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RESEARCH ARTICLE

ANATOMICAL, PHYSICOCHEMICAL, PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL INVESTIGATIONS OF *TAGETES MINUTA* L. AERIAL PARTS

Saleh Kassem Algfri[1,*], Gulia Ahmed Naser[1], Rasheed Saeed Rageh[2], Ahmed Bin Shuaib[1], Amna Haitham Nasser[3]

[1] *Dept. of Pharmacognosy, Faculty of Pharmacy, University of Aden, Yemen.*
[2] *Dept. of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Aden, Yemen.*
[3] *Nahda Makers Organization, Aden.*

***Corresponding author: Saleh Kassem Algfri; E-mail: algfri_s25@yahoo.com; algfri_s25@pharm.adenuniv.com; algfris25@gmail.com; Mobile No: (+967)773752084**

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Abstract

Tagetes minuta L., which grows in different regions of Yemen, is used to treat skin diseases and has not been adequately studied, so the aim of the present research is to evaluate its anatomical, physicochemical, phytochemical, antioxidant and antimicrobial properties of its aerial parts, using accepted standard methods. The microscopic and physicochemical parameters were determined. Phytochemical analysis was performed on petroleum ether, 80% methanol and water extracts, and carbohydrates, phenols, flavonoids, tannins, terpenes and sterols were identified. The total phenolic content was found to be 205.14 and 91.2 mg/g gallic acid equivalent and the total flavonoid content was found to be 106.46 and 18.15 mg/g quercetin equivalent of the dry extract of 80% methanol and aqueous extracts, respectively. The antioxidant activity was determined by using in vitro DPPH method. The IC50 values of quercetin, petroleum ether, 80% methanolic and water extracts were found to be 13.54, 151.04, 21.36 and 47.31 µg/ml respectively, so the 80% methanolic extract showed very high antioxidant activity compared to quercetin. The antimicrobial activity was tested by using well diffusion method, all concentrations used for the 80% methanolic extract showed very high antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*, while they showed significant of inhibition zone against *Klebsilla pneumoniae*, and *Candid albicans*. All concentrations used for the aqueous extract showed appearance of inhibition zone against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, while no effect was shown against the *Klebsiella pneumoniae*, *proteus vulgaris* and *Candid albicans*. The above results indicate that the aerial parts of *Tagetes minuta* could be a useful natural antioxidant and antibacterial agent, and may be used for further study on the plant.

Keywords: *Tagetes minuta, Anatomical, Phytochemical, Antioxidant, Antimicrobial*.

1. Introduction

Plants are an important source of medicines and pharmaceutical materials, play a major role in global health, and have been used for thousands of years [1,2]. Nowadays, approximately 80% of antimicrobial drugs, cardiovascular drugs, immunosuppressants and anti-cancer drugs are of plant origin [3], and natural products and their derivatives represent more than 50% of all the drugs in modern therapeutics, of which higher plants contribute no less than 25% of the total [4,5]. In addition, WHO supports, suggests and encourages traditional/herbal remedies in national healthcare programs as these medicines are easily available, low cost, safe and people rely on them [6]. The WHO also supports and encourages the study of plants and extracting medicines from them, with the initial process usually begins with identifying plant species of interest, especially those with documented traditional use [7], so establishing pharmacognostic standards are very important parameters for evaluation of medicinal plants [6,8]. This is followed by extraction, fractionation, and isolation of the bioactive compound where applicable. In addition, it comprises determination of quantity and

quality of bioactive compounds [9,6]. During the last three decades antioxidant-based drugs for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared [10]. Phenolic compounds such as phenolic acid, flavonoid and tannin are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity [11,12]. The antimicrobial properties of plants are due to the presence of phytochemicals such as phenolic and tannin compounds in them [13,14].

Tagetes is an important genus belonging to the Asteraceae family and consists at least of 56 species [15]. These plants contain a high number of bioactive compounds, including polyphenolic, carotenoids, and terpenes that have biological properties such as antibacterial, antioxidant, antiviral, and anticancer activities, among others [16, 17]. Volatile oils extracted from the leaves and aerial parts of *Tagetes minuta* have been widely studied in many countries [18, 19]. In Yemen, medicinal plants are widely used, although many of them have not been studied [20, 21]. *Tagetes minuta* L. grows in different regions of Yemen, including Al-Dhalea (Ashaib) [22], and is used to treat skin diseases. Previous investigation have reported cytotoxic, antioxidant and antimicrobial activities of the essential oil of Tagetes minuta from Dhamar [23]. Despite the traditional uses of *Tagetes minuta* L., there is a lack of adequate studies investigating the pharmacognostical, phytochemical, antioxidant, and antimicrobial properties of its aerial parts. The aim of this study is to evaluate the anatomical, physicochemical, phytochemical, antioxidant, and antimicrobial properties of the aerial parts of *Tagetes minuta* L.

2. Material and Methods

2.1. Collection and identification of plant material

Aerial parts (leaves, flowers and soft stems) of *Tagetes minuta* Linn. (family Asteraceae) were collected in May 2023 from Al-Dhalea (Ashaib), Republic of Yemen, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant specimen was identified by Othman S. S. Al-Hawshabi, Department of Biology, Faculty of Science, University of Aden.

2.2. Anatomical study

The microscopic characteristics of leaves and stem were studies according to WHO guidelines [8]. Sections were cleared by heating with chloral hydrate solution and examined under microscope. Photomicrographs were taken with Leica USA model 2000ATC (ocular: CPL W10X; objective: 4X, 10X and 40X). Various identifying characters, such as type of trichomes, type of stomata and epidermal cells were recorded, and then photomicrography was done [24]. Photographs were taken with the help of digital camera (Sony 16 MP).

2.3. Physicochemical study

The physicochemical parameters of the aerial parts like moisture content, percentage extractives in different solvents, ash content, acid insoluble ash, water soluble ash and moisture content by loss on drying were determined by standard methods as in WHO guidelines [8].

2.4. Phytochemical Study

2.4.1. Preparation of the extracts

The dried powder of the aerial parts (50 gm) were defatted with petroleum ether (boiling point 60-80 0C) in Soxhlet extractor. The petroleum ether extract was filtered. The marc left after petroleum ether extract was dried completely in hot air oven below 50°C and then packed well in Soxhlet apparatus and extracted with 80% methanol (80-90°C), until the extraction was completed. The 80% methanol extract was filtered., after that the marc was extracted with distill water. The aqueous extract was filtered. All the obtained extracts were separately evaporated to dryness by rotary evaporator and the percentage yield was calculated for each extract. The dried crude extracts were stored in air tight bottle at 4°C for farther study [25, 26].

2.4.2. Qualitative phytochemical analysis

Various qualitative tests were performed to determine the chemical composition of the extracts according to standard methods [25, 26].

2.4.3. Determination of total phenolic content (TPC)

The total phenolic content of the 80% methanolic and water extracts was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth [27]. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of the plant extract (1 mg/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 765 nm using UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid (20, 40, 60, 80, 120, and 140 µg/ml). The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract. All the samples were analyzed in three replications.

2.4.4. Determination of total flavonoid content (TFC)

Total flavonoid content of 80% methanolic and water extracts was determined in extracts according to colorimetric method described by [28] with some modification. Briefly 0.5 ml extract (1 mg/ml) was added in three bijou bottles and mixed with 2 ml of distilled water. Subsequently add 0.15 ml of sodium nitrite ($NaNO_2$, 5% w/v) into each bottles and the reaction mixture was allowed to stand for 6 min. Then 0.15 ml aluminium trichloride ($AlCl_3$, 10%) was added and allowed to stand for 6 min, followed by addition of 2 ml of sodium hydroxide (NaOH, 4% w/v) to the reaction mixture. Then distilled water was added to the mixture to bring the final volume up to 5 ml. the reaction mixture was mixed thoroughly and allowed to stand for another 15 min. Then absorbance was measured at 513 nm against the same mixture but without extract as a blank using spectrophotometer. Methanol was used as blank. The final absorbance of each sample was compared with a standard curve plotted from quercetin (2.5–80 µg/ml). The total flavonoid content was expressed in mg of quercetin per gram of extract. The whole experiment was conducted in three replicates.

2.5. Thin layer chromatography of extracts

Thin layer chromatography of prepared extracts was performed for separation of spots. Various solvent systems were tested to obtain best results [29]. TLC plates were viewed in UV chamber and *Rf* values of separated spots were calculated.

2.6. Antioxidant Study

2.6.1. TLC-bioautography identification of antioxidants

About 2 µg of each extract was loaded on TLC plate (20 cm x 20 cm). The plate were developed in toluene-ethyl format-formic acid (8.5:4.5:1.5) to separate different constituents. The antioxidant constituents were analyzed by DPPH technique [30]. For this 0.05% of DPPH solution in methanol was sprayed on the developed TLC plate and incubated for 10 min at room temperature. The active antioxidant constituents of the extracts were detected in sunlight as yellow spots produced via reduction of DPPH by resolved bands against purple back ground on the TLC plate. The colour of the spots was noted and *Rf* values were calculated [29].

2.6.2. DPPH Radical Scavenging Activity

The free radical – scavenging activity of each extracts was determined as described by Chan et al. with slight modification [31]. Different dilutions of the extract (10, 20, 40, 60 and 80 µg/ml) were prepared. DPPH solution was also prepared by dissolving 6.0 mg of DPPH in 100 ml methanol. Then, 1 ml of extract from each dilution was added into the test tube containing 2 ml of DPPH solution. Control was prepared by adding 1 ml of methanol to 2 ml of DPPH solution. The mixture was shaken vigorously and was left to stand in the dark for 30 min. Quercetin was taken as reference compound. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. The scavenging activity of extract on DPPH radical was calculated using the following equation:

Inhibition % = [(A0 –A1) /A0] x 100;

A0 is the absorbance of control and A1 is absorbance of test.

2.6.3. Calculate IC50 value

The 50% inhibition (IC50) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radical's activity under these conditions [32]. The effective concentration of sample required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations. The data were presented as mean values ± standard deviation (n = 3).

2.7. Antimicrobial Activity

2.7.1. Agar well Diffusion Assay

Antimicrobial activity of 80% methanolic and aqueous extracts of aerial parts of *Tagetes minuta* has been determined against four bacterial strains; *Staphylococcus aureus, Proteus vulgaris., Pseudomonas aeruginosa* and *Klebsilla pneumoniae*, also one fungi (*Candid albicans*), which were clinically isolated samples and identified by microbiologist Dr. Abdullah Omer the head of microbiological section in the National Center for General and Central Laboratories in Aden.

2.7.2. Preparation of Nutrient Agar

Nutrient agar was prepared according to the manufacturer's instructions on the bottle. 28g of NA was suspended in 1000 ml of distilled water and boiled briefly to dissolve the ingredients, then sterilized by autoclaving at 121 °C. The agar was allowed to cool, then was poured into sterile petri dishes.

2.7.3. Preparation of Mueller Hinton Agar

The Mueller Hinton agar was prepared according to the manufacturer's Instructions on the bottle. 38g of MHA was suspended in 1000 ml of distilled water and boiled briefly to dissolve the ingredients, then sterilized by autoclaving at 121 °C. The agar was allowed to cool, then was poured into sterile petri dishes.

2.7.4. Preparation of Inoculums

Few isolated bacterial colonies were transferred to a tube of sterile normal saline and the turbidity of the bacterial suspension was adjusted to 1.5 108 CFU/ml by

comparison with 0.5 McFarland standard against a sheet of white paper with black stripes. A 0.5 McFarland standard was prepared by mixing 50 µl of 1.175% barium chloride dehydrate (BaCl2.2H2O) with 9.95 ml of 1% sulfuric acid (H_2SO_4). [33].

2.7.5. Antibacterial Assay

Antibacterial activity of the 80% methanolic and water extracts was tested by using well diffusion method according to [33, 34]. In Agar Well Diffusion Assay the Mueller Hinton agar plates were inoculated with respective bacteria (*Staphylococcus aureus, Proteus vulgaris., Pseudomonas aeruginosa* and *Klebsilla pneumoniae*) and left to dry for 10 minutes. 5 wells of 5mm diameter were made on each agar plate using sterile pipette tips. The wells were labeled as test wells and control wells. The test wells were filled with 50 µl of different concentrations of the stock solution prepared from the extracts. The concentrations used were (150, 200, 250 and 300 mg/ml). The control wells were filled with dimethyl sulfoxide for the plates containing the methanol and aqueous extracts. The antibiotic Imipenem (10 µg/disc), Ciprofloxacin (5 µg/disc), Augmentin (30 µg/disc), Gentamycin (10 µg/disc) and Amikacin (30 µg/disc) The antibacterial activity was determined after 24 hours following incubation of plates at 37 °C, by measuring the diameter of the inhibition zone (in mm) around both the wells and disks using a ruler. All experiments were performed in triplicate.

2.7.6. Antifungal Assay

Pure culture for fungi was prepared by spreading fungi suspension on Sterile physiological solution on Mueller Hinton Agar. Then the same steps were done as the antibacterial assay. Use Nystatin (100 µg/disc) Fluconazle (25 µg/disc) and Voriconazole (1 µg/disc) were used as positive control. The experiment was carried out in triplicate [35].

2.8. Statistical Analysis

Analysis of variance of data was evaluated by Student's t test P-values less than 0.05 was considered to be statistically significant.

3. Results and Discussion

3.1. Anatomical study

Epidermis of leaflet: In surface view of both upper and lower surfaces, it contains polygonal epidermal cells, isodiametric with sinuous undulating anticlinal walls, anomocytic stomata and very rarely uniseriate non glandular trichomes (Figure 1 A and B).The lower surface of the leaf contains orange, round and elliptical glands, the smallest of which are located along the leaf blade around the midrib, and the largest in the basal region of the teeth (Figure 1 C).

The venation pattern leaflet: The venation pattern was observed under a microscope, with the primary vein (midrib) appears massive with a straight course. Secondary veins were decurrent, alternated, forming straight to acute angles with the main vein. Tertiary veins were irregularly reticulated, forming venous islands with terminal veins, often with simple or branches (Figure 1 C).

Microscopical characteristics of stem:

In surface view, polygonal and rectangular epidermal cells with anomocytic stomata and non-glandular trichomes were found (Figure 2).

Transverse section of stem

The outer line of the stem's transverse section was rounded with bends and ridges. The epidermis is single layer. The Cortex occurs between the epidermis and the vascular tissues. It contains some collenchyma near the epidermis and parenchyma near the vascular tissues. 1-3 layers of sub epidermal angular collenchyma. Two to four layers of thin-walled, relatively large parenchyma. In the cortical parenchyma, alternating with the vascular bundles, secretory schizogenous ducts were found. Collateral open vascular bundles are found, where xylem is arranged towards the inner side and thus the phloem is arranged towards the surface. The pith was formed by thick walled parenchymatic cells, larger than cortex cells (Figure 3 A and B).

Plant-based drugs may be used directly as crude drugs or their chief constituents/active principles might be isolated and employed as medicines [36]. Macroscopic and microscopic examinations can have great taxonomic value in the correct identification of different plant taxa [37, 38], are the first step toward determining the identity and purity of plant material and should be performed before any other tests [8]. The present studies investigated the microscopic characteristics of the leaflets such as polygonal epidermal cells, isodiametric with sinuous undulating anticlinal walls, anomocytic stomata, uniseriate non-glandular trichomes, round and elliptical glands and venation pattern as well as microscopic characteristics of the stem such as epidermal cells, anomocytic stomata and non-glandular trichomes, which are in agreement with what was published in a previous research [39].

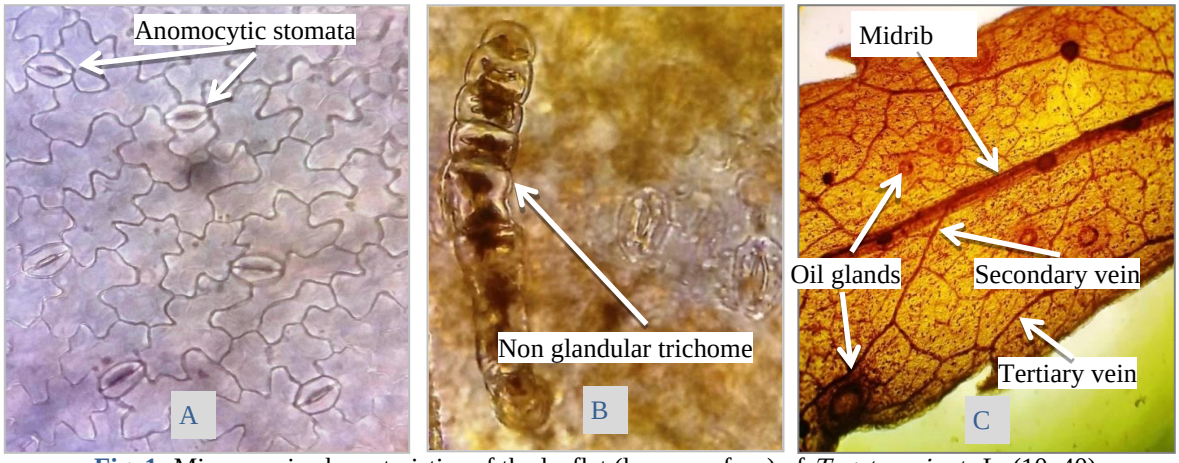

Fig. 1: Microscopic characteristics of the leaflet (lower surface) of *Tagetes minuta* L. (10x40); (A) Epidermal cells with stomata, (B) non glandular trichome, (C) venation pattern and oil glands.

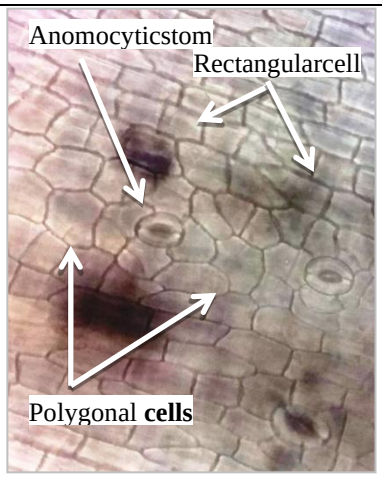

Fig. 2: Surface view of the stem epidermis of *Tagetes minuta* L. (10x40)

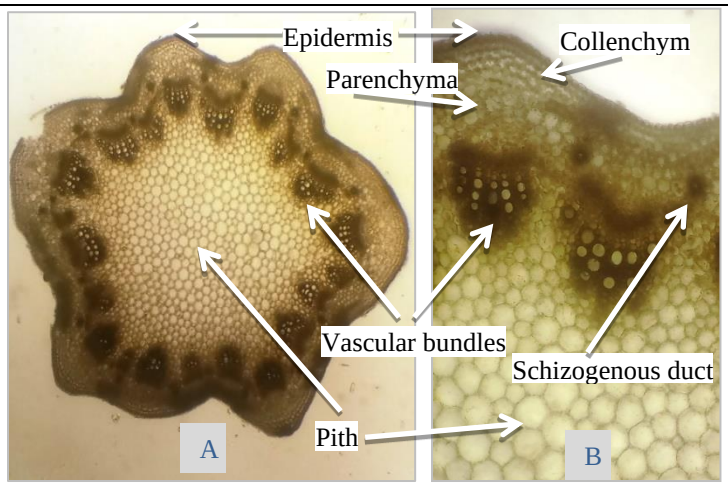

Fig. 3: Transverse section of the stem of *Tagetes minuta* L. under 10x20 magnification (A) and under 10x40 magnification (B)

3.2. Physicochemical Analysis

The physicochemical analysis provides crucial insights into the quality, purity, and authenticity of crude drugs. The **ash values** (total, acid-insoluble, and water-soluble ash) reflect the inorganic components and impurities. The results of the present study showed that the total ash value (13.30%) indicates the overall mineral content, while the acid-insoluble ash (1.40%) suggests minimal contamination with siliceous matter, ensuring the plant's quality, and the water-soluble ash (5.56%) supports the presence of water-soluble inorganic compounds, which may have biological significance. The **extractive values** highlight the solubility of active compounds in different solvents. The high water-soluble extractive value (27.33%) underscores the plant's potential for water-based extractions, the ethanol-soluble value (11.86%) suggests the presence of moderate polar compounds like flavonoids and phenols, while the petroleum ether extractive value (2.13%) points to a low concentration of non-polar compounds, such as lipids or sterols. The **moisture content** (7.02%) suggests the plant material is adequately dried, reducing the risk of microbial growth and spoilage, ensuring its stability and storage quality. The above mentioned physicochemical parameters are at an acceptable level (Table 1).

A high ash value is indicative of contamination, substitution, adulteration [40]. Acid insoluble ash indicates contamination with silica. Water soluble ash is a good indicator of the water soluble salts in the drug. Extractive values are representative of the presence of the polar or nonpolar extractable compounds in a plant material. Moisture is an inevitable component of crude drugs, and high moisture content leads to spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles [40]. The shelf life of the drug also increases with lowering the moisture contents [41]. Analysis of physicochemical parameters is important in determination of adulterants, quality and purity of a crude drug [8, 42, 43]. The physicochemical parameters obtained from this studt are important for the standardization of the aerial parts of *Tagetes minuta*.

Table 1: Physicochemical parameter of the aerial parts of *Tagetes minuta* L.

Physicochemical parameters	Value of physicochemical parameters (%w/w)
Ash values:	
1) Total ash	13.30±0.08
2) Acid insoluble ash	1.40±0.04
3) Water soluble ash	5.56±0.02
Extractive values:	
1) Water soluble	27.33 ±0.16
2) Ethanol soluble	11.86 ±0.09
3) Petroleum ether	2.13 ±0.07
Moisture content	
Loss on drying at 110ºC	7.02 ±0.03

3.3. Phytochemical Analysis

3.3.1. Qualitative phytochemical studies

The phytochemical screening methods are employed for identification of the species-based specific compounds in herbal medicines [44, 45]. The existence of secondary plant metabolites including alkaloids, flavonoids, glycosides, tannins, steroids, etc. might be attributed to the various pharmacological effects such as anti-inflammatory, antibacterial, antiviral, antioxidant and anticancer [46, 47].The percentage yield of petroleum ether, 80%methanol, and water extracts of the aerial part of *Tagetes minuta* was 2.305%, 16.29 % and 6.22 % respectively. Phytochemical screening of the studied extracts was represented in Table 2. Phytoconstituents like carbohydrates, phenols, flavonoids, tannins, triterpenes and sterols were identified, while saponins and alkaloids were not found. The genus *Tagetes* is known to contain a large number of biologically active compounds, including polyphenols, carotenoids, and terpenes [16, 17].

3.3.2. Determination of the total phenolic and flavonoid content

Estimation of total phenols and flavonoids was performed in this study. The content of the phenolic compounds of 80% methanolic and water extracts of *Tagetesminuta L.* was determined from regression equation of calibration curve (y=0.0028x-0.0112, R^2 = 0.9936) of qallic acid (20–140 µg/mL) and expressed in mg gallic acid equivalent (GAE) per gram dry extract. The result was 205.14 ±3.30 mg/g and 91.2 ±2.50 mg/g equivalent (GAE) of dry extract in 80% methanolic and water extracts respectively. The total flavonoid content of 80% methanolic and water extracts was determined from regression equation of calibration curve (y=0.0065x+0.0067, R^2 =0.9908) of quercetin (2.5-60 µg/mL) and expressed in mg quercetin equivalent (QE) per gram dry extract. The result was 106.46 ±4.11 mg/g and 18.15 ±2.30 mg/g equivalent (QE) of dry extract in 80% methanolic and water extracts respectively. Standard calibration curves for qallic acid and quercetin are shown in Figure 4 and Figure 5, respectively. Phytochemical analyses highlight the therapeutic potential of *Tagetes minuta* L. The methanolic extract has been shown to be the most potent, rich in phenols and flavonoids, known for its antioxidant and therapeutic properties.

Table 2: Results of phytochemical screenings of successive extracts of the *Tagetesminuta*L.

Phytochemicals	Tests	Petroleum Ether extract	Methanol extract	Water extract
Alkaloids	Wagner's test	-	-	-
	Mayer's test	-	-	-
	Dragendorff's reagent	-	-	-
Phenols	Ferric chloride test	-	+++	+
Flavonoids	Shinoda test	-	++	+
	NaOH Test	-	++	+
	Lead acetate test	-	++	+
	Aluminium solution test	-	+	+
Saponins	Foam test	-	-	-
Sterols/ Triterpenes	Salkowski test	+++	+++	+
	Liebermann-Burchard test	+++	+++	+
Carbohydrates	Benedict's test	-	+	-
	Fehling's test	-	+	-

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.

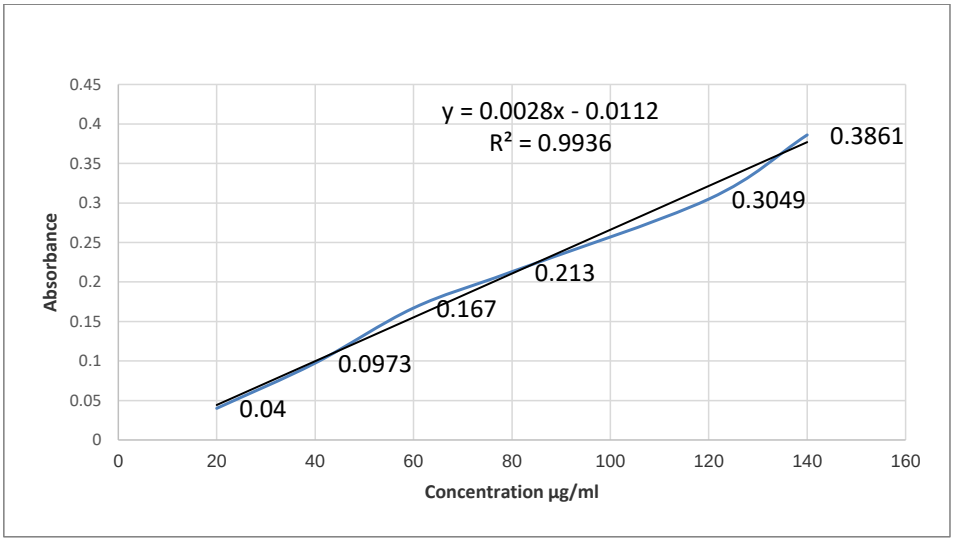

Fig.4: Calibration curve of qallic acid

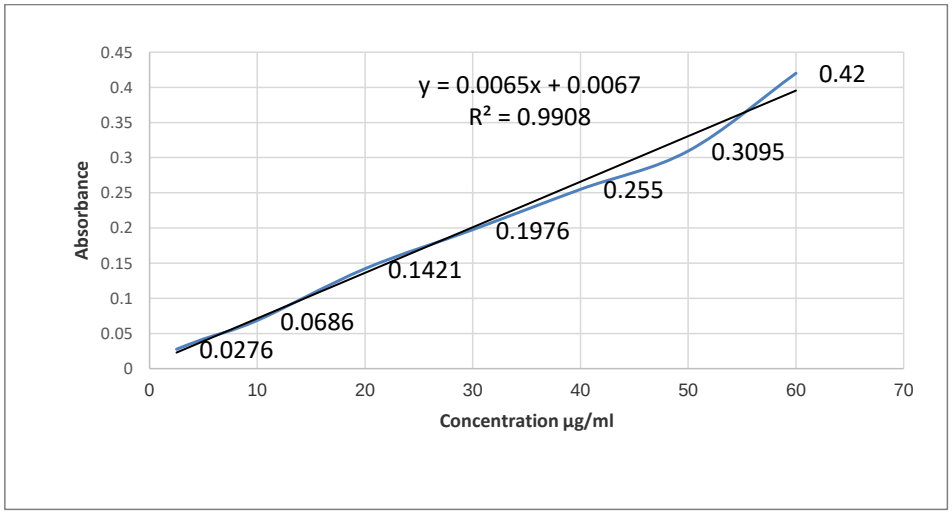

Fig. 5: Calibration curve of quercetin

3.3.3. Thin layer chromatography studies

The presence of phytoconstituents in the petroleum ether, 80% methanolic and water extracts was further confirmed by thin layer chromatography. A number of developed solvent systems were tested, the best of which was toluene-ethyl formate-formic acid (8:4:1), which revealed 9 spots (*Rf Value*s 0.26, 0.41, 0.50, 0.52, 0.60, 0.62, 0.70, 0.80, 0.83), 11 spots (*Rf Value*s 0.26, 0.27, 0.41, 0.50, 0.52, 0.60,0.62, 0.68, 0.70, 0.80, 0.83) and 7 spots (*Rf Value*s 0.16, 0.26, 0.60, 0.68, 0.70, 0.80, 0.83) inpetroleum ether, 80% methanolic and water extracts respectively (Figure 6). The TLC profiles reinforce the plant's chemical diversity, validating its traditional use in herbal medicine.

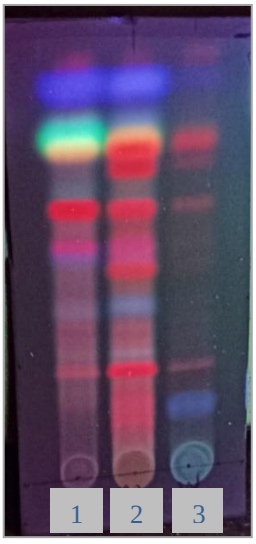

Fig. 6: TLC plate of petroleum ether (1), 80% methanolic (2) and water (3) extracts, in toluene - ethyl format-formic acid (8:4:0.2)under UV 365

3.4. Antioxidant Study

3.4.1. TLC bioautography assay

A TLC bioautography method was employed to detect the antioxidant substances of petroleum ether, 80% methanolic and water extracts of the aerial parts of *Tagete sminuta* L. After developed TLC plat was sprayed with 0.05% DPPH reagent and observed with visible light (Figure7). Isolated spots with free radical scavenging activity acquired yellow colors on a purple background [48]. Four spots (*Rf Value*s 0.09, 0.20, o.25 and 0.32) were detected in 80% methanolic extract and one spot (*Rf Value* 0.27) in water extract. The eluted spots showed yellow colour corresponding with antioxidant behavior. The result showed that the predominance of active spots in the methanolic extract underscores its richness in antioxidant phytochemicals, aligning with its higher total phenolic and flavonoid content. The single active spot in the water extract indicates a more limited range of antioxidant compounds but highlights the extract's pharmacological relevance. The petroleum ether extract did not exhibit antioxidant spots, consistent with its lower content of phenols and flavonoids.

Fig. 7: TLC bioautography of petroleum ether (1), 80% methanolic (2) and water (3) extracts, in toluene - ethyl format-formic acid (8:4:0.2) indaylight.

3.4.2. DPPH radical scavenging activity assay

The antioxidant activity of petroleum ether, 80% methanolic and water extracts of the aerial part of *Tagetes minuta*L was determined by using In Vitro DPPH method. The free radical DPPH in solution is purple in color with its odd electron and gives a strong absorption maximum at 517 nm and the color of DPPH turns from purple to yellow (Figures 8-11). Result of antioxidant activity test can be seen in Table 3 and Figure 12. The antioxidant activity was increased by increasing the extract concentration in a dose-dependent manner, the greater the color change, the greater the antioxidant capacity, which is represented by the lower IC50 value. The IC50 values of quercetin, petroleum ether, 80% methanolic and water extracts were found to be 13.54, 151.04, 21.36 and 47.31 µg/ml respectively. The result indicates that the methanolic extract has the strongest antioxidant activity among the extracts, and this result is consistent with its high phenolic and flavonoid content and the presence of multiple antioxidant spots in the TLC bioimaging assay. The water extract demonstrated moderate activity, reflecting its lower phenolic and flavonoid content, while the petroleum ether extract showed weak antioxidant activity. The findings align with earlier studies that reported the antioxidants activity in the *Tagetes* genus [18, 19], especially in the essential oil (IC50 value 36 µg/ml) of the leaves of *Tagetes minuta* L., collected from Dhamar city [23].

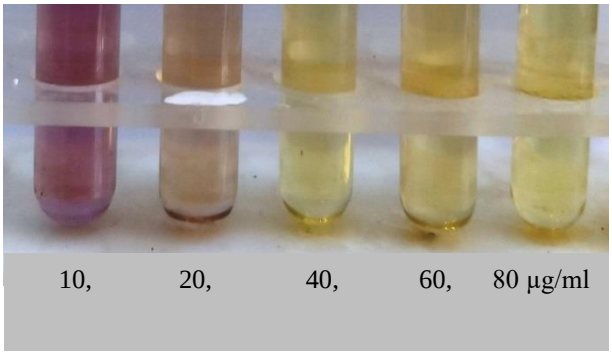

Fig. 8: Dose dependent decolorization of DPPH from purple to yellow by quercetin with different concentrations (10, 20, 40,60 and 80 µg/ml).

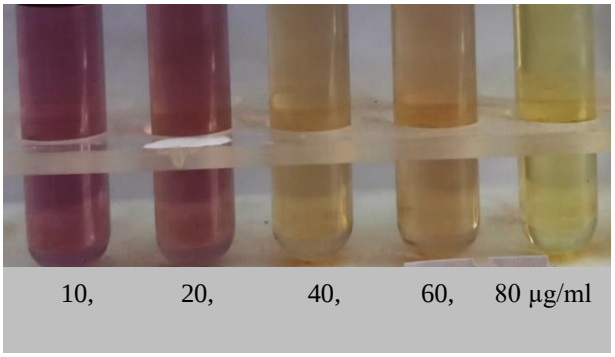

Fig. 9: Dose dependent decolorization of DPPH from purple to yellow by methanolic extract with different concentrations (10, 20, 40, 60 and 80 µg/ml).

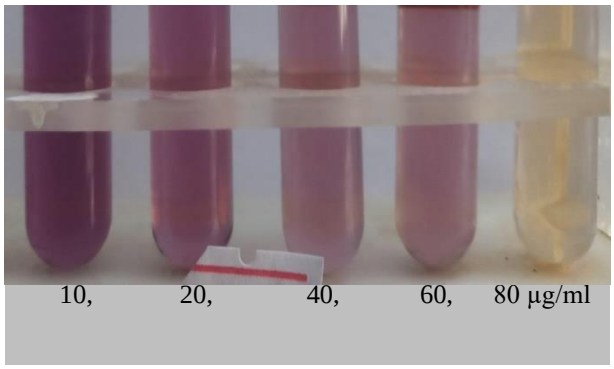

Fig. 10: Dose dependent decolorization of DPPH from purple to yellow by water extract with different concentrations (10, 20, 40, 60 and 80 µg/ml).

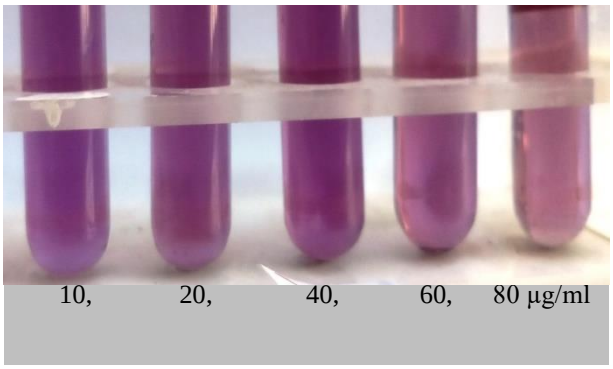

Fig. 11: Dose dependent decolorization of DPPH from purple to yellow by petroleum ether extract with different concentrations (10, 20, 40, 60 and 80 µg/ml).

Table 3: Result of DPPH free radical scavenging by the studied extracts and quercetin.

Concentrations (µg/ml)	% of scavenging			
	Qurcetine	Petroleum ether	Methanol	Water
10	45.1±2.33	11.32±4.12	39±2.51	16.83±3.21
20	54.3±3.41	16.1±3.41	49±3.30	24.09±2.42
40	73.2±4.01	21.2±2.32	67±4.02	39.49±3.52
60	87.3±2.62	26.7±3.22	82±3.12	57.94±4.02
80	96.4±3.23	30.2±4.11	92±2.32	86.01±3.11
IC50 (µg/ml)	13.54	151.04	21.36	47.31

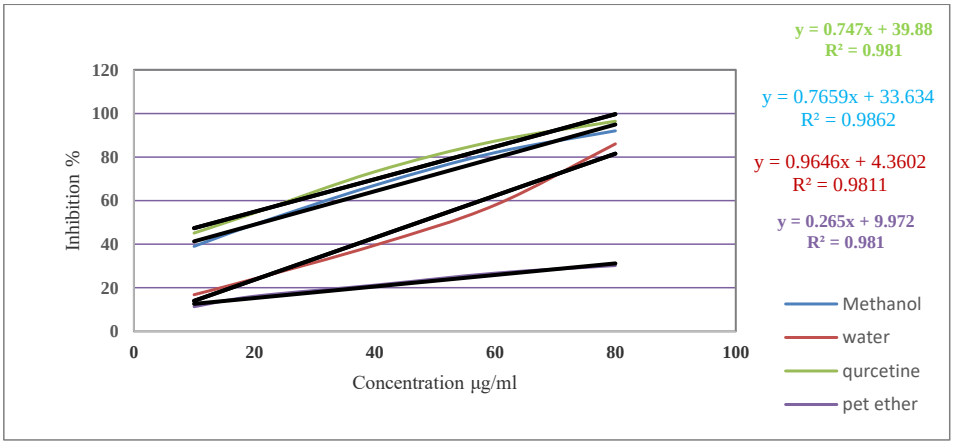

Fig. 12: The DPPH free radical scavenging activity of petroleum ether, 80% methanolic and water extracts and

3.5. Antimicrobial activity

3.5.1. Agar well Diffusion Assay

Methanolic (80%) and aqueous extracts of the aerial parts of *Tagetes minuta* showed antimicrobial activity when compared with standard antibiotics against the tested microorganisms, and the results are represented in Tables 4, 5 and 6, and Figures 13 and 14. The antimicrobial activity was determined by the presence or absence of inhibition zone around the wells. The extracts of *Tagetes minuta* scored smallest inhibition zone from lowest concentration (150, 200, 250 and 300 mg/ml) with increasing inhibition zone diameters as increasing concentrations. The 80% methanolic extract showed higher activity at different concentrations than the aqueous extract. All concentrations used for the 80% methanolic extract showed very high antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*, while they showed significant of inhibition zone against *Klebsilla pneumoniae*, and *Candid albicans*. All concentrations used for the aqueous extract showed appearance of inhibition zone against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, while they showed no effect against the *Klebsiella pneumoniae*, *proteus vulgaris*. and *Candid albicans*.The 80% methanolic extract at 250 and 300mg/ml showed highest activity against *Staphylococcus aureus* and *Proteus vulgaris*; producing inhibition zone diameter 35 and 38 mm; 37and 38mm respectively, followed *Pseudomonas aeruginosa* 34 and 35 mm respectively, with increasing significant compared to the rest concentrations. The aqueous extract at concentrations of 250 and 300 mg/ml showed high activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* resulting in an inhibition zone of 25 and 27 mm diameter for each of them, respectively. These results indicate that the methanolic extract is more effective as an antimicrobial agent than the aqueous extract, likely due to its higher concentration of bioactive compounds (flavonoids, phenols), supporting previously published literature [16, 17]. In addition, the observed antimicrobial activity of the methanolic extract against both gram-positive and gram-negative bacteria, as well as fungi, indicates its potential as a broad-spectrum antimicrobial agent. The aqueous extract's limited efficacy highlights the role of solvent polarity in extracting bioactive compounds. Also the antimicrobial activity of *Tagetes minuta* extracts, especially the methanolic extract, is in line with previous research highlighting the antimicrobial properties of its essential oils. For example, the essential oil of *Tagetes minuta* collected from Yemen showed significant activity against methicillin-resistant *Staphylococcus aureus* (23 mM) and *Candida albicans* (26 mM), confirming the findings of the present study on the efficacy of the plant against these pathogens [23]. Medicinal plants are considered new sources for producing antimicrobial agents that could act as alternatives to antibiotics in the treatment of antibiotic-resistant bacteria [49].

3.5.2. Sensitivity test of studied Microbes against some used Antibiotics

The results in Tables 5 and 6 showed the sensitivity of all studied bacteria against all antibiotics used except for augmentin, which did not show an inhibitory effect against *Klebseilla pneumonia* and *Pseudomonas aeruginosa*. Ciprofloxacine and impeneme also had a resistance effect against *Pseudomonas aeruginosa* and *Proteus vulgaris*, respectively. It was found that the diameters of the inhibition zone for antibiotics ranged between 17 - 40 mm, as *Proteus vulgaris*was less sensitive to the antibiotic gentamicin (17 mm), while *Proteus vulgaris*was more sensitive to the antibiotic ciprofloxacin (40 mm).*Candid albicans* showed sensitivity against nystatin, fluconazole and voriconazole ranged from (17-30 mm) with high sensitivity against voriconazole (30 mm).

Table 4: Inhibition effect (mm inhibition zones diameter) of A *Tagetes minuta* (aerial part) against microbial tested by Agar well diffusion at concentration (150, 200, 250, 300 mg/ml)

Extract / Microorganisms	80% Methanolic extract				Aqueous extract			
Concentration (mg/ml)	150 (mm)	200 (mm)	250 (mm)	300 (mm)	150 (mm)	200 (mm)	250 (mm)	300 (mm)
Klebsiella pneumoniae	24 ±0.40	25 ±0.25	17.83 ±0.7	26 ±0.3	-	-	-	-
Pseudomonas aeruginosa	32 ±0.3	33 ±0.3	34 ±0.29	35 ±0.4	20 ±0.4	23 ± 0.3	25 ±0.2	27 ±0.5
Staphylococcus aureus	30 ±0.3	32 ±0.6	35 ±0.3	38 ±0.3	20 ±0.40	23 ±0.5	25 ±0.4	27 ±0.3
Proteus vulgaris	34 ±0.3	36 ±0.5	37 ±0.2	38 ±1.4	-	-	-	-
Candida albicans	21 ± 0.5	22 ±0.2	22.5 ±0.25	23 ± 0.1	-	-	-	-

Table 5: Sensitivity test (mm) of microorganisms tested against some antibiotics

Antibiotic drugs / Microorganisms	Augmentin (Amc)	Ciprofloxacin (Cip)	Gentamycin (CN)	Amikacin (Ak)	Imipenem (IPM)
Klebsiella pneumoniae	resistance	24± 0.5	19 ± 0.58	18±0.2	24± 0.4
Pseudomonas aeruginosa	intermediate resistance	resistance	20± 0.5	16±0.2	33± 0.3
Staphylococcus aureus	21±0.3	30±0.58	25± 0.3	23±0.4	39± 0.2
Proteus vulvaris	21± 0.1	40± 0.29	17± 0.5	18±0.3	resistance

Table 6: Sensitivity test (mm) of fungi tested against some antifungal drugs

Antifungal drugs / Fungi	Nystatin (NS)	Fluconazle (FLC)	Voriconazole (VRC)
Candida albicans	17mm	27mm	30mm

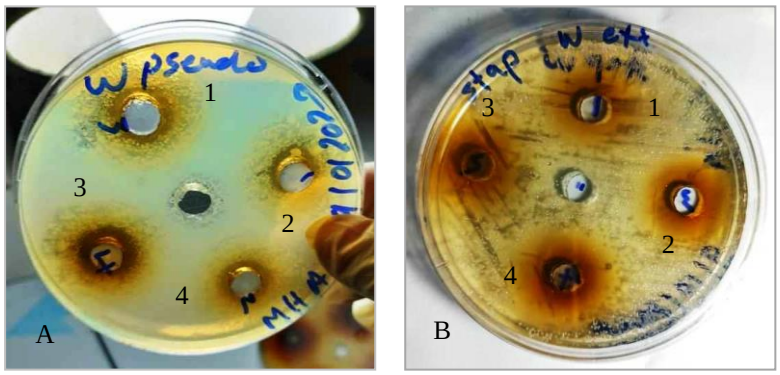

Fig. 13: A culture plate showing diameter of zones of inhibition of microbial growth for Aqueous extract of A: Pseudomonas aeruginosa, B: Staphylococcus aureus with concentrations of 1 = 150 mg/ml 2 = 200 mg/ml 3 = 250 mg/ml 4= 300 mg/ml

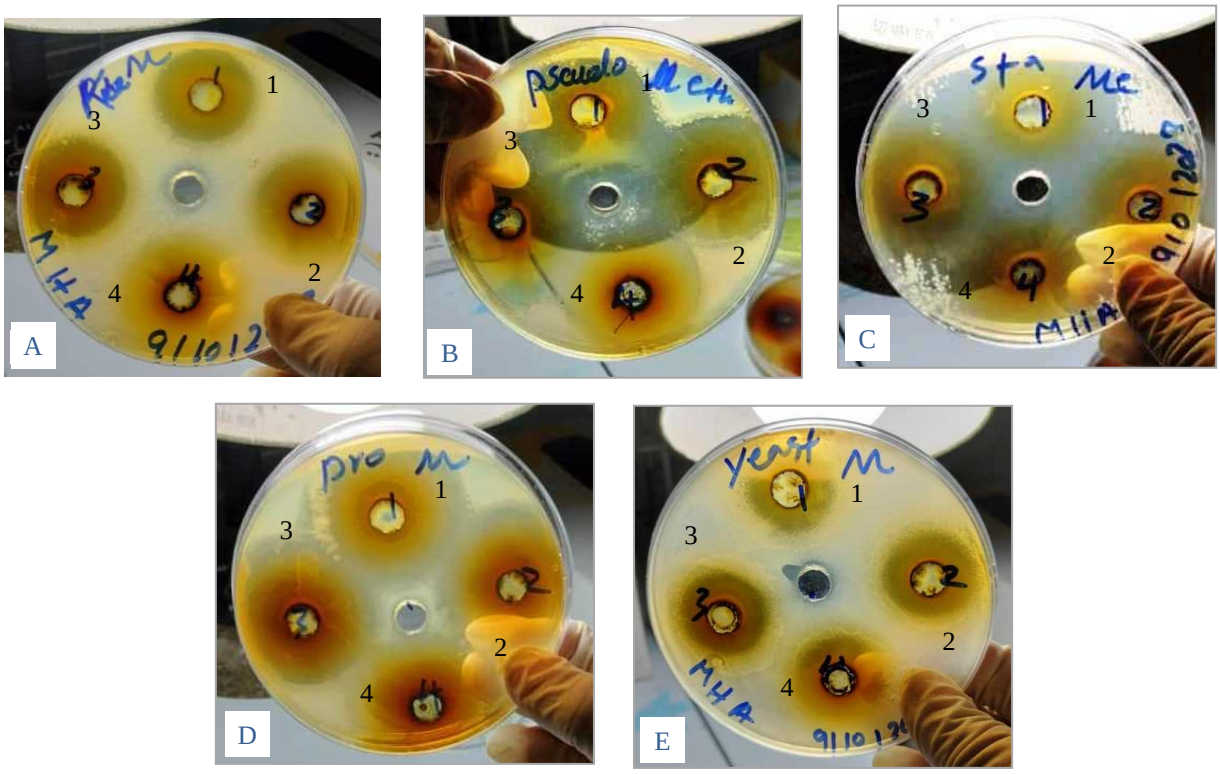

Fig. 14: A culture plate showing diameter of zones of inhibition of microbial growth for 80% Methanol extract of A: *Klebsiella pneumoniae*, B: *Pseudomonas aeruginosa*, C: *Staphylococcus aureus*, D: *Proteus vulvaris*. and E: *Candid albicans*, with concentrations of 1 = 150 mg/ml 2 = 200 mg/ml 3 = 250 mg/ml 4= 300 mg/ml

Conclusion

Tagetes minuta is a medicinal plant widely used in Yemen. Its aerial parts have been studied, and according to the results obtained, the aerial parts of the *Tagetes minuta* plant have promising potential as a source of antioxidants and antimicrobials.

Acknowledgments

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Researcher Information

ORCID
Saleh Kassem Algfri: 0000-0003-3752-2251

مقالة بحثية

دراسات تشريحية، فيزيكيميائية، كيميانباتية، مضادات أكسدة ومضادات ميكروبات للأجزاء الهوائية من نبات الرنجس

صالح قاسم الجفري[1،*]، جوليا أحمد ناصر[1]، رشيد سعيد راجح[2]، أحمد بن شعيب[1]، آمنة هيثم ناصر[3]

[1] قسم العقاقير، كلية الصيدلة، جامعة عدن، اليمن.
[2] قسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة عدن، اليمن.
[3] منظمة صناع النهضة، عدن.

* الباحث الممثل: صالح قاسم الجفري؛ البريد الالكتروني: algfri_s25@yahoo.com؛ algfri_s25@pharm.adenuniv.com؛ algfris25@gmail.com؛ رقم الجوال: (+967)773752084

استلم في: 06 ديسمبر 2024 / قبل في: 26 ديسمبر 2024 / نشر في 31 ديسمبر 2024

المُلخّص

يستخدم نبات الرنجس (*Tagetesminuta* L.) الذي ينمو في مناطق مختلفة من اليمن لعلاج أمراض الجلد ولم تتم دراسته بشكل كافٍ، لذا فإن الهدف من البحث الحالي هو تقييم خصائصه التشريحية، الفيزيكيميائية النباتية، مضادات الأكسدة ومضادات الميكروبات لأجزائه العلوية باستخدام طرق قياسية مقبولة. تم تحديد المعايير المجهرية والفيزيكيميائية. تم إجراء التحليل الكيميائي النباتي على مستخلصات البترول الإيثرية والميثانول بنسبة 80% والماء، حيث وجد فيهما الكربوهيدرات والفينولات والفلافونويدات والعفص والتربينات والستيرولات. تم تحديد أن المحتوى الفينولي الكلي 205.14 و 91.2 ملغ / غرام مكافئ حمض الجاليك ووجد أن المحتوى الفلافونويدي الكلي 106.46 و 18.15 ملغ / غرام مكافئ كيورسيتين للمستخلص الجاف للميثانول بنسبة 80 % والمستخلصات المائية على التوالي. تم تحديد النشاط المضاد للأكسدة باستخدام طريقة DPPH في المختبر. وجد أن قيم IC50 للكويرسيتين، إيثير البترول، 80 % مستخلصات الميثانول والمائية كانت 13.54، 151.04، 21.36 و 47.31 ميكروجرام/مل على التوالي، لذا أظهر المستخلص الميثانولي 80% نشاطًا مضادًا للأكسدة مرتفعًا جدًا مقارنة بالكويرسيتين. تم اختبار النشاط المضاد للميكروبات باستخدام طريقة الانتشار البئري، أظهرت جميع التركيزات المستخدمة للمستخلص الميثانولي 80% نشاطًا مضادًا للبكتيريا مرتفعًا جدًا ضد *Staphylococcus aureus* , *Pseudomonas aeruginosa* و *Proteus vulgaris*، بينما أظهرت منطقة تثبيط كبيرة ضد *Klebsilla pneumoniae,* و *Candid albican*. أظهرت جميع التركيزات المستخدمة في المستخلص المائي ظهور منطقة تثبيط ضد *Pseudomonas aeruginosa* و*Staphylococcus aureus*، في حين لم يظهر أي تأثير ضد *Klebsiella pneumoniae* و*proteus vulgaris* و*Candid albicans*. تشير النتائج المذكورة أعلاه إلى أن الأجزاء الهوائية من نبات الرنجس (*Tagetes minuta*) يمكن أن تكون مضادة للأكسدة ومضادة للبكتيريا بشكل طبيعي، ويمكن استخدامها لمزيد من الدراسات على النبات.

الكلمات المفتاحية: الرنجس، تشريح، كيميانباتي، مضاد للأكسدة، مضاد للميكروبات.

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