) Ethnobotanical, phytochemical and pharmacological properties of *Galinsoga parviflora* (Asteraceae): A review. *Trop. J. Pharm. Res.*, vol. 16. No. 12, pp. 3023 – 3033. http://dx.doi.org/10.4314/tjpr.v16i12.29

22. Bazylko A., Boruc K., Borzym J., Kiss A.K. (2015) Aqueous and ethanolic extracts of *Galinsoga parviflora* and *Galinsoga ciliata*. Investigations of caffeic acid derivatives and flavonoids by

HPTLC and HPLC-DAD-MS methods. *Phytochem. Lett.*, vol. 11, pp. 394-398. https://doi.org/10.1016/j.phytol.2014.11.005

23. Bazylko A., Borzym J., Parzonko A. (2015) Determination of *in vitro* antioxidant and UV-protecting activity of aqueous and ethanolic extracts from *Galinsoga parviflora* and *Galinsoga quadriradiata* herb. *J. Photochem. Photobiol. B: Biol.*, vol. 149, pp. 189-195. https://doi.org/10.1016/j.jphotobiol.2015.06.010

24. Cliffe S., Fawer M.S., Maier G., Takata K., Ritter G. (1994) Enzyme assays for the phenolic content of natural juices. *J. Agric. Food Chem.*, vol. 42, no. 8, pp. 1824-1828. https://doi.org/10.1021/jf00044a048

25. Dewanto V., Wu X., Adom K.K., Liu R.H. (2002) Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *J. Agric. Food Chem.*, vol. 50, no. 10, pp. 3010-3014. https://doi.org/10.1021/jf0115589

26. Bhat R., Sridhar K.R., Tomita-Yokotani K. (2007) Effect of ionizing radiation on antinutritional features of velvet bean seeds (*Mucuna pruriens*). *Food Chem.*, vol. 103, no. 3, pp. 860-866. https://doi.org/10.1016/j.foodchem.2006.09.037

27. Koh P.H., Mokhtar R.A.M., Iqbal M. (2012) Antioxidant potential of *Cymbopogon citratus* extract: alleviation of carbon tetrachloride-induced hepatic oxidative stress and toxicity. *Hum. Exp. Toxicol.*, vol. 31, no. 1, pp. 81-91. https://doi.org/10.1177/0960327111407226

28. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med*., vol. 26, no. 9-10, pp. 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

29. Ebrahimzadeh M.A., Nabavi S.F., Nabavi S.M. (2009) Antioxidant activities of methanol extract of *Sambucus ebulus* L. flower. *Pak. J. Biol. Sci.*, vol. 12, no. 5, pp. 447-450. https://doi.org/10.3923/pjbs.2009.447.450

30. Ruch R.J., Cheng S.J., Klaunig J.E. (1989) Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, Vol. 10, no. 6, pp. 1003-1008. https://doi.org/10.1093/carcin/10.6.1003

31. Bkhaitan M.M., Mirza A.Z., Abdalla A.N., Shamshad H., Ul-Haq Z., Alarjah M., Piperno A. (2017) Reprofiling of full-length phosphonated carbocyclic 2′-oxa-3′-aza-nucleosides toward antiproliferative agents: Synthesis, antiproliferative activity, and molecular docking study. *Chem. Biol. Drug. Des.*, vol. 90, no. 5, pp. 679-689. https://doi.org/10.1111/cbdd.12987

32. Kaneko N., Ago H., Matsuda R., Inagaki E., Miyano M. (1997) Crystal structure of annexin V with its ligand K-201 as a calcium channel activity inhibitor. *J. Mol. Biol.* Vol. 274, no. 1, pp.16-20. https://doi.org/10.1006/jmbi.1997.1375

33. Tria G.S., Abrams T., Baird J., Burks H.E., Firestone B., Gaither L.A., Hamann L.G., He G., Kirby C.A., Kim S., Lombardo F., Macchim K.J., McDonnell D.P., Mishina Y., Norris J.D., Nunez J., Springer C., Sun Y., Thomsen N.M., Wang C., Wang J., Yu B., Tiong-Yip C., Peukert S. (2018) Discovery of LSZ102, a potent, orally bioavailable selective estrogen receptor degrader (SERD) for the treatment of estrogen receptor positive breast cancer. *J. Med. Chem.*, vol. 61, no. 7, pp. 2837-2864. https://doi.org/10.1021/acs.jmedchem.7b01682

34. Kakiashvili D.M., van Rhijn B.W.G., Trottier G., Jewett M.A.S., Fleshner N.E., Finelli A., Azuero J., Bangma C.H., Vajpeyi R., Alkhateeb S., Hanna S., Kostynsky A., Kuk C., Van Der Kwast T.H., Zlotta A.R. (2011) Long-term follow-up of T1 high-grade bladder cancer after intravesical bacille Calmette-Guérin treatment. *BJU Int.*, vol. 107, no. 4, pp. 540-546. https://doi.org/10.1111/j.1464-410X.2010.09572.x

35. Takahara P.M., Rosenzweig A.C., Frederick C.A., Lippard S.J. (1995) Crystal structure of double-stranded DNA containing the major adduct of the anticancer drug cisplatin. *Nature*, vol. 377, no. 6550, pp. 649-652. https://doi.org/10.1038/377649a0

36. Wendorff T.J., Schmidt B.H., Heslop P., Austin C.A., Berger J.M. (2012) The structure of DNA-bound human topoisomerase II alpha: conformational mechanisms for coordinating inter-subunit interactions with DNA cleavage. *J. Mol. Biol.*, vol. 424, no. 3-4, pp. 109-124. https://doi.org/10.1016/j.jmb.2012.07.014

37. Irwin J.J., Shoichet B.K. (2005) ZINC− a free database of commercially available compounds for virtual screening. *J. Chem. Inf. Model*., vol. 45, no. 1 pp. 177-182. https://doi.org/10.1021/ci049714+

38. El-Hachem N., Haibe-Kains B., Khalil A., Kobeissy F.H., Nemer G. (2017) AutoDock and AutoDockTools for protein-ligand docking: Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1) as a case study. In: Kobeissy F.H., Stevens S.M. (eds) Neuroproteomics. Humana Press. New York, United States, pp. 391-403. https://doi.org/10.1007/978-1-4939-6952-4_20

39. O'Boyle N.M., Banck M., James C.A., Morley C., Vandermeersch T., Hutchison G.R. (2011) Open Babel: An open chemical toolbox. *J. Cheminformatics*, vol. 3, no. 1, pp. 1-14. https://doi.org/10.1186/1758-2946-3-33

40. Cornell W.D., Cieplak P., Bayly C.I., Gould I.R., Merz K.M., Ferguson D.M., Spellmeyer D.C., Fox T., Caldwell J.W., Kollman P.A. (1995) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.*, vol. 117, no. 19, pp. 5179-5197. https://doi.org/10.1021/ja00124a002

41. Halgren T.A. (1996) Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *J. Comput. Chem.*, vol. 17, no. 5-6, 490-519. https://doi.org/10.1002/(SICI)1096-987X(199604)17:5/6<490::AID-JCC1>3.0.CO,2-P

42. Halgren T.A. (1999) MMFF VI. MMFF94s option for energy minimization studies. *J. Comput. Chem.*, vol. 20, no. 7, pp. 720-729. https://doi.org/10.1002/(SICI)1096-987X(199905)20:7<720::AID-JCC7>3.0.CO,2-X

43. Trott O., Olson A.J. (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem*., vol. 31, no. 2, pp. 455-461. https://doi.org/10.1002/jcc.21334

44. Kuntz I.D., Chen K., Sharp K.A, Kollman P.A. (1999) The maximal affinity of ligands. *Proc. Natl. Acad. Sci.*, vol. 96, no. 18, pp. 9997-10002. https://doi.org/10.1073/pnas.96.18.9997

45. Rezaei F., Jamei R., Heidari R. (2018) Evaluation of volatile profile, fatty acids composition and *in vitro* bioactivity of *Tagetes minuta* growing wild in northern Iran. *Adv. Pharm. Bull.*, vol. 8, no. 1, pp. 115. http://dx.doi.org/10.15171/apb.2018.014

46. Ranilla L.G., Kwon Y.I., Apostolidis E., Shetty K. (2010) Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. *Bioresour. Technol.*, vol. 101, no. 12, pp. 4676-4689. https://doi.org/10.1016/j.biortech.2010.01.093

47. Duarte N., Kayser O., Abreu P., Ferreira M.J.U. (2008) Antileishmanial activity of piceatannol isolated from *Euphorbia lagascae* seeds. *Phytother. Res.*, vol. 22, no. 4, pp., 455-457. https://doi.org/10.1002/ptr.2334

48. Giordani R., Trebaux J., Masi M., Regli P. (2001) Enhanced antifungal activity of ketoconazole by *Euphorbia characias* latex against *Candida albicans*. *J. Ethnopharmacol.*, vol. 78, no. 1, pp. 1-5. https://doi.org/10.1016/S0378-8741(01)00295-1

49. Studzińska-Sroka E., Dudek-Makuch M., Chanaj-Kaczmarek J., Czepulis N., Korybalska K., Rutkowski R., Łuczak J., Grabowska K., Bylka W., Witowski J. (2018) Anti-inflammatory Activity and Phytochemical Profile of *Galinsoga Parviflora* Cav. *Molecules*, vol. 23, no. 9, pp. 2133. https://doi.org/10.3390/molecules23092133

50. Dudek M.K., Dudkowski Ł., Bazylko A., Kaźmierski S., Kiss A.K. (2016) Caffeic acid derivatives isolated from the aerial parts of *Galinsoga parviflora* and their effect on inhibiting oxidative burst in human neutrophils. *Phytochem. Lett.*, vol. 16, pp. 303-310. https://doi.org/10.1016/j.phytol.2016.05.007

51. Wang S., Mateos R., Goya L., Amigo-Benavent M., Sarriá B., Bravo L. (2016) A phenolic extract from grape by-products and its main hydroxybenzoic acids protect Caco-2 cells against pro-oxidant induced toxicity. *Food Chem. Toxicol.*, vol. 88, pp. 65-74. https://doi.org/10.1016/j.fct.2015.12.005

52. Farhoosh R., Johnny S., Asnaashari M., Molaahmadibahraseman N., Sharif A. (2016) Structure–antioxidant activity relationships of o-hydroxyl, o-methoxy, and alkyl ester derivatives of p-hydroxybenzoic acid. *Food Chem.*, vol. 194, pp. 128-134. https://doi.org/10.1016/j.foodchem.2015.08.003

53. Soni M.G., Carabin I.G., Burdock G.A. (2005) Safety assessment of esters of p-hydroxybenzoic acid (parabens). *Food Chem. Toxicol*., vol. 43, no. 7, pp. 985-1015. https://doi.org/10.1016/j.fct.2005.01.020

54. Kiliç I., Yeşiloğlu Y. (2013) Spectroscopic studies on the antioxidant activity of p-coumaric acid. *Spectrochim. Acta A. Mol. Biomol. Spectrosc.*, vol. 115, pp. 719-724. https://doi.org/10.1016/j.saa.2013.06.110

55. Masek A., Chrzescijanska E., Latos M. (2016) Determination of antioxidant activity of caffeic acid and p-coumaric acid by using electrochemical and spectrophotometric assays. *Int. J. Electrochem. Sci.*, vo. 11, no. 12, pp. 10644-10658. https://doi.org/10.20964/2016.12.73

56. Sharma S. H., Rajamanickam V., Nagarajan S. (2018) Antiproliferative effect of p-Coumaric acid targets UPR activation by downregulating Grp78 in colon cancer. *Chem. Biol. Interact.*, vol. 291, pp. 16-28. https://doi.org/10.1016/j.cbi.2018.06.001

57. Kim H.B., Lee S., Hwang E.S., Maeng S., Park J. H. (2017) p-Coumaric acid enhances long-term potentiation and recovers scopolamine-induced learning and memory impairments. *Biochem. Biophys. Res. Commun.*, vol. 492, no. 3, pp. 493-499. https://doi.org/10.1016/j.bbrc.2017.08.068

58. Ferreira J.C., Reis M.B., Coelho G.D., Gastaldello G.H., Peti A.P.F., Rodrigues D.M., Bas-

tos J.K., Campo V.L., Gardinassi L.G., Faccioli L.H., Sorgi C.A, Tefé-Silva C., Zoccal K.F. (2021) Baccharin and p-coumaric acid from green propolis mitigate inflammation by modulating the production of cytokines and eicosanoids. *J. Ethnopharmacol.*, vol. 278, pp. 114255. https://doi.org/10.1016/j.jep.2021.114255

59. Pandi A., Kalappan V.M. (2021) Pharmacological and therapeutic applications of Sinapic acid—an updated review. *Mol. Biol. Rep.*, vol. 48, no. 4, pp. 3733–3745. https://doi.org/10.1007/s11033-021-06367-0

60. Tewari A., Tiwari S. (2018) Synthesis of medicinal agents from plants. Elsevier. ISBN: 9780081020715

61. Kahkeshani N., Farzaei F., Fotouhi M., Alavi S.S., Bahramsoltani R., Naseri R., Momtaz S., Abbasabadi Z., Rahimi R., Farzaei M.H., Bishayee A. (2019) Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iran. J. Basic Med. Sci.*, vol. 22, no. 3, pp. 225- 237. https://doi.org/10.22038/ijbms.2019.32806.7897

62. Zahrani N.A.A., El-Shishtawy R.M., Asiri A.M. (2020) Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review. *Eur. J. Med. Chem.*, vol. 204, pp. 112609. https://doi.org/10.1016/j.ejmech.2020.112609

63. Rice-Evans C.A., Miller N.J., Paganga G. (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Biol. Med.*, vol. 20, no. 7, pp. 933-956. https://doi.org/10.1016/0891-5849(95)02227-9

64. Shabani S., Rabiei Z., Amini-Khoei H. (2020) Exploring the multifaceted neuroprotective actions of gallic acid: A review. *Int. J. Food Prop.*, vol. 23, no. 1, pp. 736-752. https://doi.org/10.1080/10942912.2020.1753769

65. Subramanian A.P., John A.A., Vellayappan M.V., Balaji A., Jaganathan S.K., Supriyanto E., Yusof M. (2015) Gallic acid: prospects and molecular mechanisms of its anticancer activity. *RSC Adv.*, vol. 5, no. 45, pp. 35608-35621. https://doi.org/10.1039/C5RA02727F

66. Mancuso C., Santangelo R. (2014) Ferulic acid: pharmacological and toxicological aspects. *Food Chem. Toxicol.*, vol. 65, pp. 185-195. https://doi.org/10.1016/j.fct.2013.12.024

67. Govindaswamy V. (2015) Therapeutical properties of ferulic acid and bioavailability enhancement through feruloyl esterase. *J. Funct. Foods*, vol. 17, pp. 657-666. https://doi.org/10.1016/j.jff.2015.06.013

68. Graf E. (1992) Antioxidant potential of ferulic acid. *Free Radic. Biol. Med.*, vol. 13, no. 4, pp. 435-448. https://doi.org/10.1016/0891-5849(92)90184-I

69. Kim H.Y., Park J., Lee K.H., Lee D.U., Jong H.K., Yeong S.K., Lee S.M. (2001) Ferulic acid protects against carbon tetrachloride-induced liver injury in mice. *Toxicology*, vol. 282, no. 3, pp. 104– 111. https://doi.org/10.1016/j.tox.2011.01.017

70. Brimson J.M., Onlamoon N., Tencomnao T., Thitilertdecha P. (2019) *Clerodendrum petasites* S. Moore: The therapeutic potential of phytochemicals, hispidulin, vanillic acid, verbascoside, and apigenin. *Biomed. Pharmacother.*, vol. 118, pp. 109319. https://doi.org/10.1016/j.biopha.2019.109319

71. Yang J., Chen J., Hao Y., Liu Y. (2021) Identification of the DPPH radical scavenging reaction adducts of ferulic acid and sinapic acid and their structure-antioxidant activity relationship. *LWT*, vol. 146, pp. 111411. https://doi.org/10.1016/j.lwt.2021.111411

72. Bautista-Hernández I., Aranda-Ledesma N.E., Rojas R., Tafolla-Arellano J C., Martínez-Ávila G.C. (2021) Antioxidant activity of polyphenolic compounds obtained from *Euphorbia antisyphilitica* by-products. *Heliyon*, vol. 7, no. 4, pp. e06734. https://doi.org/10.1016/j.heliyon.2021.e06734

73. Mostafa I., Abd El-Aziz E., Hafez S., El-Shazly A. (2013) Chemical constituents and biological activities of *Galinsoga parviflora* Cav. (Asteraceae) from Egypt. *Z. Naturforsch. C. J. Biosci.*, vol. 68, no. 7-8, pp. 285-292.

74. Bazylko A., Stolarczyk M., Derwińska M., Kiss A.K. (2012) Determination of antioxidant activity of extracts and fractions obtained from *Galinsoga parviflora* and *Galinsoga quadriradiata*, and a qualitative study of the most active fractions using TLC and HPLC methods. *Nat. Prod. Res.*, vol. 26, no. 17, pp. 1584-1593. https://doi.org/10.1080/14786419.2011.582469

75. Al-Musayeib N.M., Mohamed G.A., Ibrahim S.R., Ross S.A. (2014) New thiophene and flavonoid from *Tagetes minuta* leaves growing in Saudi Arabia. *Molecules*, vol. 19, no. 3, pp. 2819-2828. https://doi.org/10.3390/molecules19032819

76. Tanase C., Coșarcă S., Muntean D.L. (2019) A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. *Molecules*, vol. 24, no. 6, pp. 1182. https://doi.org/10.3390/molecules24061182

77. Braga G.C., Melo P.S., Bergamaschi K.B., Tiveron A.P., Massarioli A.P., Alencar S.M.D. (2016) Extraction yield, antioxidant activity andphenolics from grape, mango and peanut agro-industrial by-products. *Cienc. Rural.*, vol. 46, pp. 1498-1504. https://doi.org/10.1590/0103-8478cr20150531

78. El-Zayat M.M., Eraqi M.M., Alfaiz F.A., Elshaer M.M. (2021) Antibacterial and antioxidant potential of some Egyptian medicinal plants used in traditional medicine. *J. King Saud Univ. Sci.*, vol. 33, no. 5, pp. 101466. https://doi.org/10.1016/j.jksus.2021.101466

79. Esmaeili A.K., Taha R.M., Mohajer S., Banisalam B. (2015) Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from *in vivo* and *in vitro* grown *Trifolium pratense* L. (Red Clover). *Biomed Res. Int.*, pp. 1-11. https://doi.org/10.1155/2015/643285

80. Lee C.C., Houghton P. (2005) Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. *J. Ethnopharmacol.*, vol. 100, no. 3, pp. 237-243. https://doi.org/10.1016/j.jep.2005.01.064

81. Ibrahim S.R.M., Mohamed G.A.A. (2017) Tagetones A and B, new cytotoxic monocyclic diterpenoids from flowers of *Tagetes minuta*. *Chin. J. Nat. Med.*, vol. 15, no. 7, pp. 546-549. https://doi.org/10.1016/S1875-5364(17)30081-X

82. Pan Z.H., Zhao L., Huang R., Ma G.Y., Li Z.Q. (2007) Terpenes and sterols from *Galinsoga parviflora*. *J. Yunnan Uni. Nat. Sci.*, vol. 29, no. 6, pp. 613 – 616. https://doi.org/10.1080/23802359.2019.1623106

83. Zakšauskas A., Čapkauskaitė E., Paketurytė-Latvė V., Smirnov A., Leitans J., Kazaks A., Dvinskis E., Stančaitis L., Mickevičiūtė A., Jachno J., Jezepčikas L., Linkuvienė V., Sakalauskas A., Manakova E., Gražulis S., Matulienė J., Tars K., Matulis D. (2022) Methyl 2-Halo-4-Substituted-5-Sulfamoyl-Benzoates as High Affinity and Selective Inhibitors of Carbonic Anhydrase IX. Int. *J. Mol. Sci.*, vol. 23, no. 1, pp. 130. https://doi.org/10.3390/ijms23010130

84. El-Shamy N.T., Alkaoud A.M., Hussein R.K., Ibrahim M.A., Alhamzani A.G., Abou-Krisha M.M. (2022) DFT, ADMET and Molecular Docking Investigations for the Antimicrobial Activity of 6,6′-Diamino-1,1′,3,3′-tetramethyl-5,5′-(4-chlorobenzylidene)bis[pyrimidine-2,4(1H,3H)-dione]. *Molecules*, vol. 27, no. 3, pp. 620. https://doi.org/10.3390/molecules27030620

85. Giardina E., Pietrangeli I., Martone C., Asili P., Predazzi I., Marsala P., Gabriele L., Pipolo C., Ricci O., Solla G., Sineo L., Spinella A., Novelli G. (2007) *In silico* and *in vitro* comparative analysis to select, validate and test SNPs for human identification. *MC Genom.*, vol. 8, no. 1, pp. 1-7. https://doi.org/10.1186/1471-2164-8-457

86. Leedale J., Sharkey K.J., Colley H.E., Norton Á.M., Peeney D., Mason C.L., Sathish J.G., Murdoch C., Sharma P., Webb S.D. (2018) A combined *in vitro*/*in silico* approach to identifying off-target receptor toxicity. *Iscience*, vol. 4, pp. 84-96. https://doi.org/10.1016/j.isci.2018.05.012

87. Chinnasamy S., Selvaraj G., Selvaraj C., Kaushik A.C., Kaliamurthi S., Khan A., Singh S.K., Wei D.Q. (2020) Combining *in silico* and *in vitro* approaches to identification of potent inhibitor against phospholipase A2 (PLA2). *Int. J. Biol. Macromol.*, vol. 144, no. 1, pp. 53-66. https://doi.org/10.1016/j.ijbiomac.2019.12.091

88. Kuntz I.D., Chen K., Sharp K.A., Kollman P.A. (1999) The maximal affinity of ligands. *Proc. Natl. Acad. Sci.*, vol. 96, no. 18, pp. 9997-10002. https://doi.org/10.1073/pnas.96.18.9997

89. Defant A., Dosi F., Innocenti N., Mancini I. (2022) Synthesis of Nucleoside-like Molecules from a Pyrolysis Product of Cellulose and Their Computational Prediction as Potential SARS-CoV-2 RNA-Dependent RNA Polymerase Inhibitors. *Int. J. Mol. Sci.*, vol. *23*, no. 1, pp. 518. https://doi.org/10.3390/ijms23010518

90. Sharma D., Kumar S., Narasimhan B. (2018) Estrogen alpha receptor antagonists for the treatment of breast cancer: a review. *Chem. Cent. J.*, vol. 12, no. 1, pp. 1-32. https://doi.org/10.1186/s13065-018-0472-8

91. Kaur R., Kaur G., Gill R.K., Soni R., Bariwal J. (2014) Recent developments in tubulin polymerization inhibitors: an overview. *Eur. J. Med. Chem*., vol. 87, pp. 89-124. https://doi.org/10.1016/j.ejmech.2014.09.051