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Research Paper

Qualitative Phytochemical Characterization and Antimicrobial Activity of Nigella Sativa Seeds

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Abstract
Nigella sativa popularly known as black seed has gained popularity for a wide range of medicinal applications due to its seeds which are rich in phytochemicals. Different extracts of Nigella sativa seeds were obtained by maceration and extraction using four solvents (water, methanol, ethylacetate and hexane). The obtained extracts were tested for the presence of phytochemicals. The four extracts were found to contain alkaloids, steroids and terpenoids. Other phytochemicals such as flavonoids, phenols and tannins were only detected in aquesous and methanol extracts. The disc diffusion method was used to investigate the antimicrobial activity of N. sativa seeds extract against pathogenic bacterial strains (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis). All the extracts at 100 percent concentration were active against all the five bacteria except aqueous extract which is active against B. subtilis at 100 and 50 percent.Therefore the seeds of N. sativa can be used a natural source of antimicrobial agents in the pursuit of searching for new antibiotics against human pathogen.

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I. Introduction

Nigella sativa belong to the family Ranunculaceae, the genus is relatively small and contains about 18 species, all Nigella species are annuals [1]. Nigella sativa is the most well known member in this genus, it grows between 20-90cm in height and has threadlike leaves [2]. The plant has pale-blue to pale-purple flowers that bloom in the spring and produce fruit that contain numerous black seeds [3]. It is commonly known as black seed, black cumin and black caraway [4]. There is an evidence of human use of *N. sativa* seed from 1324BCE [5]. *N. sativa* seed is widely used in the traditional Ayurvedic system of medicine [6] and it is also used in the traditional Arabic Islamic medicine and its medicinal uses were also described in the hadith literature attributed to prophet Muhammed that Nigella seed is a medicine for every disease except death [7].

Medicinal plants from time immemorial have always been natural factories of phytochemicals such as alkaloids, flavonoids, tannins, phenols and terpenoids which are responsible for their biological activities [8]. Plants products (phytochemicals) are generated from leaves, flowers, fruits, seeds. Barks and roots and many bioactive constituents of plant have been detected and they have been characterized using different standard analytical methods [8].

Nigella sativa is one of the medicinal plant species that gained popularity for a wide range of medicinal applications due to its seeds which are rich in phytoconstituents. [9,10,11,12] reported that the major constituents in the seeds have been reported to have appetizer, antidiabetic, antimicrobial, antioxidant, analgesic and antimicrobial properties [8,11,13]. Although several works has been done on the chemical composition and pharmacological properties of *N. sativa* seeds but [14] reported that the complete chemical profile of *N. sativa* seed extracts is not yet determined. In this view, it is necessary to evaluate the therapeutic properties such as the promising antimicrobial effects of herbal extracts due to the escalating search of natural products that confer desirable antibacterial or antimicrobial properties against antibiotic resistance [15] and hence the need for this research.

Investigation pertaining to the analysis and antimicrobial properties of the phytoconstituents of natural products such as the extracts of *N. sativa* seeds offer new opportunities to discover and formulate effective

antibiotics as alternative treatment in the case of drug resistant pathogenic bacterial strains [16,17,18]. This study therefore aimed to investigate the chemical composition of the extracts of *N. sativa* seeds in four different solvent systems and to infer from these results the phytoconstituents that are responsible for their corresponding antimicrobial activity against various clinically resistant bacteria as well as to compare these findings with those reported worldwide.

II. Materials and Methods

Collection of sample and preparation of the extract.

N. sativa seeds were purchased from a herbal shop in a local market in Oyo town, Oyo state, Nigeria. It was washed and allowed to dry at room temperature before pulverization into a powder. The powder was used for the preparation of medicinally active extracts.

Preparation of different solvent extract

50g of *N. sativa* seed powder were soaked separately with 250ml of each solvent (water, methanol, ethylacetate and hexane) for 24 hours at room temperature and the filtrates were separated from the solid residue using whatman No 1 filter paper. All the filtrates were pre-concentrated under reduced pressure using rotary evaporator at 40-60^{o}C to yield crude extract (aqueous, methanol. Ethylacetate and hexane). All the extracts were stored at 4^{o}C in a refrigerator until analysis.

Qualitative Phytochemical Screening

The extracts obtained were subjected to phytochemical screening using standard methods [8,19,20].

- Test for flavonoids: Three drops of ferric chloride hexahydrate ($FeCl_36H_2O$) solution were added to 2.0ml of each extract. The formation of an intense green color indicates the presence of flavonoid.
- Test for phenols: Three drops of 5% $FeCl_36H_2O$ solution were added to 2.0ml of each extract. The presence of phenol was shown by a deep blue-black color.
- Test for tannin: Two ml of each extract was mixed with 3.0ml of distilled water. To this mixture, 2.0ml of 5% $FeCl_36H_2O$ solution and the resulting brownish-green or dark-green solution confirms the presence of tannin.
- Test for saponins: Each extract was diluted with distilled water and shaken in a test tube for 15 minutes. The presence saponin is indicated by the formation of a layer of foam.
- Test for terpenoids: Two ml of $CHCl_3$ were mixed with 1.0ml of extract and 3.0ml concentrated H_2SO_4 were carefully added to form later. The presence of terpenoids was indicated by a reddish-brown coloration at the interface.
- Test for steroids: 5ml of chloroform ($CHCl_3$) and 2.0ml acetic anhydride ($(CH3CO)_2O$) were added to 2.0ml of each extract followed by concentrated H_2SO_4. The reddish-brown coloration at the interface shows the presence of steroids.

Antimicrobial Activity

The disc diffusion method was used to investigate the antimicrobial activity of *N. sativa* seeds extract against pathogenic bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus and Bacillus subtilis*). The bacterial strains cultures were subcultured onto the fresh nutrient agar [11]. Five colonies were picked from the fresh nutrient agar culture to prepare the bacteria suspension of each strain to be tested and inoculated in 3ml of distilled water. After five minutes, 100µL of each strain suspension was spread onto the plates of nutrient agar. Paper discs (5mm) prepared from Whatman no. 1.5 filter papers were placed on top of the inoculated plates. The extracts prepararion of *N. sativa* seeds, extracted were tested for their inhibitory activity against the pathogenic bacteria by pippeting 10µL of each preparation or extract onto the 5mm paper discs. The volume of each preparation delivered onto each disc was selected on the basis that plants may exhibit antimicrobial properties when assayed at an adequate amount [17,25]. Distilled water was used as a negative control in addition to the extracts while 5mm discs of a known antibiotic (Streptomycin) was selected among others and used as a positive control for comparision. All plates were incubated at 37^{o}C for 18-24 hours. The zones of inhibition were observed, measured and recorded..

III. Results and Discussion

Qualitative Phytochemical Screening

The qualitative phytochemical screening og aqueous, methanol, ethylacetate and hexane extracts of *N. sativa* seed is as shown in figure 1. All the four extracts were found to contain alkaloids, steroids and terpenoids. Other phytochemicals such as flavonoids, phenols and tannins were only detected in aquesous and methanol extracts. Saponin was only detected in aqueous extract. Aqueous and methaniol extracts of *N. sativa* were found to contain phytochemicals in a large amount compared to other extracts. The result of the present study showed

that *N. sativa* seeds are great reservoirs of medically active ingredients and this is consistent with the earlier reports [21]. Alkaloids, flavonoids and tannins are known to elicit broad antimicrobial activity against bacteria, fungi and parasites [8,19]. *N. sativa* seeds possess these important phytochemical constituents to which their pharmacological activities are ascribed; this signifies their potential use as medicine against microbial infection [21].

Table 1: Qualitative Phytochemical Screening of Aqueous, methanol, ethylacetate and hexane extracts of *N. sativa* seeds.

		Results			
Phytochemical Constituents	Test	Aqueous Extract	Methanol Extract	Ethylacetate Extract	Hexane Extract
Saponin		+	-	-	-
Alkaloid	Mayer and Wagner test	+	+	+	+
Phenol & Tanin		+	+	-	-
Steroids & Terpenoid		+	+	+	+
Flavonid	Lead acetate test	+	+	-	-

Antimicrobial activities of *N. sativa* seeds in different solvents.

The antimicrobial activity of *N. sativa* seed extracts was investigated against some common clinically resistant Gram negative (*E. coli, P. mirabilis, P. aeruginosa)* and Gram positive bacteria *(S. aureus and B. subtilis*). The zone of inhibition from different extracts were measured using streptomycin as positive control and distilled water as negative control as shown in table 2. All the extracts at 100 percent concentration were active against all the five bacterial except aqueous extract which is active against B. subtilis at 100 and 50 percent. Concentration activity of the methanol extracts at 100 percet followed the following order. P. auruginosa>P. mirabilis> E. coli> S. aureus> B. subtilis. The aqueous extract only showed antibacterial activity against B. subtilis. These observations are in agreement with similar works by shafodino [21].

Table 2: Antimicrobial activities of *N. sativa* seed in different solvent

		Gram negative		Gram positive		
Extracts	Concentration in %	*E. coli*	*P. mirabilis*	*P. aeruginosa*	*S. aureus*	*B.. subtilis*
N.S $_{aq}$	100	-	2	-	-	10
	50	-	-	-	-	8
	25	-	-	-	-	7
N.S $_{meth}$	100	12	14	15	11	10
	50	10	12	13	10	8
	25	6	11	8	6	4
N.S $_{Ea}$	100	8	10	12	10	11
	50	6	6	10	8	8
	25	3	2	6	4	4
N.S $_{hex}$	100	8	2	10	8	8
	50	4	-	6	6	4
	25	2	-	4	3	2
Positive treatment	Streptomycin	-	25	29	14	26
Negative treatment	Distilled water	-	-	-	-	-

Keynote: -- Resistant. < 8mm – not sensitive. 9-14mm – sensitive. 15 – 19mm ----- very sensitive. > 20mm ultra sensitive.

IV. Conclusion

The qualitative phytochemical composition and antimicrobial activity of N. sativa seeds was carried out using different extracts. . Different component which are medicinally active were successfully extracted using four solvents (water, methanol, ethylacetate and hexane). All the four extracts obtained above were found to contain alkaloids, steroids and terpenoids. Other phytochemicals such as flavonoids, phenols and tannins were only detected in aqueous and methanol extract. All the extracts at 100 percent concentration were active against al the five clinically resistant bacteria except aqueous extract which is active against *B. subtilis* at 100 and 50 percent concentration. The phytochemicals and/or the complex interactions among other constituents present in

the extracts may be responsible for their in dispensable antimicrobial activity. Therefore, the seeds of *N. sativa* can be used a natural source of antimicrobial agents in the pursuit of searching for new antibiotics against human pathogen.

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