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EVALUATION OF PHYTOCHEMICALS, ANTIOXIDANTS AND ANTIBACTERIAL ACTIVITY OF *NIGELLA SATIVA* SEED EXTRACTS

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ABSTRACT:

Nigella sativa (Family Ranunculaceae) is one of the medicinal plant species that has become well-known for a variety of medical uses due to its rich source of phytoconstituents. Ongoing scientific research on *N. sativa* seeds is necessary to have a deeper comprehension of its numerous medical applications. The present study is focused on analyzing the phytochemicals present in the *Nigella sativa* (NS)seeds, and its anti-oxidant and anti-microbial activity. Crude extract of *Nigella sativa* of solvents extracts (acetone, petroleum ether, chloroform, diethyl ether, hexane, and ethanol) were analyzed for their phytochemical properties by using standard methods. Based on the qualitative phytochemicals profile, ethanol, acetone, petroleum ether, and hexane solvent extracts were analyzed for their anti-oxidant properties by DPPH assay and antimicrobial activity against human pathogenic organisms (*Staphylococcus aureus, E.coli*, *Pseudomonas auroginosa* and *Enterobacter sps)*by well diffusion method. Results of phytochemical studies reveal the presence of terpenoids, saponins, alkaloids, flavonoids, carbohydrates, steroids, phytosterol, cardiac glycosides, anthraquinones, polyphenol but glycosides were absent in all six solvent extracts. The antioxidant activity of selected solvent extracts shows close anti-oxidant properties to reference compounds when using higher concentrations of extracts. Each solvent extract shows potential inhibitory activity against the selected pathogenic microorganism. Therefore, this study confirms that *N.sativa* seeds extract contains a variety of chemical components and active functional groups linked to their antioxidant properties and antimicrobial activity.

Keywords: *Nigella sativa,* Quantitative analysis, Phytochemicals, Medicinal properties, Nigella sativa seeds

1. INTRODUCTION:

The important resources of pharmaceuticals for conventional medical systems, nutraceuticals, dietary supplements, contemporary medications, pharmaceutical intermediates, folk remedies, and chemical entities for synthetic drugs are found in medicinal plants. The World Health Organization (WHO) estimates that up to 80% of people get their medication from traditional medicinal plants (Arunkumar and, Muthuselvam, 2009). Nearly all species of medicinal plants include many active compounds, and understanding their composition is essential before conducting additional research. *Nigella sativa,* an indigenous herbaceous plant that is a member of the Ranunculaceae family and is more often recognized as the fennel flower plant, is one example of a therapeutic plant. This plant may reach a maximum height of roughly 60 centimeters and features finely divided foliage in addition to blue flowers that yield black seeds. The plant is known by many other names e.g. kalonji (Urdu), habba-tu sawda (Arabic), black cumin (English), shonaiz (Persian), kalajira (Bangali) (Khan, 1999).

Black cumin (*Nigella sativa* L.) seeds have been used for medicinal purposes for more than 2000 years. *Nigella sativa* L. seed contains essential oils, saponins, polyphenols, fatty oils, fatty acids and bitter nigelon, nigelin and thymokinon substances are responsible for various pharmacological activities of the plants (Abbas *et al.,*2021, Shah *et al.,* 2011, Zaman *et al.,* 2012). Naturally, the *Nigella sativa (NS)* seed is a rich source of antioxidants. The effectiveness of currently available artificial antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), in postponing cell degeneration is frequently documented (Kahl *et al*., 1993). The biological legacy of the seed extracts is rich, therefore it's plausible that one or more active components are targeted to treat individual diseases. This range of therapeutic applications can be explained by the diversity of secondary metabolites (Saleh *et al*.,2017 Bourgaud *et al.,* 2001, Grech-Baran and Pietrosiuk, 2012). NS is referred to as "El Habba Saouda" in Islamic culture and is utilized in traditional medicine by the adage that it is "a drug for all diseases except death." These terms remained mysterious until scientific analysis was able to identify the medicinal potentials of this plant (Avicenne *et al.,* 1999).

Living organisms can synthesize these phytochemicals by primary or secondary metabolism. Nonpolar solvents like hexane, petroleum ether, and chloroform are limited to extracting nonpolar substances like lipids (fatty acids). Conversely, polar solvents like acetone, diethyl ether, and ethanol can be used to extract polar substances like flavonoids and polyphenols. The objective of this investigation was to concurrently extract, and assess the antioxidant and antibacterial activities of these secondary metabolites. Secondary metabolites are varied chemically and taxonomically classified substances with unclear functions. These seeds have been demonstrated to boost the body's defenses against infection, avoid blockages in blood vessels, decrease cholesterol, enhance heart performance, improve memory and concentration, boost the hormone bioactivity, nullify the toxic effects, reduce stress, warm the stomach, enhance the digestive tract, anti-inflammatory, promote the breast milk secretion, refresh the skin cells and have anti-tumor activity (Iran, 2014). Therefore, the goal of the current study is to assess the phytochemicals, antioxidant, and antibacterial properties found in the *Nigella sativa* seeds.

2. MATERIALS AND METHODS:

2.1. Collection of plant materials

The *Nigella sativa* L. seeds were purchased from a local market in Chengal Pattu District, Tamil Nadu, India. To remove the clinging contaminants, the black seeds were thoroughly rinsed with plenty of water. To prepare the extract, only the clean seeds were used..

Fig-1: ***Nigella Sativa*** **seeds**

Fig-2: ***Nigella sativa*** **Seed Powder**

2.2. Preparation of the extract:

The seeds of *Nigella sativa* were shade-dried at room temperature and then powdered using a blender (Fig-1&2). The most popular solvents for removing solid and essential oils from NS seeds are methanol, hexane, chloroform, and acetone (Wajs *et al*.,2008, Singh *et al.,* 2005, Soto *et al*., 2007, Bourgou *et al*., 2010, Rao *et al*., 2007 and Zúñiga *et al*.,2003). In the present study, 10gm of dried powdered seeds were subjected to extraction with 50 ml of six different solvents like hexane, acetone, ethanol, chloroform, petroleum ether, and diethyl ether in different conical flasks. Kept in a shaker for 28-48 hours with continuous shaking. Then the extract was filtered by using Whatman filter paper and then the samplewas stored in a clean bottle at a low temperature for further use (Fig-3).

Fig-3: Extraction of solvents using Whatman No .1 filter paper

2.3. Preliminary Phytochemical Screening:

For each type of chemical family compound (steroids, alkaloids, and flavonoids), phytochemical assays require appropriate revealers. By either directly spraying the revealers onto TLC plates or adding a few drops of the revealers to the solution, this method's basic idea is the visual observation of color shift. Several authors in the literature provide detailed descriptions of these techniques (Belfekih *et al*., 2017, Louiz *et al.,*2003). To find out the different preliminary phytochemical constituents such as Terpeniods, Carbohydrates, Phytosterol, Alkaloids, Quinone, Saponin, Steroids, Flavonoids, Cardiac glycosides, Glycosides, Anthroquinones, Polyphenols analysis was done by using standard methods described by Harborne, 1998 and Thangaraj, 2016.

a) Terpenoids Test:

In a test tube 0.5ml of extracts, 2ml of chloroform, and concentrated sulphuric acid was added. The formation of a red-brown color indicated the presence of terpenoids.

b) Carbohydrates Test:

In a test tube 2ml of extracts, added 2 drops of Molish's reagent. The mixture was shaken well and added few drops of concentrated sulphuric acid. The appearance of a reddish color indicated the presence of carbohydrates.

c) Phytosterol Test:

In a test tube 1ml of extracts, added a few drops of chloroform and concentrated sulphuric acid were added. The appearance of a brown color ring indicated the presence of Phytosterol.

d)Alkaloids Test:

In a small test tube 2ml of extracts, were added 2ml of concentrated sulphuric acid, and a few drops of Wagner's reagent. The presence of green color or white precipitate indicated the presence of alkaloids.

e) Quinones Test:

In a test tube 1ml of extracts, and 1ml of concentrated sulphuric acid was added. The formation of red colour indicated the presence of quinines.

f) Saponin Test:

In a test tube, about 2ml of extracts were taken and mixed properly with 5 ml of distilled water and it was vigorously shaken for 15 min, if stable foam appeared, then it indicated the presence of saponin.

g) Steroids Test:
Two ml of the sample were taken and dissolved in five ml of chloroform in a test tube. An equal volume of strong sulfuric acid (5 ml) was then cautiously poured via the test tube's walls. If the top layer becomes red and the sulfuric acid layer becomes yellow with a faint green fluorescence, suggesting that steroids are present

h) Flavonoids Test:
2 ml of plant extract and 1 ml of 2N sodium hydroxide were put into a test tube. The presence of flavonoids was indicated by the yellow color.

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i)Cardiac Glycosides Test:

2 ml of glacial acetic acid, around 0.5 ml of extract, and a few drops of 5% ferric chloride were added. Then one ml of strong sulfuric acid was added. The brown ring formation suggested the presence of cardiac glycosides.

j) Glycosides Test:
In a test tube 2ml of extract, 3ml of Chloroform, and 2ml of ammonia solution were added. The formation of pink colour indicated the presence of Glycosides.

k)Anthroquinones Test:
1ml of extracts added a few drops of 10% ammonia solution. The appearance of pink color indicated the presence of Anthraquinone.

l)Polyphenols Test:
In a test tube 1ml of extract, 2ml of distilled water, and a few drops of 10% Ferric chloride were added. The formation of a blue color indicated the presence of Polyphenols.

2.4. Anti-oxidant activity by DPPH assay:
The DPPH test is widely used in antioxidant research. This assay is a popular, quick, and affordable approach for the measurement of anti-oxidant properties in crude plant extracts. This assay is based on the theory that an antioxidant donates hydrogen. It measures chemicals that remove radicals from the body. The process by which DPPH takes up hydrogen from an antioxidant is seen in Figure 4 below. DPPH is one of the few stable organic nitrogen radicals that can be bought. The antioxidant effect is indicated by the rate at which DPPH disappears in test samples. The most widely used method for measuring DPPH has been using a UV spectrometer because of its accuracy and simplicity of use. DPPH has a strong absorption maximum at 517 nm (purple). When hydrogen from an antioxidant is absorbed, the color changes from purple to yellow, and DPPH is formed. It is a stoichiometric process in terms of the quantity of absorbed hydrogen atoms. Consequently, it is simple to assess the antioxidant action by monitoring the decline in UV absorption at 517 nm

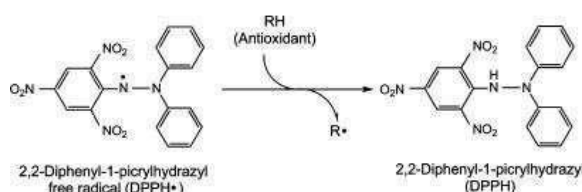

Fig-4 DPPH Mechanism of reaction

PROCEDURE:

1. Prepare the 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of Samples respectively at different concentrations (5, 10,15,20 µg/ml).
2. The mixes must be quickly mixed and then left to stand for half an hour at room temperature.
3. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference

4. Lower absorbance values of the reaction mixture indicate higher free radicalscavenging activity.

5. The capability of scavenging the DPPH radical can be calculated by using the following formula.
6.DPPH scavenging effect (% inhibition) = [(absorbance of control- absorbance of reaction mixture)/absorbance of control] X 100.

2.5. Antibacterial activity:

The crude extracts were subjected to a preliminary antibacterial screening utilizing the agarwell diffusion assay technique. Plant extracts were dissolved in 100% v/v dimethyl sulfoxide (DMSO) to yield working quantities of 20 mg/mL. Using sterile cotton swabs, aseptically disseminate standardized broth cultures of test bacterial isolates (*E. Coli, Enterobacter Sps, Staphylococcus aureus,* and *Pseudomonas aeruginosa*) onto the Mueller Hinton Agar (MHA) platesurface. After letting each culture plate dry for roughly five minutes, sterilized cork borers (8 mm in diameter) were used to create agar wells. Two hundred microliters of each of the crude extracts and controls were put into each of these wells. After one hour at room temperature to enable the agents to permeate into the agar media, the plates were appropriately incubated. The antibacterial test employed, DMSO (100% v/v) as the negative control and gentamycin (50 µg/mL) as the positive control. After that, the MHA plates were incubated for 24 hours at 37 °C. Measurements were made of the inhibition zone diameters (IZDs).

3. RESULT AND DISCUSSION:

3.1. Phytochemical Screening of *Nigella Sativa* seeds:

Nigella sativa is one of the medicinal plant species that gained popularity for a wide range of medicinal applications due to its seeds, generally known as black seeds, which are rich in phytoconstituents.

Phytochemical analysis of the seed extract of *Nigella Sativa* was investigated in different solvent extracts (Acetone, Petroleum ether, Diethyl ether, Hexane, chloroform and ethanol). The analysis of different solvent extracts showed the presence of Terpenoids, Carbohydrates, Phytosterol, Alkaloids, Quinone, Saponin, Steroids, Flavonoids, Cardiac Glycosides, Anthroquinones, and Polyphenols. Glycosides were absent in all the solvent extracts (Table -1). According to the current research study findings, which are in line with past phytochemical screening investigations done using various solvent extracts of *N. sativa* seeds are excellent sources of phytochemicals, or medicinally active substances. The study reported that the *N.sativa* seeds contain many active phytoconstituents such as carbohydrates, proteins, dietary minerals (such as Fe and Zn), vitamins, crude fiber, alkaloids, saponins, steroid, terpenoids, p-cymene, limonene, and fatty acids (Mengesha, 2015, Srinivasan, 2018 and Hadi *et al*., 2016).

Conferring to a previous study, bioactive components of *N. sativa* seeds, including phenols, tannins, alkaloids, flavonoids, and terpenoids, are known to elicit wide antimicrobial responses against a variety of pathogens, including viruses, fungi, bacteria, and parasites (Hasan Khan *et al*.,2019, Francois *et al*., 2020, Kalaichelvi *et al.,* 2017).

Table -1: Phytochemical analysis of *Nigella Sativa* seed extracts

Phytochemical tests	Solvent extracts						Observation
	Acetone	Petroleum ether	Diethy lether	Hexane	Chloroform	Ethanol	
Terpenoids test	++	++	-	++	+	++	red-brown colour
Carbohydrates	+	+	+	+	+	--	Reddish Colour
Phytosterol	++	+	-	+	+	++	brown colour
Alkaloids	+	++	+	+	-	+	Green colour
Quinones	++	++	-	++	+	++	red colour
Saponin	+	+	-	++	+	+	Stable foam formation
Steroids	-	++	+	++	-	+	Upper layer red color
Flavonoids	+	+++	-	++	-	++	Yellow colour
Cardiac glycosides	+	+	+	+	+	--	brown ring
Glycosides	-	-	-	-	-	-	Pink colour
Anthroquinones	+	++	-	+	-	+	pink colour
Polyphenols	++	+++	-	++	-	+	blue colour

(-) Absence, (+) Presence, (++) Moderate presence , (+++) High presence

3.2. DPPH Radical Scavenging Activity:

The scavenging capacity of different solvent extracts of *Nigella sativa* seeds were analyzed using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay.

Table -2 Hexane extracts of *Nigella sativa* seeds:

CONCENTRATION	PERCENTAGE OF INHIBITION
5 µg/ml	4
10 µg/ml	12
15 µg/ml	18
20 µg/ml	23

Table -3 Ethanolic extracts of *Nigella sativa* seeds:

CONCENTRATION	PERCENTAGE OF INHIBITION
5 µg/ml	9
10 µg/ml	13
15 µg/ml	19
20 µg/ml	24

Table -4 Acetone extracts of *Nigella sativa* seeds:

CONCENTRATION	PERCENTAGE OF INHIBITION
5 µg/ml	9
10 µg/ml	15
15 µg/ml	22
20 µg/ml	29

Table -5 Petroleum ether extracts of *Nigella sativa* seeds:

CONCENTRATION	PERCENTAGE OF INHIBITION
5 µg/ml	11
10 µg/ml	18
15 µg/ml	28
20 µg/ml	37

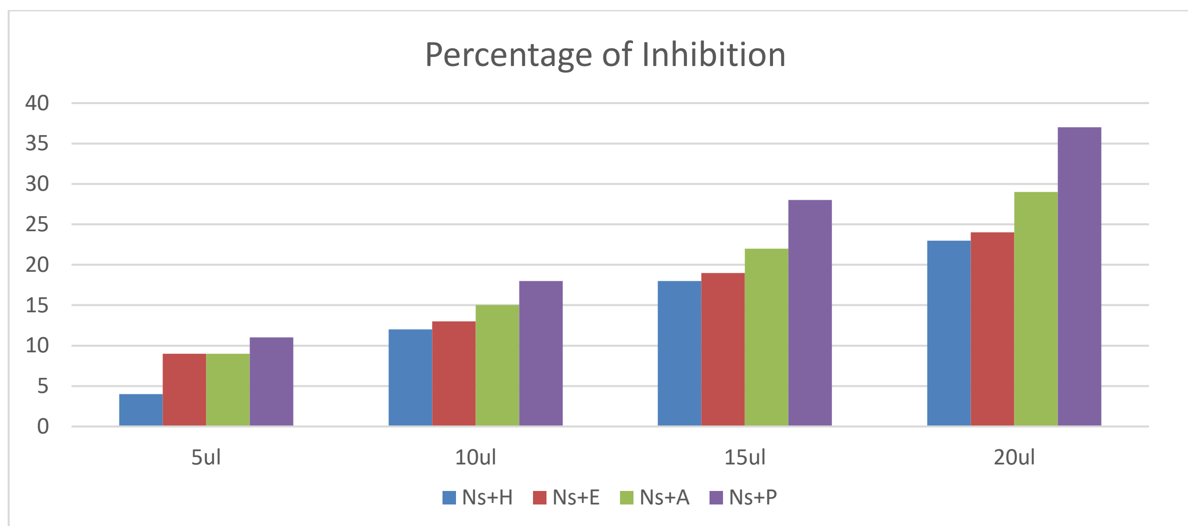

Fig-5: Antioxidant properties of different solvent extracts of *Nigella sativa* seeds

Ns+H (*Nigella sativa seed* + Hexane), Ns+E(*Nigella sativa seed* + Ethanol) , Ns+A(*Nigella sativa seed* + Acetone), Ns+ P(*Nigella sativa seed* +Petroleum ether)

The DPPH antioxidant tests' results on the radical scavenging activity are compiled in Table 2 – 5 (Fig-5). It was done for the extracts of hexane, ethanol, acetone, and petroleum ether they were all compared to the ascorbic acid reference value. It was observed that the petroleum ether and acetone extracts showed strong anti-oxidant scavenging activity followed by ethanol and hexane extracts. This may be due to the presence of active secondary metabolites. A study report by Tiji *et al* (2021) the antioxidant qualities of NS seed hexane and acetone extracts and fractions were compared. The presence of fractions with varying degrees of antioxidant activity in the extracts was confirmed by the authors; this unique character possibly depends on precise secondary metabolites present in the fractions, such as polyphenols, terpenoids etc. The methanolic extract from NS seeds has been shown to have stronger antioxidant activity than the aqueous extract in another investigation by Pop *et al.,* (2020)· According to Ouattar *et al*., (2022)· NS seed extracts have dose-dependent antiradical action, with crude extract exhibiting less activity than extracts made of n-butanol or ethyl acetate.

3.3. Anti-bacterial activity:

(A)

(B)

Fig – 6 : Inhibitory effect of solvent extracts of Nigella sativa seeds
A) Anti-bacterial activity against *E.coli* B) Antibacterial activity against *Enterobacter* C) Antibacterial activity against *Pseudomonas aeruginosa* D)Antibacterial activity against *Staphylococcus aureus*

The antibacterial activity of *Nigella sativa* or black cumin seed extracts was studied against a few common pathogenic microorganisms (i.e. *E.coli, Enterobacter* Sps, *Staphylococcus aureus, Pseudomonas aeruginosa*). The observed zones of inhibition from different extracts were measured and confirmed using Gentamycin (standard antibiotic) as a positive control, and DMSO as a negative control (Fig-6). The black cumin (or) *N. sativa* seed solvent extracts exhibited a variety of complex antibacterial properties with varying degrees of potency against harmful bacterial strains. A similar result was reported by Festus *et al.,*(2022) regarding the antimicrobial activity of undiluted extracts of *N. sativa* seeds using the disc diffusion assay on nutrient agar plates. The order of the bacteria that the extract from hexane oil inhibited was *B. subtilis > S. aureus > E. coli > P. aeruginosa.*

Methanol and aqueous extracts of *N. sativa* seeds contain therapeutically active components like alkaloids, tannins, phenols, and flavonoids or other phytochemicals that have been recognized as antimicrobial compounds. As a result, their synergism mechanism against predation by pathogenic microorganisms can serve as a defense, as demonstrated by earlier phytochemical investigations conducted in previous studies (Kalaichelvi K and Dhivya, 2017., Chudobova *et al.,*2014, Costa *et al.,*2013).

Another research by Sivanandham (2015) and Shikwambi *et al*., (2021) found that aqueous extracts had less inhibitory activity than extracts from organic solvents. Since they were unable to inhibit the majority of the tested microorganisms. They concluded that most water-soluble phytochemicals, including flavonoids, may not have any antibacterial properties and that phenolic compounds may only be significant as antioxidants. Furthermore, it was revealed that while water is a common solvent utilized by conventional healers, organic solvents like methanol, ethanol, and hexane have been shown to extract more of the antibacterial agents or bioactive constituents to consistently exhibit antimicrobial activity. In our study also observed a similar kind result that, different solvent extracts show possible Zone of inhibition against the selected pathogenic organism.

4. CONCLUSION:

The medicinal qualities and phytochemicals of *Nigella sativa* have been investigated since the 1950s. From the present study it was concluded that different solvent extracts of *Nigella sativa* seed show the presence of many phytochemicals like Terpenoids, Carbohydrates, Phytosterol, Alkaloids, Quinone, Saponins, Steroids, Flavonoids, Cardiac Glycosides, Anthroquinones and Polyphenols, this ascertained their medicinal value. Glycosides were absent in all the solvent extracts. Based on the phytochemical analysis and previous literature four solvent extracts (Acetone, petroleum ether, Hexane and Ethanol) were used for the antioxidant activity by DPPH assay. The petroleum ether and acetone extracts showed potent anti-oxidant scavenging activity followed by ethanol and hexane extract when increasing the concentration of extracts. Important secondary metabolites and a dependable source of antioxidant chemicals can be found in *Nigella sativa* L. Each solvent extract shows potential inhibitory activity against the selected pathogenic microorganism, this may be due to the presence of active secondary metabolites. These findings could be utilized to create multifunctional medicinal products, and their use as dietary sources of antioxidants for the prevention and treatment of disease should be given careful consideration.

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CONFLICT OF INTEREST:

The authors declare no conflict of interest.

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REFERENCES:

1. Arunkumar S, Muthuselvam M. (2009), Analysis of phytochemical constituents and antimicrobial activities of Aloe vera L against clinical pathogens. World J. Agric. Sci, 5, 572–576.

2. Khan MA. (1999), Chemical composition and medicinal properties of Nigella sativa Linn. Inflammopharmacol , 7(1), 15–35.

3. Abbas RZ, Colwell DD, Iilleard J. (2021), Botanicals: An alternative approach for the control of avian coccidiosism. World Poultry Sci J, 6 8, 203–215.

4. Shah AJ, Zaidi MA, Sajjad H, Hamdullah, Gilani AH. (2011), Antidiarrheal and Antispasmodicactivities of the extract of Vincetoxicum stocksii are mediated through calcium channelblockade. Bangl. J. Pharmacol, 6, 46–50.

5. Zaman MA, Iqbal Z, Abbas RZ, Khan MN. (2012), Anticoccidial activity of herbal complex inbroiler chickens challenged with Eimeria tenella. Parasitol ,139, 237–243.

6. Kahl, Kappus R. (1993), Toxicology of the synthetic antioxidants BHA and BHT in comparisonwith the natural antioxidant vitamin E, Zeitschrift fur Leb. Untersuchung und -forsch, vol., 196,329–338.

7.Saleh FA, El-Darra N, Raafat K, Ghazzawi IE. (2017), Phytochemical analysis of nigella sativa L. Utilizing GC-MS exploring its antimicrobial effects against multidrug-resistant bacteria. Pharmacognosy Journal, vol. 10(1), 99–105.

8. Bourgaud F, Milesi S, Gontier E. (2001), Production of plant secondary metabolites: a historicalperspective. Plant Science, vol. 161(5), 839–851.

9. Grech-Baran M, Pietrosiuk A. (2012), Artemisia species in vitro cultures for production of biologically active secondary metabolites. BioTechnologia, vol. 4, 371–380.

10.Avicenne BS, Al-Sheikh IS, Al-Hussein Ben Ali RA, Le Canon de la M´edecine.(1999), Les medicaments simples (non composes). Avicenna Canon of Medicine, Kazi Publi- ´ cations, Chicago, IL, USA.

11. Iran J. (2014), Black cumin (Nigella sativa) and its constituent (thymoquinone) a review on antimicrobial effects. Basic Med Sci, 17(12), 929–38.

12. Wajs A, Bonikowski R, Kalemba D. (2008), Composition of essential oil from seeds of Nigellasativa L. cultivated in Poland. Flavour Fragr. J, 23, 126–132.

13. Singh G, Marimuthu P, De Heluani CS, Catalan C. (2005), Chemical constituents and antimicrobial and antioxidant potentials of essential oil and acetone extract of *Nigella sativa* seeds. Journal of the Science of Food and Agriculture, 85(13), 2297–2306.

14. Soto C, Chamy R, Zuniga M. (2007), Enzymatic hydrolysis and pressing conditions effect on borage oil extraction by cold pressing. Food Chem,102: 834–840.

15. Bourgou S, Bettaieb I, Saidani M, Marzouk B. (2010), Fatty Acids, Essential Oil, And PhenolicsModifications of Black Cumin Fruit under NaCl Stress Conditions. J. Agric. Food Chem, 58, 12399–12406.

16. Rao MV, Al-Marzouqi AH, Kaneez FS, Ashraf SS, Adem A. (2007), Comparative Evaluationof SFE and Solvent Extraction Methods on the Yield and Composition of Black Seeds (Nigella sativa). J. Liq. Chromatogr, Relat. Technol, 30, 2545–2555.

17. Zúñiga ME, Soto C, Mora A, Chamy R, Lema JM.(2003), Enzymic pre-treatment of Guevina avellana mol oil extraction by pressing. Process Biochem, 39, 51–57.

18. Belfekih F, El O, Mariam Y, Lella C. (2017), Screening phytochimique D’Arbutus unedo L. HeAmerican Journal of Innovative Research & Applied Sciences, vol. 5(3), 237–245.

19.Louiz I, Sellem F, Tekitek A, Langar H, El Abed A.(2003), Etude des saponines isol ´ ees d’uneesp ´ ece d’holothurie ` Holothuria tubulosa de la lagune de Bizerte. Bulletin de L’INSTM Salammbo, vol. 30, 115–119.

20. Harborne JB. (1998), Textbook of Phytochemical Methods. A Guide to Modern Techniques ofPlant Analysis. 5th Edition. Chapman and Hall Ltd, London, 21-72.

21. Thangaraj P. (2016), Pharmacological Assays of Plant-Based Natural Products. Springer, Geneva.

22. Mengesha Yessuf A. (2015), Phytochemical Extraction and Screening of Bio-Active Compounds from Black Cumin (Nigella Sativa) Seeds Extract. Am J Life Sci, 3(5), 358.

23. Srinivasan K. (2018), Cumin (Cuminum cyminum) and black cumin (Nigella sativa) seeds: Traditional uses, chemical constituents, and nutraceutical effects. Food Qual Saf , 2(1), 1–16.

24. Hadi MY, Mohammed GJ, Hameed IH. (2016), Analysis of bioactive chemical compounds ofNigella sativa using gas chromatography-mass spectrometry.J Pharmacogn Phyther, 8(2), 8–

25. Hasan Khan N, Yoke Hwa C, Perveen N and Paliwal N. (2019), Phytochemical screening, antimicrobial and antioxidant activity determination of Trigonella foenum-graecum seeds. Pharm Pharmacol Int J , 7(4).

26. Francois N, Charity NM, Fred O, Meryl C, Nicholas A, Jones OM. (2020), Antimycobacterialactivities, cytotoxicity and phytochemical screening of extracts for three medicinal plants growingin Kenya, J Med Plants Res, 14(4),129–43.

27. Kalaichelvi K, Dhivya SM. (2017), Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of Micrococca mercurialis (L.) Benth. Int J Herb Med 2017, 5(6), 40–4.

28. Tiji S, Benayad O, Berrabah M, El Mounsi I, Mimouni M.(2021), Phytochemical Profile and Antioxidant Activity of Nigella sativa L Growing in Morocco, Sci. World J, 20, 6623609.

29. Pop RM, Bocsan IC, Buzoianu AD, Chedea VS, Socaci SA, Pecoraro M, Popolo A. (2020), Evaluation of the Antioxidant Activity of Nigella sativa L. and Allium ursinum Extracts in a Cellular Model of Doxorubicin-Induced Cardiotoxicity. Molecules, 25, 5259.

30.Ouattar H, Zouirech O, Kara M, Assouguem A, Almutairi SM, Al-Hemaid FM, Rasheed RA, Ullah R, Abbasi AM, Aouane M.(2022), In Vitro Study of the Phytochemical Composition and Antioxidant, Immunostimulant, and Hemolytic Activities of Nigella sativa (Ranunculaceae) and Lepidium sativum Seeds. Molecules, 27, 5946.

31. Festus S. Shafodino, Julien M. Lusilao, Lamech M. Mwapagha I.(2022), Phytochemical characterization and antimicrobial activity of Nigella sativa seeds. PLOS ONE, 17(8), e0272457.

32. Chudobova D, Dostalova S, Blazkova I, Michalek P, Ruttkay-Nedecky B, Sklenar M. (2014), Effect of ampicillin, streptomycin, penicillin and tetracycline on metal resistant and non-resistant Staphylococcus aureus. Int J Environ Res Public Health, 11(3), 3233–55.

33.Costa EV, Da Cruz PEO, De Lourenc¸o CC, De Souza Moraes VR, De Lima Nogueira PC, Salvador MJ. (2013) , Antioxidant and antimicrobial activities of aporphinoids and other alkaloidsfrom the bark of Annona salzmannii A. DC. (Annonaceae). Nat Prod Res, 27(11), 1002–6.

34. Sivanandham V and Institutions TM. (2016), Phytochemical techniques—a review 2016.

35. Shikwambi NC, Onywera H, Mwapagha LM. (2021), An in vitro investigation of the phytochemical contents of Marsdenia macrantha root and its antibacterial activity against selectedfoodborne pathogens. Pan Afr Med J, 40.