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Phytochemical Investigation and Spectrophotometric Studies of Piperine and Thymoquinone Compounds in Methanolic Extracts of *Nigella sativa* and *Piper nigram* spices

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Abstract

This study was carried out on some spice samples including *Nigella sativa* and *Piper nigram*. Phytochemical screening was applied to the methanolic extraction of both the studied plants. The results showed that methanolic extract of *Nigella sativa* and *Piper nigram* revealed the presence of various chemical constituents, such as Alkaloide, Carbohydrates, Glycosides, Tannins, Flavones, Saponins. Piperine is characterizing compound present in fruits of *piper nigram* used as a bioavailability enhancer. Some spectrophotometric methods as (U.V) and I.R were applied to identify the major chemical compounds of the studied extracts. The results recorded that the Piperine and Thymoquinone compounds were found in the studied plant extracts.

Keywords: *Piper Nigram*, *Nigella sativa*, Piperine Thymoquinone, Spectrophotometric

Introduction

The Plants are significant wellsprings of medication utilizing in all societies from antiquated occasions. The different native plants are being utilized in the analysis, counteraction, and disposal of physical, mental, or social irregularity [1]. Restorative plants are the foundation of customary medication and are vital to the wellbeing of people and networks [2]. The restorative estimation of these plants lies in some synthetic substances that produce a distinct physiological activity on the human body [3].

The medications are gotten from the entire plant or from various organs, similar to leaves, stem, bark, root, blossom, seed, and so on. Some medications are set up from excretory plant items, for example, gum,

pitches, and latex. Phytochemistry is a part of science with photochemical, for example, synthetics acquired from the plant source. These mixes are known as auxiliary plant metabolites and have organic properties, for example, cancer prevention agent movement, antimicrobial impact, tweak of detoxification proteins, incitement of the safe framework, reduction of platelet collection, and adjustment of chemical digestion and anticancer property [4].

The phytochemical study helps in finding the elective wellspring of remedial synthetic substances of significance. The FT-IR and UV have demonstrated to be an important apparatus for portrayal and ID of mixes or useful gatherings (substance securities) present in an obscure combination of plant remove [5,6]. It is a quick, non-dangerous strategy with the least example readiness essential [7]. Nigella sativa is a native herbaceous plant that has a place with the Ranunculaceae family that is all the more usually known as the fennel bloom plant. This plant has finely separated foliage and blue blossoms, which produce dark seeds. In Islamic medication, the utilization of the dark seeds is suggested in everyday use since it is viewed as probably the best type of mending medication accessible Nigella sativa seeds, as the healthful and restorative plant. It has generally been utilized for years as people medication and a portion of its dynamic mixes were accounted for against numerous afflictions.

It was accounted for that the seeds of Nigella sativa and oil constituents, specifically thymoquinone (TQ), that have demonstrated likely restorative properties; they display intense mitigating impacts on a few aggravations based models including test encephalomyelitis, colitis, peritonitis, edema, and joint pain through concealment of the incendiary go-between prostaglandins and leukotrienes [8]. The oil and dynamic element of TQ demonstrated advantageous invulnerable modulator properties, enlarging the T cell and regular executioner cell-interceded resistant reactions [9].

Distinctive pharmacological impacts, for example, gastric ulcer recuperating [10], hostile to microbial impact [11], against disease action [12]. cardiovascular disorders [13], calming and antitumor impacts [1], antitussive impact [14], hostile to uneasiness impact [15], against asthmatic impact, mitigating impacts in pancreatic malignancy cells [16], hostile to helicobacter movement [17], tumor development concealment [18] and hostile to viral action against cytomegalovirus [19] have been accounted for this therapeutic plants.

The product of Piper nigrum is one of the most seasoned and most mainstream flavors on the planet, native to the Malabar shoreline of India and utilized as a sweet-smelling energizer in cholera, dyspepsia, fart, hostile to intermittent in malarial fever, and joint pain infection. *Piper nigrum* is all the more notable species on account of its high business, monetary and restorative properties [20]. The *phytochemistry* of the variety flutist is rich where the examinations have uncovered the sufficient presence of terpenes, amides, and alkaloids [21-23]. It is realized that *Piper nigrum* has natural action, for example, central nervous system depressant, cell reinforcement, extremist rummaging, against *insecticidal*, *antibacterial*, *allelopathy*, a*nticonvulsant*, hostile to *tubercular*, *antipyretic*, *mitigating*, *antioxidant*, and *exteroceptive (24, 25).* Dark pepper has assumed a significant part in molding recorded occasions on the plants. This study was aimed to investigate *phytochemical* and *spectrophotometric* of *piperine* and *thymoquinone* compounds in *methanolic* extracts of *Nigella sativa* and *Piper nigram* spices.

Materials and Methods

Collection of Samples

The two plant species: (*Nigella sativa* and *Piper nigrum*) were collected randomly from El-Beida City market. The plants were washed with refined water and air dried at room temperature. Then samples were dried then grinded by mortar.

Preparation of the Extracts

The Extraction was done by the method described by [26]. Twenty-five grams of powdered plants were gauged and blended with 100 ml methanol in and kept in a rotatory shaker at 150 rpm for 4 hours. At that point, separate were dissipated under diminished tension utilizing turning evaporator contraption and permitted to dry in the incubator till total dryness.

Phytochemical Screening of Spices

Phytochemical screening was carried out on methanol extract of spices. The following tests were performed to detect various phytochemical constituents which may be present in the studied plant extracts.

Screening for Alkaloids (Mayer's Test)

To 2 ml of the extract was boiled with dilute hydrochloric acid and the mixture filtered and to the filtrate a few drops of Mayer's reagent. A cream or white color precipitate produced immediately indicates the presence of alkaloids**.**

Screening for Carbohydrate Test

To 1ml of extract, 1ml of Benedict's reagent was added. The mixture was heated on a boiling water bath for 2 minutes solution appeared green showing the presence of reducing sugar.

Screening for Glycosides (Keller Kilianin Test)

5ml of each extract was added with 2 ml of glacial acetic acid which followed by the addition of few drops of ferric chloride solution and 1ml of concentrated Sulphuric acid. The formation of the brown ring at the interface confirms the presence of glycosides.

Screening for Terpanoids (Salkowski Test)

5 ml of extract was taken in a test tube and 2 ml of chloroform added to it followed by the addition of 3ml of concentrated sulphuric acid. The formation of the reddish-brown layer at the junction of two solutions confirms the presence of terpenoids.

Screening for Flavonoids (Alkaline Reagent Test)

2 ml of extracts were treated with few drops of 20% sodium hydroxide solution formation of intense yellow color, which becomes colorless on the addition of dilute hydrochloric acid, which indicates the presence of flavonoids.

Screening for Saponins (Foam Test)

2ml of the extract was taken in a test tube and 6ml of distilled water added to it. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Screening for Steroids

1ml of the extract was dissolved in 10ml of chloroform and an equal volume of concentrated sulphuric acid added by the sides of the test tube. The upper layer turns red and the sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids.

Screening for Tannins

2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicated the presence of tannins.

Results and Discussion

The results of the phytochemical investigation demonstrated the presence of wide compounds of natural products. The phytochemical screening showed the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, phytosterol, saponin, and tannins, (Table:1). The presence of alkaloid (natural compound with nitrogen-containing bases that has different therapeutic properties) piperine bio enhancer is a specialist which expands bioavailability and bioefficacy of a specific medication with which it is joined; it doesn't have any commonplace pharmacological movement of its own at the portion utilized. Piperine a significant constituent is an individual from the Lipids family.

Lipids are a gathering characterized as comprising of fats or fat-like substances. As a fragrant zest, the dark pepper is described by a slight and recognizable stale smelling fragrance; this smell is a direct result of the presence of numerous unstable oils fundamentally in the fragile living creature and skin of the natural product. The entirely recognizable and sharp "chomp" of the dark pepper is because of the presence of two essential alkaloids in the pepper, are the mixes piperine and the compound piperidine, the tart nibble additionally comes somewhat due to the numerous particular plant tars which are found in the seeds of the spice. Piperine was separated from dark pepper by utilizing methanol. After disengagement, recognizable proof of the item was finished by softening point, IR, and UV noticeable spectrophotometer Analysis of secluded Piperine Crystal.

Table (1): The results of phytochemical screening.

Plant species	**Metabolite**							
	1	2	3	4	5	6	7	8
Nigella Sativa	+	+	+	+	+	–	+	–
Piper nigrum	+	+	+	+	+	++	+	–

Where :1= Flavonoids 2 = Alkaloids , 3= Charbohydrats , 4 = Cardiac glycosides , 5= Steroid , 6= Terpenoids , 7= Saponins and 8= Tanins.

Chemical tests

All Chemical Tests for alkaloids were positive and especially with concentrated H2SO4, where red color was obtained. The reddish-brown precipitate was obtained with Dragendroff's Reagent. The U.V spectra of Piperine: was showed in figure (1).

The presence of fragrant C – H extending indicated assimilation over 3000 cm^{-1} and around 2872.64 cm^{-1} for aliphatic C–H extending. Our finding concurred with the reference information. A solid retention band in the area around 1700 cm^{-1} is because of the C=O extending and the presence of this band shows that the ketonic gathering (C=O) might be available in the compound. Piperine demonstrated ingestion at 1688.19 cm^{-1} which showing the presence of ketonic (C=O) gathering. An ether gathering (methylene ether, – O– CH_2 – O –) is particularly obvious from the solid band at 1215 cm^{-1} whereas the trial information found at 1179 $cm^{-1}$and 1215 cm^{-1} for symmetric and unbalanced extending of a similar ether gathering, individually.

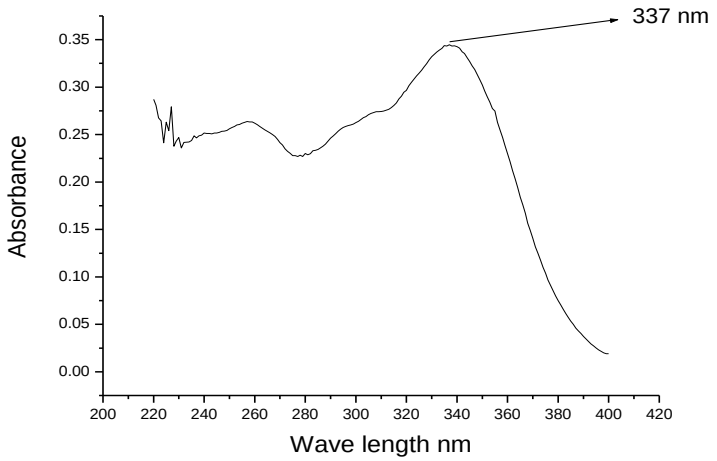

Figure (1): The λ_{max} of Piperine.

Also, the chemical structure of piperine was shown in figure (2).

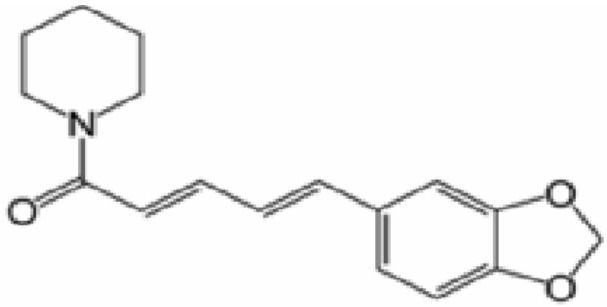

Figure (2): Structure of piperine.

Whereas the I.R spectra were shown in figure (3).

U.V. Spectra

The wavelengths of absorption spectra can be correlated with the types of bonds in a given molecule and are valuable in determining the functional groups present within a molecule. Thymoquinone is characterized by the presence of a prominent peak (λ_{max}) at 257 nm, in this study, one peak was appear at 258 nm, this mainly gave indication of presence Thymoquinone in the studied extract, (Figure, 4)

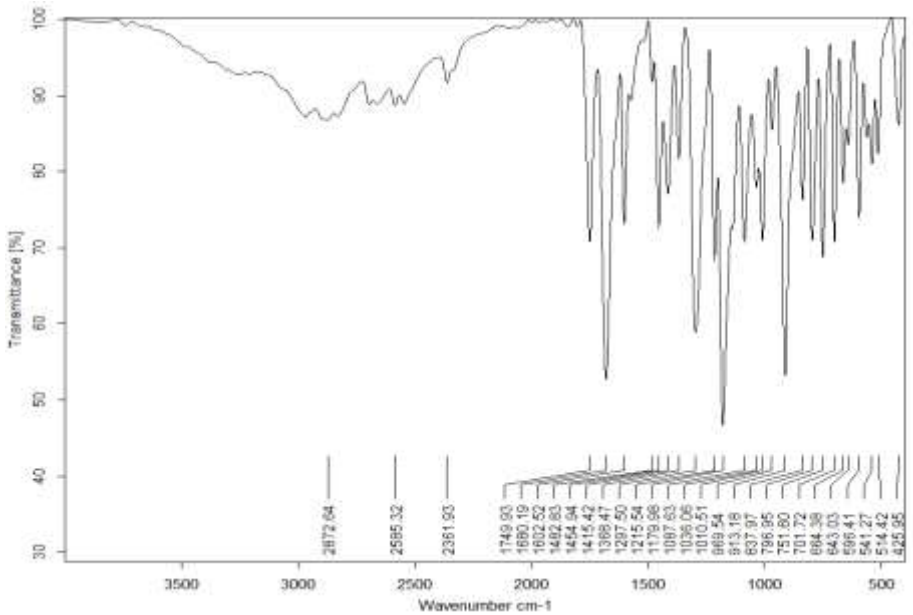

Figure (3): The I.R spectra of piperine.

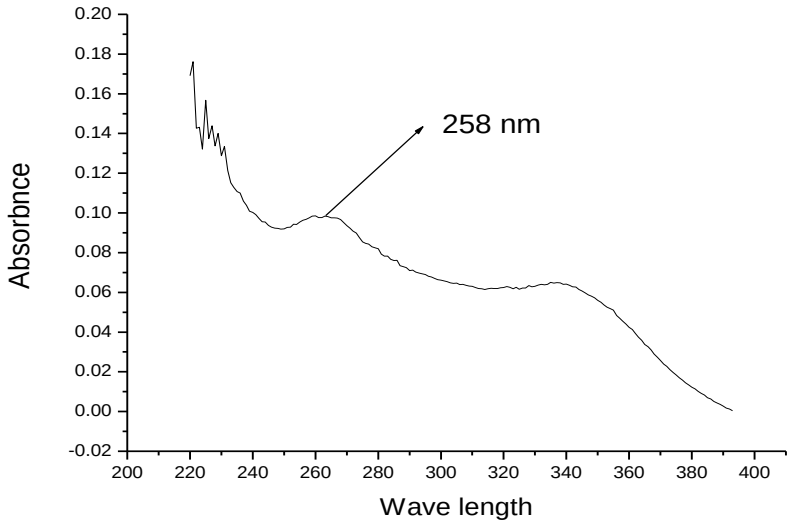

Figure (4): The UV analysis of Thymoquinone.

The structure of Thymoquinone in Figure (5) as following:

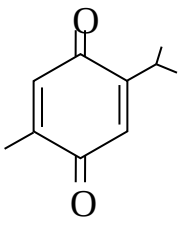

Figure (5): Structure of Thymoquinone.

Also, the I.R analysis was used for the extract, the I.R spectra are in harmony with the U.V analysis (Figure 6).

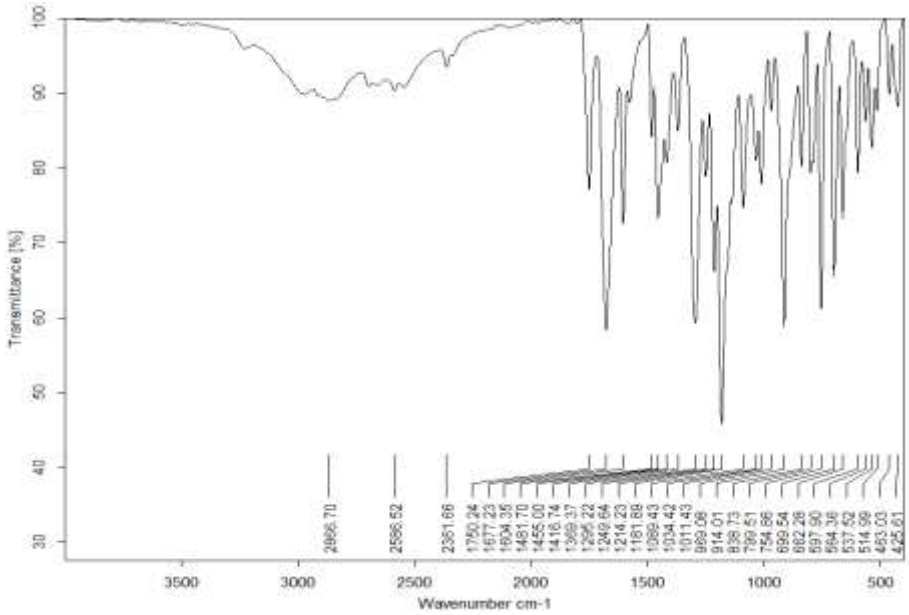

Figure (6): I.R OF Thymoquinone.

The IR assimilation spectra of Thymoquinone (THQ) were gotten. The (IR) spectra showed a strong extending band of the carbonyl gathering of a cyclohexadiene is seen at the wavenumber 1677cm-1, which is upheld by the qualities detailed for thymoquinone (1650 cm-1) and 1,4 benzoquinone (1661cm-1). The extraordinary band present at (2866. Cm-1) compares to the C-H extending of aliphatic gatherings, and the worth recently revealed was (2969 cm-1). The more fragile band saw at a higher wavenumber (~3040 cm-1) was allotted to the extending seen in the vinylic C - H in the C = C-H gatherings, which had recently been accounted for at wave number (3041 cm-1). This component can be seen all the more plainly as a detached band in the range of 1,4-benzoquinone (with-out aliphatic C-H stretching's) at (3058 cm-1).

The C = C extending (1640-1675 cm^{-1}) yielded a separated and decently solid band at (1640 cm^{-1}) in 1, 4-cyclohexadiene. The C = C extending band can't be unambiguously distinguished on the grounds that the solid carbonylic extending band in thymoquinone is available in this recurrence range. Likewise, the force of the C = C band is relied upon to be lower than both the carbonylic band and the C = C extending in 1, 4 cyclohexadiene, which contains no methyl and isopropyl substituent's; nonetheless, it should be noticed that there is a change at (1673 cm^{-1}) marginally isolated from the carbonylic band (a similar component is available at (1678 cm^{-1}) in 1, 4-benzoquinone) that could be probably attributed to the C = C extending band. The range was found to display tops like those revealed in the writing. The compound design of THQ.

Conclusion

According to the results which were obtained in this study, it could be concluded that *Nigella sativa* seeds and *pipper nigram* contains various bioactive compounds. The presence of these bioactive compounds justifies the use of the seeds for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting them to biological activity will definitely give fruitful results.

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