Study of phytochemical analysis and antimicrobial activity of ethanolic extract of *Nigella Sativa* L. and *Matricaria Chamomilla* L

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ABSTRACT

Background: *Nigella sativa* L. and *Matricaria chamomilla* L. are used widely as traditional medicine since ancient ages because they contain some substances believed to have antitumor, anti-oxidants, anti-inflammatory, and antimicrobial activities. **Objective:** to assess the quantitative assay for alkaloids, tannins, phenolic acids, saponins and flavonoids in *Nigella sativa* L. and *Matricaria chamomilla* L. extracts with evaluation of their antimicrobial activity. **Results:** the quantitative assay for *Nigella sativa* L. and *Matricaria chamomilla* L. extracts were total flavonoids (295.21±0.51 and 263.12±1.05 rutin Eq/g DW), total phenolic acids (388.60±1.02 and 302.10±0.88 µg gallic acid equivalent (GAE)/mg DW), total tannins (1.23±0.11 and 1.33±0.13%), total saponins (1.71±0.10 and 1.66±0.16%) and total alkaloids (2.01±0.20 and 1.07±0.14 %), respectively. Based on the observed results, *Nigella sativa* L. and *Matricaria chamomilla* L. extracts contain active anti-microbial substances. Diameters of the inhibition zones of *Nigella sativa* L. and *Matricaria chamomilla* L. extracts were about (17 and 15 – 14 and 18 – 8 and 8 – 12 and 10 – 15 and 13 – 16 and 14 mm) against *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli, Klebsiella pneumoniae, Candida albicans* and *Candida glabrata* respectively. The minimum inhibitory concentrations MICs of *Nigella sativa* L. against the bacterial and candida strains ranged from 250 µg/ml to 500 µg/ml and MLCs ranged from 500 µg/ml to 1000 µg/ml. where the MICs values of *Matricaria chamomilla* L. against the bacterial and candida strains ranged from 500 µg/ml to 1000 µg/ml and minimum lethal concentrations MLCs ranged from 1000 µg/ml to 2000 µg/ml. **Conclusion:** this study demonstrates the *Nigella sativa* L. *Matricaria chamomilla* L. efficacy as an therapeutic agents in the treatment of many microbial diseases to provide a comprehensive guide for pathologists

Key words: Black cumin, Chamomile, Alkaloids, Tannins, Phenolics, Saponins, Flavonoids.

INTRODUCTION

Nigella sativa (family: Ranunculaceae) in Asia is known as black cumin but in Western countries known as black seeds. In many Arabic countries, it is called habbatussauda, and in India it is known as kalonji (Ahmad *et al.*, 2013). *N. sativa* used widely as traditional medicine since ancient ages because it contains some substances that are believed to have antitumor, anti-oxidants, anti-inflammatory, and antimicrobial activities (Gholamnezhad *et al.,* 2016). The ulcers caused by diabetes tend to be wet with a lot of dead tissues. The dead tissues are a favorite nutrient medium for bacterial and fungal growth (Bale *et al.*, 2004). The difficult infection that makes ulcers caused by diabetes is difficult to be treated. Many research results recorded that the bacteria causing an infection in diabetic ulcers are *Pseudomonas aureginosa*, *Streptococcus*, *Pseudomonas Mirabilis*, *Staphylococci*, *Escherichia coli* and *Klebsiella pneumoniae* (Sutjhajo *et al.*, 2012). Many studies reported that *Nigella sativa* seeds extracts are used as an antibacterial agent against bacteria causing diabetic ulcers infections and removing renal stone and treating coughs and has an important role in cancerous cells growth inhibition. Moreover, it is used for the treatment of diarrhea and abdominal pain (Enomoto *et al.,* 2001). In Pakistan, *N. Sativa* is also used to manage lactation and bacterial infections (Aziz *et al.*, 2017), while in Morocco, the seeds are suggested for urological, nephrological, and otolaryngological conditions, as well as treating infections of the respiratory system, skeleton-muscular system hyper-sensibility, and urological conditions (Jamila and Mostafa, 2014). It has been suggested to use bee honey along with *N. sativa* seeds to enhance its anti-rheumatic capabilities (Khabbach *et al.*, 2012). In addition, seeds (in fresh, dried, and powdered forms) and leaves are used in Ethiopia and Malaysian traditional medicine to cure malaria (Al-Adhroey *et al.,* 2012) and in Ethiopia, leaves and seeds are used in (fresh, dried, and powdered) forms. The use of *Nigella sativa* is even more common, and it is regarded as a panacea for its curative effects in Bangladesh and Qatar (Jennings *et al.,* 2015 and Alrawi *et al.,* 2017).

One of the most significant traditional herbal remedies is the *Matricaria chamomilla* plant. This herbal plant, which belongs to the Asteraceae family, is still utilized in a variety of biomedical fields, including the cosmetic and pharmaceutical industries (Roby *et al.*, 2013 and Agatonovic-Kustrin *et al.*, 2015). The *Matricaria chamomilla* plant has very potent antibacterial and antioxidant properties (Stanojevic *et al.*, 2016). Numerous studies have shown that chamomile extract and essential oil have strong antibacterial properties against a wide range of bacteria (both Gram +ve and Gram -ve), including *Salmonella typhimurium*, *E. coli*, *Bacillus*, and *S. aureus*. The high concentration of several phenolic chemicals in this plant also contributes to its remarkable antibacterial action. Terpenoids, flavonoids, phenolic, matricin and apigenin chemicals are also present in the chamomile plant (Ismail *et al.*, 2013). The primary cause of chamomile extract's antioxidant action is the presence of flavonoids. Antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, analgesic, anti-hyperglycemic, anti-stress, hepatoprotective, and antihypertensive qualities are only a few of the pharmacological effects of chamomile plant extract (Formisano *et al.*, 2015). Chamomile was highly regarded in monastic medicine during the middle ages. Chamomile has been shown to relieve stomach and abdominal pain, menstrual discomfort, dermatitis, mouth inflammation from gargling, and genital inflammation (Srivastava and Gupta, 2011). Avicenna recognized the use of various medicinal plants in the Canon of Medicine, and the chamomile plant is one of them. He used it for edema, headache relief, toothache, jaundice, lithiasis conjunctivitis, chronic fever, amenorrhea, aphthous ulcers, and muscle tightness. Chamomile also was advised to treat a variety of inflammatory and itchy conditions, as well as to speed the recovery of skin lesions in a variety of patients undergoing various surgeries (Mahdizadeh and Ghadiri, 2015).

MATERIALS AND METHODS

Collection and processing of the seeds

N. sativa and flowers of *M. chamomilla* were purchased from Agricultural Research Center in Cairo, Egypt. After drying, the *N. sativa* seeds and chamomile flowers were grounded into powder by an electric grinder and preserved.

Preparation of extracts:

A 100g of powdered materials were dissolved in 1liter of ethanol 70 % and kept for 24h, at 25°C. After 24 h, the materials were filtered and the filtrate was placed on water bath at 60°C for the evaporation of extra solvents. The extracts obtained were refrigerated at 4°C for further studies.

Quantitative analysis:

The quantitative assay is carried out for alkaloids, tannins, phenolic acids, saponins and flavonoids.

Estimation of total phenolic content (TPC):

The amount of total phenolic in extract was determined with the Folin Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic was expressed as μg gallic acid equivalent (GAE)/mg DW. Concentrations of 10, 20, 30, 40 and 50 μg/ml of gallic acid were prepared in methanol. Concentration of 1mg/ml of plant extract was also prepared in methanol and 0.5 ml of each sample were introduced into test and mixed with 2.5ml of a 10 fold dilute Folin Ciocalteu reagent and 2ml 0f 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760 nm spectrophotometrically. All determination was performed in triplicate. The Folin Ciocalteu reagent is sensitive to reducing compounds including polyphenols. They produce a blue color upon reaction. This blue color was measured spectrophotometrically (Chun *et al.*, 2003 & Maurya and Singh, 2010). Line of regression from gallic acid was used for estimation of unknown phenol content. From standard curve of gallic acid line of regression was found to be y = 0.0013x + 0.056 and R2 = 0.9872 where (y) was the absorbance and (**x**) was the μg GAE/mg of the extract Thus the goodness of fit was found to be good for selected standard curve. By putting the absorbance of test sample (y = absorbance) in line of regression of above mentioned (GA.).

Estimation of total flavonoid content (TFC)

The amount of total flavonoid content in extract was determined by aluminum chloride assay through colorimetric method. A 0.5ml aliquot of appropriately diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15ml of a 5% $NaNO_2$ solution. After 6 minutes, 0.15 ml of a 10% $AlCl_3$ solution was added and allowed to stand for 6 minutes, then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water

was added to bring the final volume to 5ml, then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Rutin was used as standard compound for the quantification of total flavonoid. Total flavonoid content was expressed as mg rutin/g dry weight (mg rutin Eq/g DW), through the calibration curve of rutin. All samples were analyzed in three replications (Samatha *et al.,* 2012 & Han and May 2012).

$y = 0.0012x + 0.0011$

$R2 = 0.9986$

Where (**y**) was the absorbance and (**x**) was the μg rutin/mg of the extract.

Estimation of total tannins by using gravimetric method (copper acetate method)

This method depends on quantitative precipitation of tannin with copper acetate solution, igniting the copper tannate to copper oxide and weighing the residual copper oxide (Ali *et al.,* 2011). Two grams of samples were separately extracted for about one hour with two successive quantities, each of 100 ml of acetone-water (1:1) and then filtered. The combined extract, in each case, was separately transferred into a 250 ml volumetric flask and adjusted for volume with distilled water. Each extract was quantitatively transferred to a 500ml beaker and heated till boiling, then 30ml of 15% aqueous solution of copper acetate was added with stirring. The precipitate of copper tannate was collected on ash less filter paper and the precipitate was ignited in a porcelain crucible (the crucibles were previously ignited to a constant weight at the same temperature). Few drops of nitric acid were added to the residue and reignited to constant weight. The weight of copper oxide was determined and the percentage of tannin was calculated according to the following correlation:

Each 1g of Cuo = 1.305g tannins

Estimation of total saponins

A 2g from each sample were dispersed in 20 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h. with continuous stirring at about 55°C. The mixture was filtered and the residue was re-extracted with another 200 ml of 20% of ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorous. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage (Obadoni and Ochuko, 2001 and Okwu and Ukanwa, 2007).

Estimation of total alkaloids

Two grams DW of each sample were extracted with 90% of ethanol till exhaustion (tested with Mayer's reagent). The alcoholic extract of the plant was concentrated under reduced pressure until dryness at a temperature not exceeding 40°C, acidified with HCl (3%), and filtered. The filtrate obtained was extracted with chloroform to remove acid alkaloid portion. The acidic aqueous layer was adjusted to alkaline media with ammonia and the liberated alkaloid base portion was extracted with chloroform till exhaustion (tested by Mayer and Dragendorff's reagents). The chloroform extract was filtered over anhydrous sodium sulfate and evaporated under reduced pressure till dryness, then weighed it to calculate the percentage w/w. (Woo *et al.,* 1977).

Antimicrobial property.

Tested microorganisms

***Bacteria*:** Four isolates *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli* and *Klebsiella pneumoniae* were isolated from clinical samples and identified based on culture, morphology, and biochemical analysis according to Bergey's manual (Bergey's manual, 2009). In addition, a Vitek2 system was carried out to confirm the identity (Mekky *et al.,* 2021a).

***Candida*:** Two isolates were *Candida albicans and Candida glabrata* isolated from clinical samples and identified in Mycology Lab. of the Botany and Microbiology Dep. Faculty of Science, Al-Azhar University, Cairo, Egypt.

Antibacterial activity:

Agar well diffusion method was carried out to evaluate the antibacterial activity. The nutrient agar plates were prepared and test bacterial strains were inoculated on it. Then by means of 6mm borer wells were produced on agar plates. From each extract, 100μl was transferred through micropipette into the wells in the inoculated agar plates (Ibtisam, 2011).

For better diffusion of the extracts before incubation, the plates were placed at 4°C for 3-4h. (Alam *et al.,* 2010). The bacterial inoculated plates were incubated at 37°C for 24h. After the completion of incubation period, zone of inhibition (mm) was measured. Each concentration of the extract was assessed for antibacterial activity in triplicate and their mean zone of inhibition was taken (Manjulika *et al.,* 2014). A 100 µl Dimethyl sulfoxide (DMSO) was used as negative control.

Anti-candida activity:

The potato dextrose agar (PDA) plates were prepared and the fungal strains like *C. albicans and C. glabrata* were spread on the PDA. A sterile 6mm diameter cork-borer was used to made holes in the inoculated plates (Arunkumar and Muthuselvam, 2009). A 100 µl from of each extract was applied through micropipette into the wells in the inoculated PDA plates (Ibtisam, 2011). For better absorption of the extracts before incubation, the plates were placed at 4 centigrade for 30 min. (Alam *et al.,* 2010). The fungal inoculated plates were incubated at 25°C for 48h. After the completion of incubation period zone of inhibition was measured in mm by means of scale. Each concentration of the extracts was assessed for antifungal activity in triplicate and their mean zone of inhibition was taken.

Broth microdilution assay.

The suspension was turbid to 0.5 McFarland standards (10^8 cfu / ml) produced by fresh subcultures from bacteria and yeast Mueller Hinton Broth (MHB) and Potato Dextrose Broth (PDB), respectively. The corresponding suspension was diluted to 10^6 cfu / ml. Prepared microbial inoculation (100 µl) was added to each well of a sterile flat bottom 96-well microtiter plate containing the tested concentrations of *Nigella sativa* and *Matricaria chamomilla* (100 µl / well). As a result, a final inoculation concentration of 5 x 10^5 cfu / ml was obtained from each well. The optical density was measured at 620 nm after 24 h. at 37 °C for bacteria, 48 h. at 28 °C for yeast, using an ELISA microplate reader (Sunrise ™ -TECAN, Switzerland) at Faculty of Science Al-Azhar University in Cairo, Egypt. Finally, cell concentration was converted to an average growth inhibition percentage (%). The rate of decrease in microbial growth (GR %) was estimated as follows, based on the treatment of the control group (excluding the extract). GR% = CT / C x100 where C is the treated cell concentration of the control group and T is the extraction process. Three replicas were considered. Results were reported as the mean ± SE of three experiments (Tenover 2019).

Preparation of resazurin solution.

The concentration of resazurin stain solution is 0.02% (w/v). 0.002 g of resazurin stain salt fine powder was dissolved in 10 ml of sterile distilled water and vortex. The complete mixture is filtered through a Millipore membrane filter (0.2 µm). This solution can be stored at 4°C for 2 weeks (Khalifa *et al.,* 2013).

Determination of minimum inhibitory concentration (MIC):

The minimum inhibitory concentration of *Nigella sativa* and *Matricaria chamomilla* was evaluated against bacterial and fungal strains via a method described in the guide (Clinical and Laboratory Standards Institude, 2009). The MIC test for bacteria is evaluated in a 96-well microtiter plate with round bottom by a standard broth microdilution assay. On the other hand, as described above, unicellular fungi are evaluated as bacteria (Mekky *et al.,* 2021b).

Determination of minimum lethal concentrations (MLCs)*:*

The MLCs of *Nigella sativa* and *Matricaria chamomilla* against tested pathogens was assessed by the macro broth dilution assay as described by Ansari *et al.,* (2015), with few modifications**.** All cultures were grown in media containing *Nigella sativa* and *Matricaria chamomilla* extracts, and the group with *Nigella sativa* and *Matricaria chamomilla* was taken as control. Twofold dilution of varying concentrations (*Nigella sativa* and *Matricaria chamomilla* 4000–1000 µg/ml) were selected for the treatment to determine the MLCs. The overnight-grown cultures were then streaked on agar plates from each treated concentration to determine the MLC.

Statistical analysis:

All the experiments were performed in triplicate and data were analyzed. Analyses were performed as prescribed by Kareem *et al* 2020.

RESULTS AND DISCUSSION

This study was to assess the quantitative assay for alkaloids, tannins, phenolic acids, saponins and flavonoids in *Nigella sativa* and *Matricaria chamomilla* extracts with evaluation of their antimicrobial activity. The results

obtained showed that quantitative assay for *Nigella sativa* and *Matricaria chamomilla* extracts were total flavonoids (295.21±0.51 and 263.12±1.05 g; mg rutin Eq/g DW), total phenolic acids (388.60±1.02 and 302.10±0.88 mg GAE/g DW.), total tannins (1.23±0.11 and 1.33±0.13 %), total saponins (1.71±0.10 and 1.66±0.16 %) and total alkaloids (2.01±0.20 and 1.07±0.14 %), respectively Table (1) and Figure (1 and 2). Therefore, our results were supported by many previous results recorded that methanol is the most preferred solvent for extraction of the most flavonoid and polyphenolic compounds from *Nigella sativa* and *Matricaria chamomilla* samples (Padmaja and Prasad, 2011).

Anti-bacterial and anti-candida property of Nigella and Chamomile plant extracts using the agar well diffusion and the broth micro dilution assay.

Antimicrobial impedance become the biggest health risk in many places in the world, which damages human health and increases the mortality rate and disease risk associated with major, life-threatening conditions. The antibacterial and antifungal properties of plants against multi-drug resistant bacteria are therefore being studied by numerous specialists from various nations in a contemporary manner (Sofy *et al.,* 2020).

The antimicrobial activity including the antibacterial action of *Nigella sativa* and *Matricaria chamomilla* plant extracts against bacterial strains has been determined on *S. aureus*, *S. haemolyticus*, *E. coli* and *K. pneumoniae* as bacterial strains, in addition to the antifungal property on *Candida albicans* and *Candida glabrata* as fungal strains using agar well diffusion prescribed by (Mekky *et al.,* 2021b).

Data presented in Table (2) and Figure (3, 4 and 5) demonstrate that all tested strains were susceptible to *Nigella sativa* and *Matricaria chamomilla*. These results confirm previous reports that suggested that *N. sativa* has antibacterial properties against Gram-positive bacteria due to its carvacrol and thymoquinone content (Kouidhi *et al*., 2011; Piras *et al*., 2013 and Magi *et al*., 2015).

Determination of minimum inhibitory concentrations (MICs) and minimum lethal concentrations (MLCs) of Nigella sativa and Matricaria chamomilla on bacteria:

The minimum concentrations of inhibition (MICs) values of *Nigella sativa* and *Matricaria chamomilla* against the bacterial strains ranged from 250 µg/ml to 1000 µg/ml, while the minimum concentrations of lethal (MlCs) values were ranged from 500 µg/ml to 2000 µg/ml, see Table (3) and Figure (6, 7 and 8)

Resazurin stain was used in the current study as an indicator to determine bacterial and candida cell growth (McNicholl *et al.,* 2007). Oxidoreductases enzyme inside life bacteria and candida cells reduce the resazurin salt to resorufin and change the blue non-fluorescent colour of resazurin salt to the pink and fluorescent color of resorufin See (Figure 8).

Anti-candida property of Nigella sativa and Matricaria chamomilla plant extracts were assessed using the broth micro dilution assay and the agar well diffusion method.

Data presented in Table (4) and Figure (9 and 10) demonstrate that all investigated strains of bacteria and fungi were susceptible to *Nigella sativa* and *Matricaria chamomilla.* These findings corroborate earlier research that found that the plant extracts and essential oils of these plants have strong antimicrobial properties that are effective against both Gram-positive and Gram-negative bacteria, such as *E. coli*, *S. thyphimurium*, *S. aureus*, and *Bacillus*. These plants' strong antibacterial activity is also a result of their high phenolic component content. Terpenoids, flavonoids, phenolic chemicals, matricin, and apigenin are all found in chamomile (Roby *et al.,* 2013). *Nigella sativa* is a medicinal plant believed to have diuretic, antidiabetic, antitumor, gastro protective, CNS depressant, ant spasmolytic, antioxidant, anti-inflammatory, antimicrobial, antiurolithiatic, anticonvulsant, ant nociceptive, anxiolytic, hepatoprotective, nephroprotective, antihelminthic and immunomodulatory activities (Padmaa, 2010).

Determining both the minimal lethal concentrations (MLCs) and the minimum inhibitory concentrations (MICs).

Many researchers have studied and recorded the antibacterial and antifungal activity of chamomile oil (Göger *et al*., 2018), however, the action mechanisms of sub inhibitory concentrations have not previously been studied or recorded. Our data and our results suggest an effective killing activity of chamomile on selected bacteria and fungi. It is believed that essential oils act against cell cytoplasmic membrane and induce stress in bacteria and fungi (Memar *et al.,* 2018). Inhibitory concentrations at their lowest values MICs of *Nigella sativa* and *Matricaria chamomilla* varied from 250 µg/ml to 1000 µg/ml against

the *Candida* strains, with the evaluation of the MlCs values were varied between 500 and 1000 μg/ml, see Table (5) and Figure (11 and 12).

The inhibition dependent on concentration for *S. aureus*, as Gram-positive and Gram-negative, as *P. aeruginosa*, *E. coli*, and pathogenic yeast *C. albicans* was found on filter paper discs impregnated with ethyl ether extract of *N. sativa* (25-400 μg/disc), the extract had additive antibacterial action with doxycycline, erythromycin, tobramycin, and ampicillin and antibacterial synergism with streptomycin, gentamicin, lincomycin and sulfamethoxazole trimethoprim combination (Hanafy and Hatem, 1991). Based on the observed results, Nigella and Chamomile extracts were active anti-bacterial and anti-candida substances.

CONCLUSION

The current study aimed to assess the quantitative assay for alkaloids, tannins, phenolic acids, saponins and flavonoids in *Nigella sativa* and *Matricaria chamomilla* with determination the antibacterial and anti-candida effects. The results obtained showed that quantitative assay were total flavonoids (295.21±0.51 and 263.12±1.05 rutin Eq/g DW), total phenolic acids (388.60±1.02 and 302.10±0.88 μg gallic acid equivalent (GAE)/mg DW), total tannins (1.23±0.11 and 1.33±0.13 %), total saponins (1.71±0.10 and 1.66±0.16 %) and total alkaloids (2.01±0.20 and 1.07±0.14 %), respectively. Based on the observed results, Nigella and Chamomile were an active anti-bacterial and anti-candida substance. Diameters of the inhibition zones of Nigella and Chamomile extracts were about (17 and 15 – 14 and 18 – 8 and 8 – 12 and 10 – 15 and 13 – 16 and 14 mm) against S. *aureus*, *S. haemolyticus*, *E. coli, K. pneumoniae, C. albicans* and *C. glabrata,* respectively. The MICs of *Nigella sativa* against the bacterial and candida strains were from 250 μg/ml to 500 μg/ml and minimum lethal concentrations MLCs were from 500 μg/ml to 1000 μg/ml. Where the (MICs) values of Chamomile against the bacterial and candida strains ranging from 500 μg/ml to 1000 μg/ml and MLCs rangeing from 1000 μg/ml to 2000 μg/ml. Finally, this study demonstrates *Nigella sativa* and *Matricaria chamomilla* efficacy in antimicrobial activities.

REFERENCES

Agatonovic-Kustrin, S., Ortakand, D.B., Morton, D.W. and Yusof, A.P. 2015. Rapid evaluation and comparison of natural products and antioxidant activity in calendula, feverfew, and German chamomile extracts. *J. Chromatogr*, 1385, 103–110.

Ahmad, A., Husain, A. and Mujeeb, M. 2013. Therapeutic Potential of *Nigella Sativa:* A Miracle Herb: A review. *Asian Pacific Journal of Tropical Biomedicine,* 3(5):337-352.

Al-Adhroey, A.H., Nor, Z.M., Al-Mekhlafi, H.M., and Mahmud, R. 2010. Ethnobotanical study on some Malaysian anti-malarial plants: a community based survey. *J. Ethnopharmacol* 132, 362–364.

Alam, M.M., Yasmin, M. and Nessa, J. 2010. Antibacterial activity of chloroform and ethanol extracts of cumin seeds (Nigella sativa) against multi-drug resistant human pathogens under laboratory conditions. *J. Med. Plants Res,* 18:1901–1905.

Ali, M., Husain, shahnaz, S. and Ansari, H. 2011. Composition of the volatile oil of *Zingiber officinale* roscoe rhizomes and effects of physical factors on the oil. *International research journal of pharmacy*, 2(9): 173-177.

Alrawi, S. N., Khidir, A., Elnashar, M. S., Abdelrahim, H. A., Killawi, A. K., Hammoud, M. M. 2017. Traditional Arabic & Islamic medicine: validation and empirical assessment of a conceptual model in Qatar. *BMC Complement. Altern. Med.* 17, 157

Ansari, M. A., Khan, H. M. and Alzohairy, M. A. 2015. Green synthesis of Al_2O_3 nanoparticles and their bactericidal potential against clinical isolates of multi-drug resistant *Pseudomonas aeruginosa*. *World Journal of Microbiology and Biotechnology.* 31(1):153–164.

Arunkumar, S. and Muthuselvam, M. 2009. Analysis of phytochemical constituents and antimicrobial activities of *Aloe vera* L. against clinical pathogens. *World journal of agricultural sciences,* 5(5):572–576.

Aziz, M. A., Khan, A. H., Adnan, M., and Izatullah, I. 2017. Traditional uses of medicinal plants reported by the indigenous communities and local herbal practitioners of Bajaur Agency, Federally Administrated Tribal Areas, Pakistan. *J. Ethnopharmacol*, 198, 268–281.

Bale, S, Tebble, N. and Price, P. 2004. A topical Metronidazole Gel Used to Treat Malodorous Wounds. *British Journal of Nursing*, 390-391.

Bergey's manual, 2009. Bergey's manual of systematic bacteriology. Sneath PHA, Shar M, *Elizabeth, Holt JG (Eds.) Pub*. Williams and Wilkins, 26: 1-5.

Chun, K., Kim, D., and Lee, C.Y. 2003. Superoxide Radical Scavenging Activity of the Major Polyphenols in Fresh Plums. *Journal of Agricultural and Food Chemistry* (51): 8067-8072.

Clinical and Laboratory Standards Institude, 2009. Performance standards for antimicrobial disk susceptibility tests; approved standard-tenth edn 29: M02-A10. *National Committee for Clinical Laboratory Standards* 29.

Enomoto, S., Asano, R. and Iwahori, Y. 2001. Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. *Biol Pharm Bull,* 24(3):307–310.

Formisano, C., Delfine, S., Oliviero, F., Tenore, G.C., Rigano, D. and Senatore, F. 2015. Correlation among environmental factors, chemical composition and antioxidative properties of essential oil and extracts of chamomile (*Matricaria chamomilla* L.) collected in Molise (South-central Italy). *Ind. Crop. Prod*, 63, 256–263.

Gholamnezhad, Z., Havakhah, S. and Boskabady M.H. 2016. Preclinical and Clinical Effects of *Nigella sativa* and its Constituent, Thymoquinone: A review. *Journal of Ethnopharmacology*, 190:372-386.

Göger, G., Demirci, B., Ilgın, S. and Demirci, F. 2018. Antimicrobial and toxicity profiles evaluation of the Chamomile (*Matricaria recutita* L.) essential oil combination with standard antimicrobial agents. *Ind. Crop. Prod.,* 120, 279–285.

Han, N.M. and May, C.Y. 2012. Determination of antioxidants in oil palm empty fruit bunches. *American journal of applied sciences*, 9(11): 1862-1867.

Hanafy, M.S. and Hatem M.E. 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *J. Ethnopharmacol,* 34:275–278.

Ibtisam M.A. 2011. Antimicrobial activity of ethanolic extracts from some medicinal plants. *Aust. J. basic,* 5: 678–683.

Ismail, M.C., Waleed, S., Ibrahim, K. and Fakhri, N.U. 2013. Synergistic interaction between Chamomile flower (*Matricaria chamomilla* L.) extracts and tetracycline against wound infection bacteria. *Al-Nahrain J. Sci,* 16, 191–195.

Jamila, F., and Mostafa, E. 2014. Ethnobotanical survey of medicinal plants used by people in Oriental Morocco to manage various ailments. *J. Ethnopharmacol* 154, 76–87.

Jennings, H. M., Merrell, J., Thompson, J. L., and Heinrich, M. 2015. Food or medicine the food-medicine interface in households in Sylhet. *J. Ethnopharmacol*, 167, 97–104.

Kareem, S.H., Naji, A.M., Taqi, Z.J. and Jabir, M.S. 2020. Polyvinylpyrrolidone Loaded-MnZnFe 2O4 Magnetic Nanocomposites Induce Apoptosis in Cancer Cells Through Mitochondrial Damage and P 53 Pathway. *Journal of Inorganic and Organometallic Polymers and Materials,* 30 (12):5009-5023.

Khabbach, A., Libiad, M., Ennabili, A., and Bousta, D. 2012. Medicinal and cosmetic use of plants from the province of Taza, Northern Morocco. *Boletín Latinoamericano y Del. Caribe de Plantas Medicinales y Aromáticas* 11, 46–60.

Khalifa, R.A., Nasser, M.S., Gomaa, A.A., Osman, N.M. and Salem, H.M. 2013. Resazurin Microtiter Assay Plate method for detection of susceptibility of multidrug resistant *Mycobacterium tuberculosis* to second-line anti-tuberculous drugs. *Egypt. J. Chest Dis. Tuberc*, 62: 241–247.

Kouidhi, B., Zmantar, T., Jrah, H., Souiden, Y., Chaieb, K., Mahdouani, K. and Bakhrouf, A. 2011. Antibacterial and resistance-modifying activities of thymoquinone against oral pathogens. Ann Clin Microbiol Antimicrob 10: 29.

Magi, G., Marini, E. and Facinelli, B. 2015. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant group A *Streptococci*. *Front Microbiol*, 6: 1-7.

Mahdizadeh, S. and Ghadiri M.K. 2015. Gorji Avicenna's Canon of Medicine: a review of analgesics and antiinflammatory substances. *Avicenna J. Phytomedicine,* 5, p. 182.

Manjulika, Y., Sanjukta, C. and Deepak, K.Y. 2014. Improved antibacterial efficacy of a novel formulation- synergistic effect of herbs. *Int. J. Pharm. Biol. Sci*, 4:36–39.

Maurya, S. and Singh, D. 2010. Quantitative Analysis of Total Phenolic Content in *Adhatoda vasica* Nees, *International Journal of Phamtech. Research*, 4:2403-2406.

McNicholl, B.P., McGrath, J.W. and Quinn, J.P. 2007. Development and application of a resazurin-based biomass activity test for activated sludge plant management. *Water Res.* 41(1):127-33

Mekky, A.E., Farrag, A.A., Hmed A.A. and Sofy A.R., 2021a. Preparation of Zinc Oxide Nanoparticles using *Aspergillus niger* as Antimicrobial and Anticancer Agents, *Journal Pure Applied Microbiology*, 15(3): 1547-1566.

Mekky, A.E., Farrag, A.A., Hmed, A.A. and Sofy, A.R. 2021b. Antibacterial and Antifungal Activity of Green synthesized Silver Nanoparticles Using *Spinacia oleracea* leaves Extract, *Egyptian Journal of Chemistry* 64: 5781-5792.

Memar, M.Y., Ghotaslou, R., Samiei, M. and Adibkia, K. 2018. Antimicrobial use of reactive oxygen therapy: Current insights. *Infect Drug Resist*, 11, 567–576.

Obadoni, B.O. and Ochuko, P.O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some

homeostatic plants from Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Sciences*, 8: 203-208.

Okwu, D.E. and Ukanwa, N.S. 2007. Nutritive value and phytochemical contents of fluted pumpkin (*Telfaria Occidentalis Hook* f.) vegetable grown with different levels of Turkey droppings. *African Crop Science Conference Proceedings*, 8: 1759-1964.

Padmaa, MP. 2010. *Nigella sativa* Linn.-A comprehensive review. *Indian J. Nat Prod Resour*, 1(4):409–429.

Padmaja, A., and Prasad, N. 2011. Pomegranate (*Punica granatum* L.) peel extract as a source of natural antioxidant. *Journal of Food Science and Engineering*, 1(3): 171.

Piras, A., Rosa, A., Marongiu, B., Porcedda, S., Falconieri, D., Dessì, M.A., Ozcelik, B. and Koca, U. 2013. Chemical composition and in vitro bioactivity of the volatile and fixed oils of *Nigella sativa* L. extracted by supercritical carbon dioxide. *Ind Crops Prod*, 46: 317-323.

Roby, M.H., Sarhan, M.A., Selim, K.A. and Khalel, K.I. 2013. Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). *Ind. Crop. Prod*, 44, 437–445.

Samatha, R., Shyamsundarachary, R., Srinivas, P., Swamy, N.R. and Kurz, L. 2012. Phytochemical screening and spectroscopic determination of total phenolic and flavonoid contents of Eclipta alba Linn, 5(4): 177- 179.

Sofy, A.R., Aboseidah, A.A., El-Morsi, E.S. Azmy, H.A. and Hmed, A.A. 2020. Evaluation of antibacterial and antibiofilm activity of new antimicrobials as an urgent need to counteract stubborn multidrug-resistant bacteria. *J. Pure Appl. Microbiol,* 14, 595–608.

Stanojevic, L.P., Marjanovic-Balaban, Z.R., Kalaba, V.D., Stanojevic, J.S. and Cvetkovic, D.J. 2016. Chemical composition, antioxidant and antimicrobial activity of chamomile flowers essential oil (*Matricaria chamomilla L.*). *J. Essent. Oil Bear Plants,* 19, 2017–2028

Sutjhajo, A. and Kuman K. 2012. Antibiotic di Kaki Diabetic. *Indonesian Jurnal of Clinical Pathologi and Medical Laboratory*, 20(1): 20-24.

Tenover, F.C. 2019. In Encyclopedia of Microbiology (Fourth Edition).

Woo, W.S., Chi, H.J., and Hye, S. 1977. Alkaloid screening of some Saudi Arabian plants. *Korean Journal of Pharmacognosy*, 8, (3): 109-113.

Table 1: Quantitative phytochemical analysis of *N. sativa* and *Matricaria chamomilla* extract.

Plants	Components	Total active materials
Nigella Sativa L.	Total flavonoids (g; mg rutin Eq/g DW)	295.21±0.51
	Total phenolic acids (mg GAE/g DW.)	388.60±1.02
	Total tannins (%)	1.23±0.11
	Total saponins (%)	1.71±0.10
	Total alkaloids (%)	2.01±0.20
Matricaria chamomilla L.	Total flavonoids (g; mg rutin Eq/g DW)	263.12±1.05
	Total phenolic acids (mg GAE/g DW.)	302.10±0.88
	Total tannins (%)	1.33±0.13
	Total saponins (%)	1.66±0.16
	Total alkaloids (%)	1.07±0.14

Table 2: The antibacterial activity of *N. sativa* and *Matricaria chamomilla* plant extracts using agar well diffusion method and broth micro dilution assay.

Bacterial strains	Antibacterial activity			
	Nigella sativa		*Matricaria chamomilla*	
	Diameter of Inhibition zone (mm) [a]	Mean growth Inhibition percentage (%) [b]	Diameter of Inhibition zone (mm) [a]	Mean growth Inhibition percentage (%) [b]
S. aureus	17 ± 0.44	100 ± 0.25	15 ± 0.22	100 ± 0.22
S. haemolyticus	14 ± 0.77	100 ± 0.00	18 ± 0.00	100 ± 0.55
E. coli	8 ± 0.65	100 ± 0.44	8 ± 0.43	100 ± 0.32
K. pneumoniae	12 ± 0.63	100 ± 0.45	10 ± 0.66	100 ± 0.00

a) Diameter of inhibition zone was determined by the agar well diffusion method.
b) Mean growth inhibition percentage (%) was determined by the broth micro dilution method.

Table 3: Minimum inhibitory and minimum lethal concentrations of *Nigella sativa* and *Matricaria chamomilla* plant extracts against bacterial strains.

Bacterial Strains	Minimum inhibitory (MICs) and Minimum Lethal (MLCs) concentrations against bacterial strains			
	Nigella sativa		*Matricaria chamomilla*	
	MICs µg/ml	MLCs µg/ml	MICs µg/ml	MLCs µg/ml
S. aureus	250	500	1000	2000
S. haemolyticus	500	500	1000	2000
E. coli	250	500	500	1000
K. pneumoniae	250	500	1000	2000

Table 4: The anti-candida activity of nigella and chamomile plant extracts using agar well diffusion method and broth micro dilution assay.

Candida strains	Anti-candida activity			
	Nigella sativa		*Matricaria chamomilla*	
	Diameter of Inhibition zone (mm) [a]	Mean growth Inhibition percentage (%) [b]	Diameter of Inhibition zone (mm) [a]	Mean growth Inhibition percentage (%) [b]
C. albicans	15 ± 0.72	100 ±0.33	13 ± 0.00	100 ±0.22
C. glabrata	16 ± 0.66	100 ±0.65	14 ± 0.44	100 ±0.63

a) Diameter of Inhibition zone was determined by the agar well diffusion method.
b) Mean growth inhibition percentage (%) was determined by the broth micro dilution method.

Table 5: Minimum inhibitory and minimum lethal concentrations of nigella and chamomile plant extracts against candida strains.

Candida strains	Minimum inhibitory (MICs) and Minimum Lethal (MLCs) concentrations against candida Strains			
	Nigella sativa		*Matricaria chamomilla*	
	MICs	MLCs	MICs	MLCs
C. albicans	250	500	500	1000
C. glabrata	500	1000	1000	1000

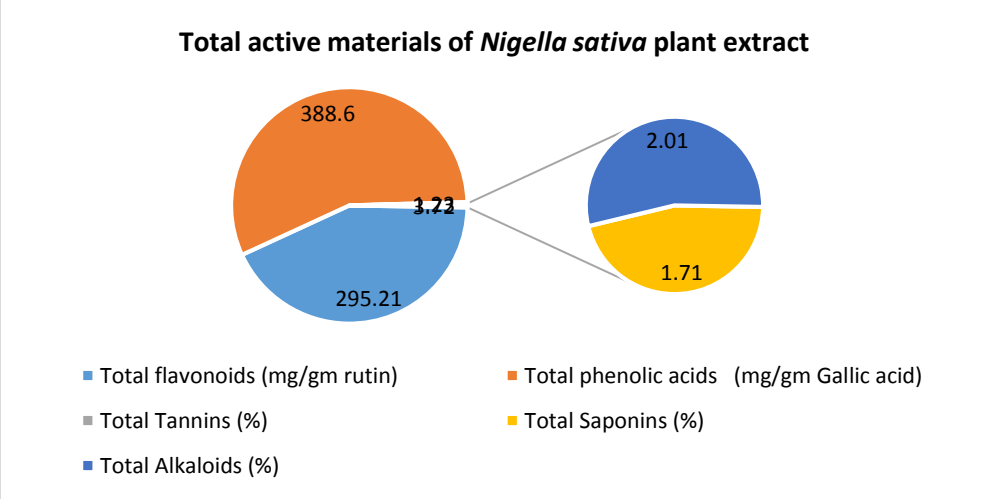

Figure 1: Total active materials histogram of *Nigella sativa*

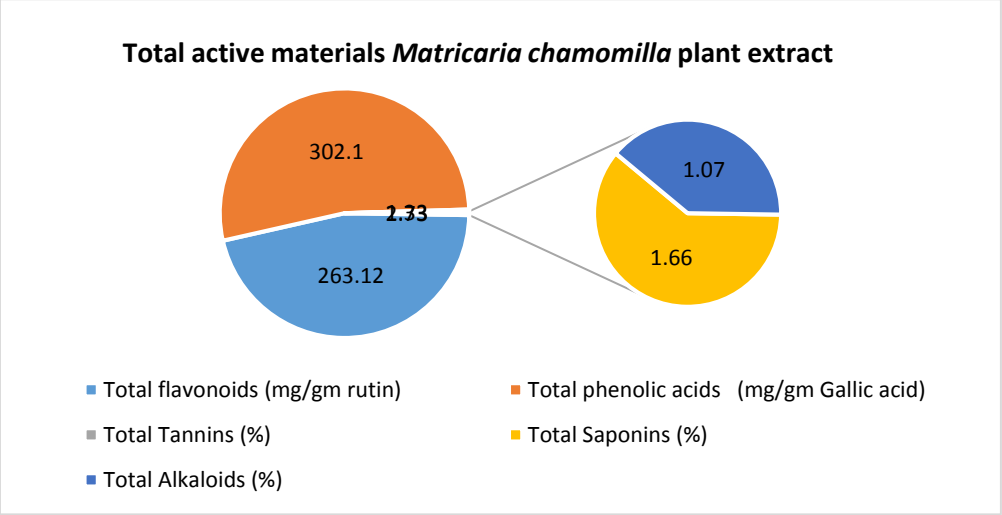

Figure 2: Total active materials histogram of *Matricaria chamomilla*.

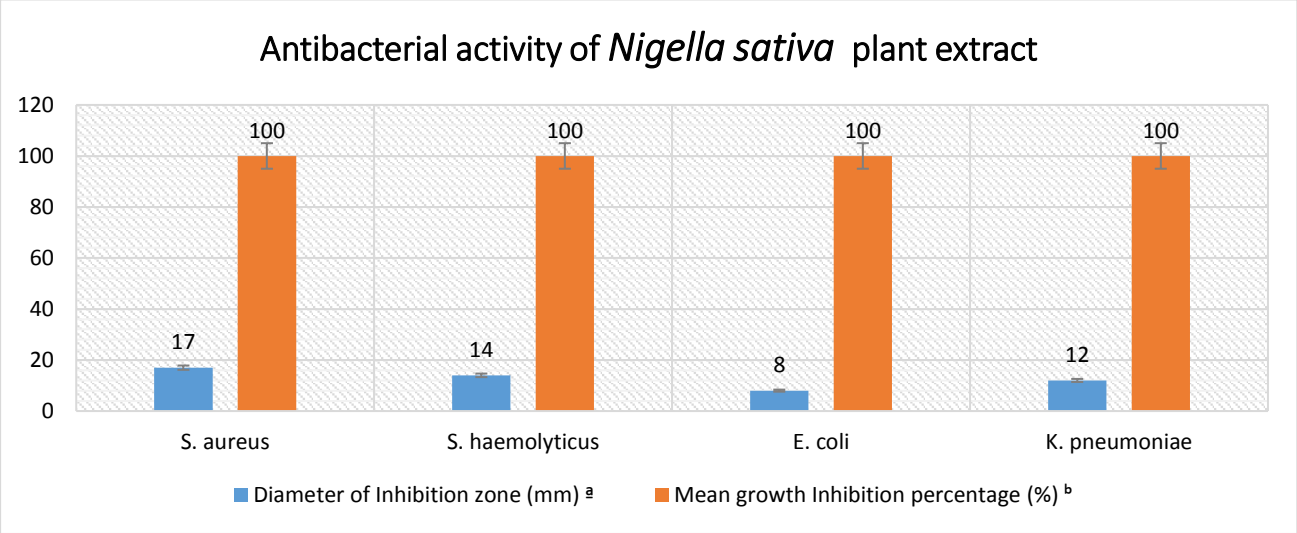

Figure 3: Anti-bacterial activity histogram of *Nigella sativa* plant extract (100 µl) against the pathogenic bacteria strains using inhibition zone diameter and Mean growth inhibition percentage (%).

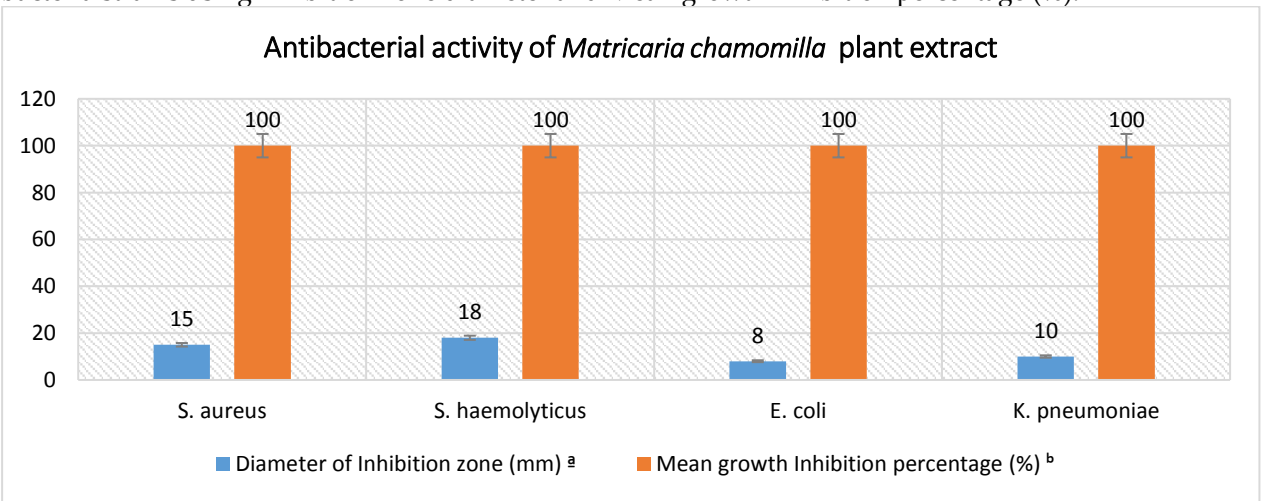

Figure 4: Anti-bacterial activity histogram of *Matricaria chamomilla* plant extract (100 µl) against the pathogenic bacteria strains using inhibition zone diameter and Mean growth inhibition percentage (%).

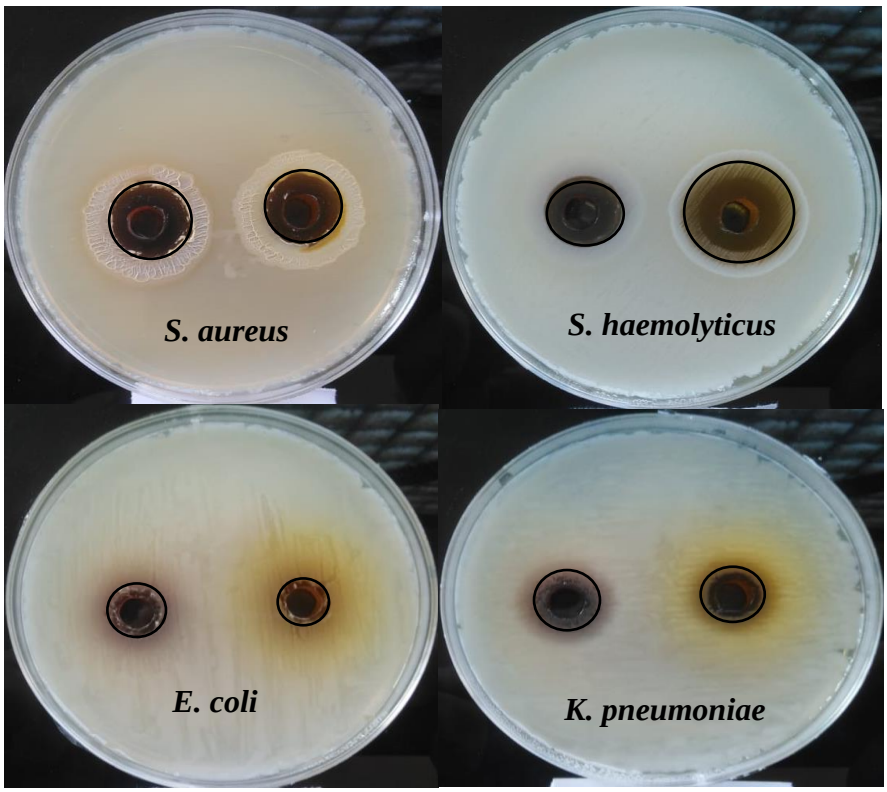

Figure 5: Inhibition zones produced by *Nigella sativa* and *Matricaria chamomilla* plant extracts against ***S. aureus***, ***S. haemolyticus***, ***E. coli*** and ***K. pneumoniae*** using agar well diffusion method (100 µl).

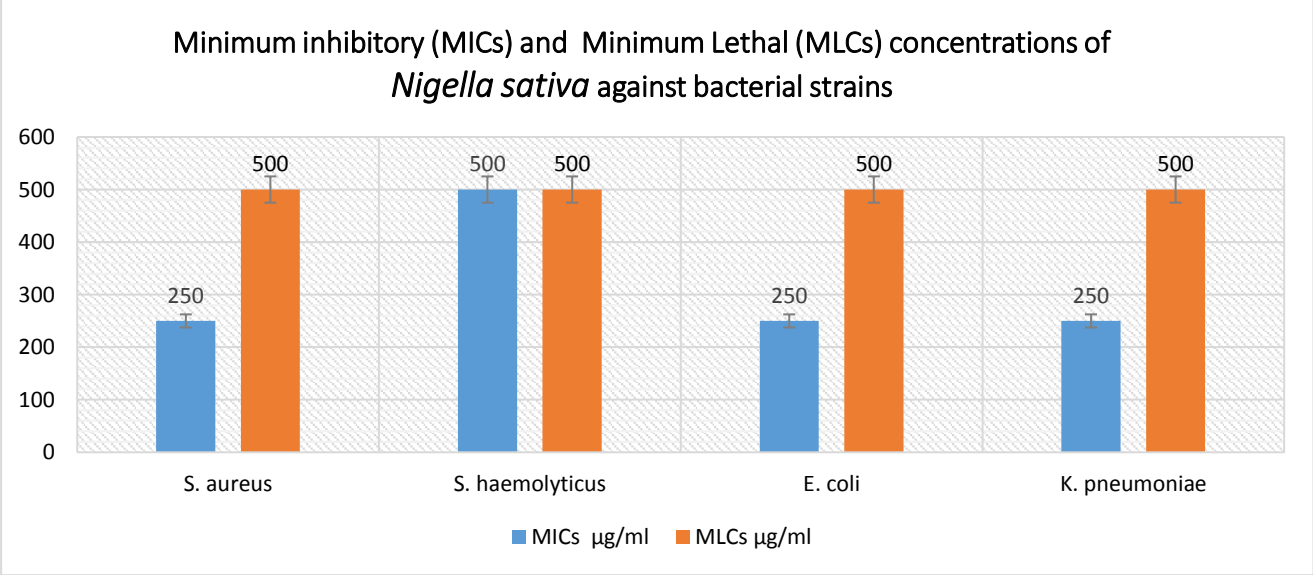

Figure 6: Minimum inhibitory (MICs) and minimum lethal (MLCs) concentrations histogram of *Nigella sativa* plant extract against bacterial strains

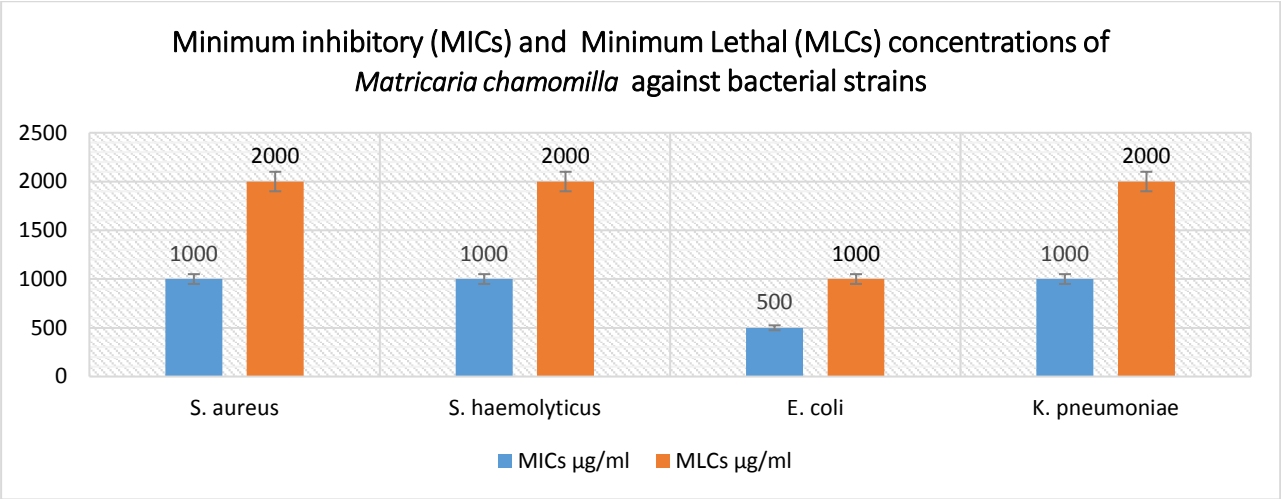

Figure 7: Minimum inhibitory (MICs) and minimum lethal (MLCs) concentrations histogram of *Matricaria chamomilla* against bacterial strains.

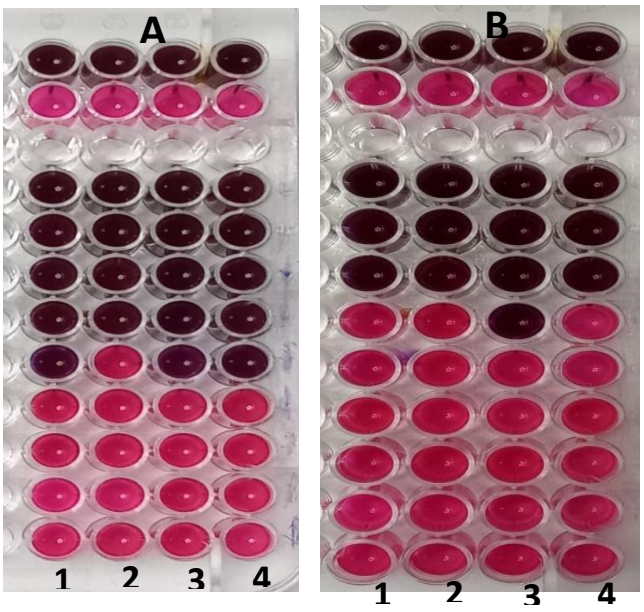

Figure 8: Ninety-six well Microtiter plates of the colorimetric-XTT assay for determination of MICs values of (A) *Nigella sativa* plant extract and (B) *Matricaria chamomilla* plant extract against 1- *S. aureus*, 2- *S. haemolyticus*, 3- *E. coli* and 4- *K. pneumoniae*.as bacterial strains using resazurin salt.

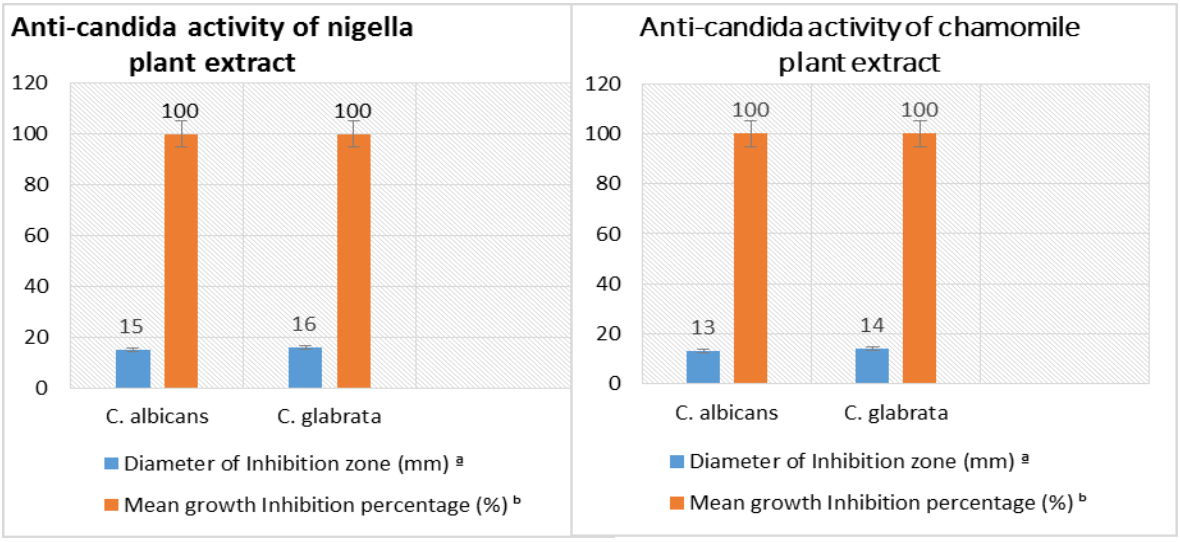

Figure 9: Anti-candida activity histogram of *Nigella sativa* and chamomile plant extract (100 µl) against the pathogenic candida strains using inhibition zone and Mean growth inhibition percentage (%).

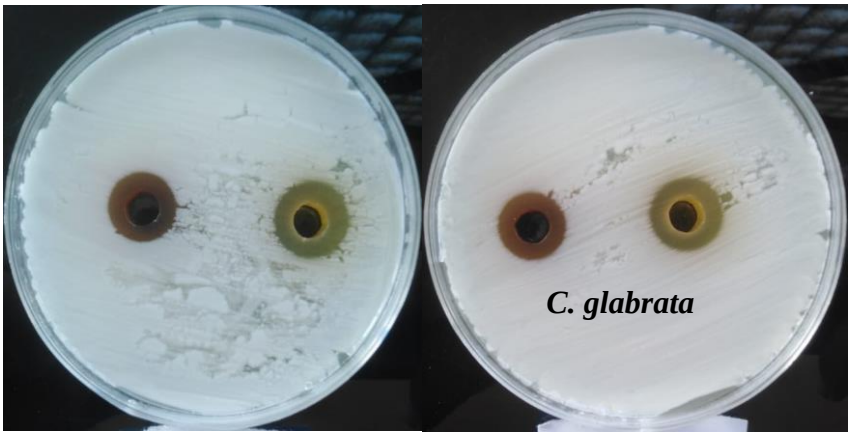

Figure 10: Inhibition zones produced by *Nigella sativa* and *Matricaria chamomilla* plant extracts against *C. albicans and C. glabrata* using agar well diffusion method (100 µl).

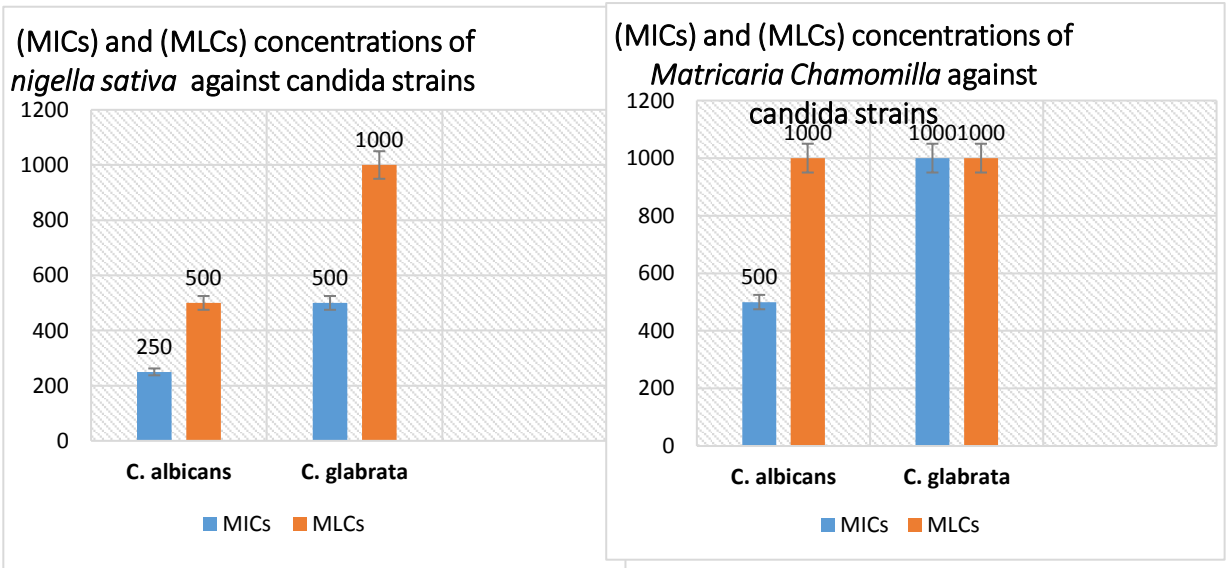

Figure 11: Minimum inhibitory (MICs) and Minimum Lethal (MLCs) concentrations histogram of *Nigella sativa* and *Matricaria chamomilla* plant extracts against candida strains.

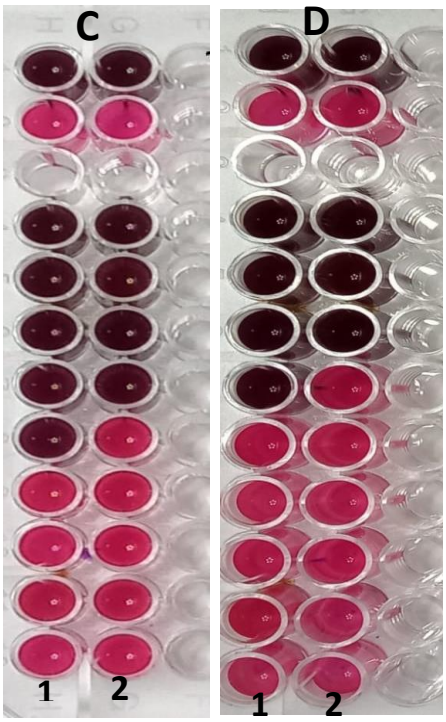

Figure 12: Ninety-six well Microtiter plates of the colorimetric-XTT assay for determination of MICs values of (A) *Nigella sativa* plant extract and (B) *Matricaria chamomilla* plant extract against 1- *C. albicans* and 2- *C. glabrata* as candida strains using resazurin salt.

دراسة التحليل الكيميائي النباتي والنشاط المضاد للميكروبات للمستخلص الإيثانولي لنباتي حبة البركة والبابونج

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الملخص العربى

خلفية البحث: تستخدم حبة البركة والبابونج على نطاق واسع باعتبارهما دواءً تقليدياً منذ العصور القديمة لاحتوائها على مضادات الأكسدة ومضادات الالتهاب ومضادات الميكروبات ومركبات مضادة للأورام. **الهدف من البحث:** التقييم الفيتوكيميائي للقلويدات والتانينات والأحماض الفينولية والصابونينات والفلافونويدات في مستخلصات حبة البركة والبابونج مع تقييم نشاطها كعوامل مضاد للميكروبات. **النتائج:** أظهرت النتائج أن التقييم الفيتوكيميائي لمستخلصات حبة البركة والبابونج كانت الفلافونويدات الكلي (٢٩٥,٢١ ± ٠,٥١ و ٢٦٣,١٢ ± ١,٠٥ مجم / جم) ، الأحماض الفينولية الكلية (٣٨٨,٦٠ ± ١,٠٢ و ٣٠٢,١٠ ± ٠,٨٨ مجم / جم) ، إجمالي التانينات (١,٢٣ ± ٠,١١ و ١,٣٣ ± ٠,١٣٪) ، إجمالي الصابونينات (١,٧١ ± ٠,١٠ و ١,٦٦ ± ٠,١٦٪) وإجمالي القلويدات (٢,٠١ ± ٠,٢٠ و ١,٠٧ ± ٠,١٤٪) على التوالي. بناءً على النتائج المرصودة ، كانت مستخلصات حبة البركة والبابونج من المواد الفعالة المضادة للميكروبات. كانت أقطار مناطق التثبيط لمستخلصات حبة البركة والبابونج حوالي (١٧ و ١٥ - ١٤ و ١٨ - ٨ و ٨ - ١٢ و ١٠ - ١٥ و ١٣ - ١٦ و ١٤ ملم) ضد مقابل *استافيلوكوكس اوريس* و *استافيلوكوكس هموليتيكس* و *ايشيريشيا كولاي* و *كليبسيلا نيومونيا* و *كانديدا البيكانس* و *كانديدا جلابراتا* على التوالي. تراوحت قيم أقل التركيزات المثبطة للنمو من حبة البركة ضد سلالات البكتيريا والكانديدا من ٢٥٠ ميكروجرام/مل إلى ٥٠٠ ميكروجرام/مل وتراوحت أقل التركيزات المميتة من ٥٠٠ ميكروجرام/مل إلى ١٠٠٠ ميكروجرام/مل. كما تراوحت قيم أقل التركيزات المثبطة للنمو للبابونج ضد السلالات البكتيرية والكانديدا من ٥٠٠ ميكروجرام/مل إلى ١٠٠٠ ميكروجرام/مل وتراوحت أقل التركيزات المميتة من ١٠٠٠ ميكروغرام/مل إلى ٢٠٠٠ ميكروجرام/مل. **الاستنتاج:** توضح هذه الدراسة فعالية حبة البركة والبابونج كعوامل علاجية بديلة في علاج العديد من الأمراض الجرثومية لتوفير دليل شامل لأخصائي علم الأمراض.

الكلمات الاسترشادية: الحبة السوداء ، البابونج ، القلويات ، التانينات ، الفينولات ، الصابونينات ، الفلافونويدات.